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Variation in germination, in response to salinity and waterlogging and the link with seed tree habitat of ten Australian native species.

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Figure 1. Shoalhaven River at Ballalaba

Showing general landscape of the upper catchment; forested mountain tops with modified rural and riparian zones, hosting a mixture of native remnant stands, pasture and degraded soils.

Unless stated otherwise, this work is entirely my own. Signed: Suzanne Gray

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ABSTRACT

The aim of this research project was to investigate whether Australian native seed sourced from different habitats showed variation in tolerances to waterlogging and salinity. This was done by assessing germination under six different treatments of salinity, waterlogging, salinity with waterlogging and salinity with the addition of a biologically active fertiliser called 'PuraCast', derived from worm castings, product of the Industry sponsor. Seed was sourced from seven habitat types in the Upper Shoalhaven Catchment and neighbouring areas, for ten species within a 100 km radius of Braidwood, NSW. Species tested were: *Eucalyptus ovata, E. pauciflora, E. dives, E. blakelyi, E. aggregata, Acacia melanoxylon, A. decurrens, Casuarina littoralis, C. stricta, and Leptospermum flavescens.*

The impetus for research began when monitoring of revegetation project's showed variation within species for seedling survival of tubestock grown in the local Landcare Nursery. This observation was particularly marked on degraded soils and appeared to be linked to the locations from which the seed had been sourced. Glasshouse germination trials were designed for seven habitat types and results showed there were highly significant differences in response to treatments, particularly to the highest treatment of salinity which equated to a field value of medium salinity – 2-5 deci-Siemans/metre ($P<0.001$). Seed from saline, waterlogged and acid habitats showed a significantly higher germination than seed sourced from benign habitats ($P<0.001$). Differences in germination response to treatments were significant between high and low salinity habitats; and other habitat types ($P<0.001$). The germination of seed from acidic habitats in the medium salinity treatment was significantly greater than other habitats for *Eucalyptus* species ($P<0.001$). There was also a significant two-way interaction between treatment and habitat for the whole dataset ($P<0.05$).

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 The Issue

Rehabilitation of degraded land is a major issue facing land managers across Australia. Soil degradation due to salinity has been identified as a major cause of erosion and economic loss in many parts of Australia (Haw *et al,* 2000). Media reports (e.g. Landline, ABC, 19.09.04) suggest that over one million hectares (ha) of land were severely salt affected in Australia by 2000, with scientific sources suggesting that nearly five times this area is actually under threat, particularly in the Western Australian wheat belt (Bell, 1999, Stirzaker *et al*, 2002, Atwell *et al*, 2010). The National Land and Water Resource Audit in 2001 found that 4.7 million ha of land were salt affected, with the possibility this will expand to 17 million ha by 2050 (NLWRA, 2001, Stirzaker *et al*, 2002; Atwell *et al*, 2010). In the National Land and Water Resource Audit 2002-2008, no new information on the extent of soil salinity was available, but the lack of a coordinated approach to assessing salinity at a national level was identified as a problem. 'There is no current national database for land salinity information' that 'while significant progress has been made, Australia is still not well equipped to report on local progress, or national soil condition and change' (NWLRA, 2009 p.27). Although the work described in this thesis took place some years ago, it still has current relevance and aims to provide some insight into how the habitat of seed trees may affect the salinity and waterlogging tolerances of progeny. The discussion of results of these trials may help land managers in their provenance selection for rehabilitation of degraded sites via revegetation.

In the early 1980's, it was estimated that NSW contained about 4000 ha of salt affected land. By 1994, the area of known salt affected land in NSW covered over 100,000 ha (Christiansen *et al*, 1994; Haw *et al,* 2000), a 25-fold increase in little more than a decade.

The cost per annum is estimated to be approximately $250 million, in terms of agricultural productivity loss and infrastructure degradation but the long term cost of salinisation of waterways, plus loss of biodiversity and soil erosion is incalculable (Stirzaker *et al*, 2002). Dryland salinity has been identified as an environmental problem that requires cooperation and partnership from a national level down to the local community (Natural Heritage Trust of Australia Act, 1997). In the 1990's, rural landholders were encouraged to join forces with government assistance, to attempt to solve the major environmental problems of land degradation through community action, education and revegetation (Natural Heritage Trust of Australia Act, 1997 revised).

1.2 Land Degradation and Dryland Salinity

The term 'land degradation' is used in this thesis to refer to the anthropogenic acceleration of land and soil changes and the consequent impacts on agriculture; as defined in Haw *et al* (2000). Dryland salinity is described as the process of a rising watertable carrying dissolved salts to the surface, which is deleterious to vegetation and can result in a scalded area (Peck, 1978; Taylor, 1991). The watertable rises once deep-rooted trees are removed from the landscape and replaced with cereal crops or pastures; and it is estimated that as a direct result of human action, the area of saline land in Australia is increasing by as much as 5% per annum in some regions, although the rate of clearing has declined in recent times (Atwell *et al,* 2010). Clearing of trees and replacement with pastures reduces water absorption from the root-zone, due to the shallower roots of grasses (Bari and Schofield, 1990; Haw *et al*, 2000; Lefroy and Stirzaker, 2002). This may increase rainfall entering the groundwater, which can cause it to rise at low points in the catchment (known as discharge areas) leading to waterlogging and soil salinity (Bari and Schofield, 1990; Haw *et al*, 2000; Lefroy and Stirzaker, 2002).

The groundwater (also known as the watertable) is at the bottom of the soil profile, above bedrock, generally several metres below the soil surface (Taylor, 1991). It is subject to hydrogeological and biophysical processes across the whole catchment, including the process of evapotranspiration, where tree roots draw in moisture which is then transpired into the atmosphere through their leaves (Peck, 1978; Taylor, 1991). The first signs of rising groundwater to the casual observer are vegetation dieback and waterlogging of low lying areas, even during dry times (Bell *et al,* 2001). Although tree removal may have occurred in patches across the catchment, rising groundwater usually becomes evident near the surface of low lying areas, but it can also seep out of hillsides where geological formations hold groundwater above impervious rock layers, known as a perched watertable (Stirzaker *et al*, 2002, Atwell *et al*, 2010). Groundwater contains varying levels of dissolved salts that have moved down through the soil profile with rainfall, via the natural process termed leaching. Groundwater salinities can vary greatly, depending on the age and type of the sediments, but the process for soil salinisation is the same, regardless of salt concentration. Once dissolved salts are carried upwards, salt precipitates out and increases in concentration, becoming toxic to soil biota, particularly fungi and microbes; and vegetation dies (Stirzaker *et al*, 2002, Atwell *et al*, 2010, Chowdhury *et al*, 2011). Sites affected by sever soil salinity have a lack of vegetation, salt deposits on the soil surface, scalding (salt scalds) and eventually, a hardpan of crusted salt on the soil surface (saltpan) (Peck, 1978; Eastham *et al*, 1993; Bell *et al,* 2001; Stirzaker *et al*, 2002).

The Australian continent has approximately 300 million ha of soils underlain with highly saline watertables that lie relatively close to the surface and clearing of native forests, particularly via clear-felling across whole landscapes has caused a rise in groundwater (Atwell *et al,* 2010, Bell, 1999). The State of Environment Report (SoE 2006), refers to the

National Land and Water Resources Audit (2001) and states that approximately 2 million ha of arable land were salinised in 2000 (NLWRA, 2001; SoE, 2006). It was suggested in the SoE 2006 that this area is likely to increase. The NWLRA 2002-2008 and State of the Environment Report 2011 do not actually quantify increases in salinity, but note increasing soil acidity. Figure 1 below shows salinity hazard.

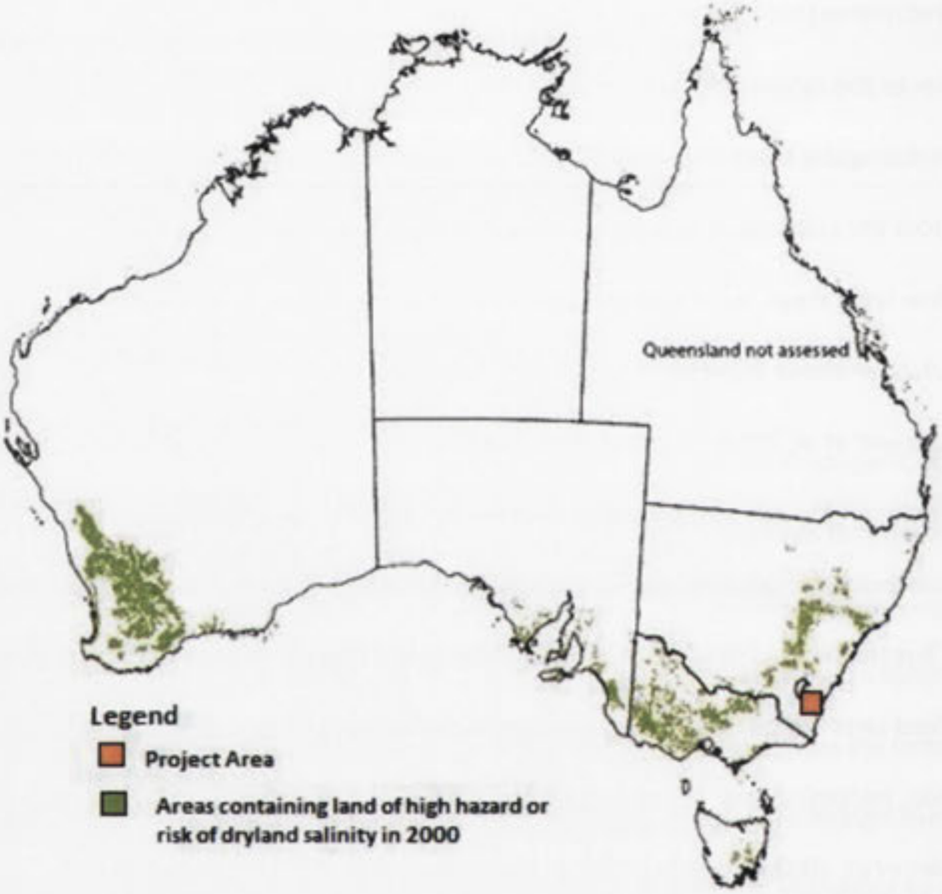


Figure 1. Area containing land of high hazard or risk of dryland salinity in 2000. Image courtesy NLWRA, 2001

Where the watertable lies within 1.5-2 m of the surface, evaporation draws moisture up and out via stable soil capillaries and when the rising groundwater is saline, salts precipitate out, depositing clumps of salt that accumulate at the soil surface mainly in the A and B horizons (Peck 1978).

The process of a tree or shrub drawing water in through root hairs and the subsequent loss of water to the atmosphere through leaf stomata via evapotranspiration helps to maintain the watertable level below the ground surface (Peck, 1978, Kumar and Rao, 1985, Taylor, 1991, Bell, 1999). Established tree roots are able to draw water from deep within the soil profile and, when in close proximity to the watertable can access groundwater directly from it, provided it is of a low salinity (generally EC < 1 deci-Sieman/metre – dS/m soil salinity, - Taylor, 1991). A transpiring forest or open woodland loses moisture to the atmosphere constantly, sourcing its water from both incident rainfall and, the watertable and this process keeps the watertable generally more than 2 m below the surface (Atwell *et al,* 2010). When the forest, open woodland or vegetative cover is removed and replaced by shallow-rooted, lower leaf area grasses and legumes, a much larger amount of incident rainfall moves down through the soil profile and, as a result, there are net gains to the water table, which then rises and leads to soil salinity (Hoy *et al*, 1994; Haw *et al*, 2000 Stirzaker *et al,* 2002, Atwell *et al*, 2010).

As soil salinity increases, seepage scalds form, vegetation dies and a crust of salt can become visible on the soil surface, a problem referred to as secondary salinity (Peck, 1978; Taylor, 1991). Salt affected, low-lying land is called a discharge area, where water is discharged from the ground, some distance downstream from the recharge area where it was absorbed (Taylor, 1991). The distance between recharge and discharge areas is at a catchment level, so the location of scalds have no relation to property boundaries, nor are they necessarily related to tree cover in the immediate vicinity; and as such, the problem cannot be dealt with purely on the basis of one property owner taking action, but needs catchment-wide action (Christiansen *et al,* 1994; Richardson and Narayan, 1995; Stirzaker *et al*, 2002). Returning salt affected land to productive use is a major challenge facing many land managers across Australia and finding salt tolerant species for these sites (Stirzaker *et al*, 2002).

1.3 Rehabilitation

If the salinity problem is not addressed in the early stages, saline, denuded soil becomes light and puffy and erosion often follows (Peck, 1978). If the problem continues to be left unattended, topsoil loss may occur (Peck, 1978; Bell 1999). Once topsoil has been lost, subsoil erosion often follows via both sheet erosion and gully erosion that cause the site to become highly degraded and costly to rehabilitate (Bell, 1999). Hay bales placed at the head of an erosion gully can alleviate the erosion problem in its early stages and plantings or successful direct seeding may arrest the soil loss (Hoy *et al*, 1994). Topsoil loss exposes the subsoil (which can be alkaline, with calcium carbonate clumps clearly visible) and the land generally becomes unproductive for agriculture. Rehabilitation then requires heavy machinery to move large quantities of soil into and away from the gullies; and ripping then mounding for planting and seeding; and undertake erosion control works such as concrete flumes, wire mesh and other highly expensive gully control measures before soil stabilisation can occur (Taylor, 1991, Ritson and Pettit, 1992, Stirzaker *et al*, 2002).

Revegetation of salinised areas with salt tolerant tree species before major erosion gullies form is a widely accepted method of rehabilitation, with plantings recommended in both scalded soils, at the midpoint or break of slope and, upslope from saltpans (Morris and Thomson, 1983, Greenwood *et al*, 1985, Taylor, 1991, Bari and Schofield, 1992, Christiansen *et al*, 1994, Dunn *et al*, 1994, Bell, 1999, Benyon *et al*, 2001).). Revegetation trial work in the 1980's and 1990's showed a preliminary reduction in watertable levels, potentially as a result of tree plantations being established nearby (Biddiscombe *et al,* 1981; Bari and Schofield, 1992; Benyon *et al*, 2001), but as this was also a time of drought, it is not fully conclusive. Some studies have shown that a single tree will depress the watertable immediately within its vicinity, and a plantation even more so (Stirzaker *et al*, 2002). Break of slope plantations have been advocated as a means of watertable control, because trees are planted out of the immediate scalded zone, but still close enough to affect the watertable (Bari and Schofield, 1992, Christiansen *et al*, 1994, Dunn *et al*, 1994, Bell, 1999).

In some areas, however, more recent findings raise questions about the long term benefit of plantations in break of slope positions, particularly where the position of the raised watertable is highly localised, for example, a plantation placed upslope from a perched watertable was found to be quite ineffective (McJannet *et al,* 2000). Success of tree plantings on saline soils in Australia has been variable, in terms of tree survival and also, the ability of plantations to lower watertables over the longer term, but revegetation is still accepted as the most cost-effective approach to improve soil and in-stream salinity (Hairsine and van Dijk, 2006).

Transpiration is highest in vigorously growing young trees, not senescing plantations so plantings into or near salt scalds, if successfully established, may only be a short term solution for lowering the watertable (McJannet *et al*, 2000). Salt tolerant tree species planted near salt scalds may be able to utilise groundwater and grow for a number of years, but over time, the constant use of groundwater leaves deposits of salt around the root zone. The salts build up and eventually can become toxic and lethal to vegetation, as described in sections above and below (Bennett and George, 1995; Stirzaker *et al*, 2002). Other studies suggest that salt tolerant shrubs, herbs and grasses might be a more effective method of returning salt scalded land to productivity, or at the very least, control soil loss.

These findings further highlight the need for identifying the most salt and waterlogging tolerant trees and shrubs to lower the watertable. Salinity and waterlogging tolerant trees can be planted closer to a scalded area than break-of-slope plantations; and even if they are only able to depress the watertable for 5-10 years as some research has shown (McJannet and Vertessy, 2002) at the very least, unproductive agricultural land can be utilised in the short-term.

A longer term, successful method for reclamation of degraded land was found in the experimental work of Bari and Schofield (1991 and 1992) where they looked at the effect of agroforestry plantings on the watertable over a ten year period and showed that plantings into moderately saline soils lowered the watertable level by approximately 10%.

Direct seeding is a much more cost effective method for revegetating degraded sites than tree planting, but both methods are recommended. The size, placement and shape of plantations, site preparation and planting methods have all been identified as key aspects of a successful rehabilitation plan, as well as appropriate choice of species and provenance selection (Bari and Schofield, 1991; Bell, 1999, Niknam and McComb, 2000; Stirzaker et al, 2002). Further investigation into the planting and direct seeding densities of selected species, with provenances tailored to suit individual sites, has also been recommended (Walsh et al, 1995; Niknam and McComb, 2000; Stirzaker et al 2002). The importance of considering local environments and climates for seedling provenance selection has been deemed an important factor during revegetation projects (Christiansen *et al,* 1994; Richardson and Narayan, 1995; Stirzaker *et al*, 2002). Provenance variation was previously identified as an issue worthy of research when selecting species for degraded agricultural land (Benyon *et al*, 2001; Khurana and Singh, 2001; Stirzaker *et al*, 2002).

Establishment of tolerant trees on degraded sites, for livestock shelter, firewood and perhaps even commercial timber could result in low productivity land becoming economically viable. If commercial tree cover is not possible, establishment of native tree and shrub shelter belts can assure at the very least, that topsoils are protected and stock animals have some additional shelter (Scott *et al*, 2000). Any form of vegetative cover on degraded sites is more acceptable to land managers than eroding topsoil and denuded landscapes, so if nothing else is possible, establishing a herb and grass layer of tolerant native species is desirable (Scott *et al*, 2000, Stirzaker *et al*, 2002).

For the purpose of soil conservation, using locally occurring native plant species that are tolerant to multiple environmental factors *e.g.* salinity and frost, could ensure resources and habitat for native fauna (Stirzaker *et al*, 2002). A multipurpose planting of local species with proven survival rates could be presented as a more acceptable solution to landholders than a single purpose planting of non-local native species and/or exotics and locating suitable populations of local species for rehabilitation is becoming increasingly important (Stirzaker *et al*, 2002). Marcar (1989) suggested a possible link between frost resistance and salinity tolerance for forest species of *Eucalyptus* and other research suggests that salt tolerant seedlings are more likely (but not necessarily) to be found from trees in saline habitats than the same species in other habitats (*e.g.* Niknam and McComb, 2000; Stirzaker *et al*, 2002) Provenance variation in salinity, waterlogging tolerances and multiple tolerances within Australian natives has been identified (Walsh *et al,* 1995; Niknam and McComb, 2000; Stirzaker *et al* 2002). Whether this variation is linked to adaptation to local environmental conditions in the seed tree habitat was the key focus of this thesis.

1.4 Development of Project

The Landcare movement began in the Upper Shoalhaven Catchment in 1992, after a public meeting which fostered the formation of Landcare groups. Government-funded planting of shelterbelts and farm forestry plots began in 1993 through Part 3 of the *Natural Heritage Trust Act* 1997 (hereafter, referred to as NHT). Many of the early NHT revegetation programs in frost-prone areas of the Upper Shoalhaven Catchment exhibited a seedling mortality of greater than 50%, mostly due to their lack of frost hardiness. The need for research into appropriate species for areas of climatic extremes became evident. (Booth, 2012; Tallaganda Shire – Ballalaba-Krawaree Landcare Group Inc. reports and meeting minutes 1993-1995). Landcare groups, the Catchment Management Committee, Office of Environment and Heritage (formerly Dept. of Land and Water Conservation), Salt Action partnered me in my role of Project Coordinator at the Braidwood Landcare Nursery, as we began to answer questions of best species for rehabilitation of degraded sites in the local area and locations of the best seed sources. This report details the seed collection sites, seed sources and germination trials that were part of the initiative.

Endemic native species were strongly favoured by NHT funding bodies for use in rehabilitation and revegetation projects (NHT Act, 1997 Section 10) and funding was obtained from Salt Action NSW to investigate suitable local native species and variation within those species. Seed trees were identified during Landcare Nursery seed collection and revegetation projects; and also via survey work (Storey and Gray, 1996). Seeds were sourced across a range of habitat types, from ten local species. The germination project was conducted at the Landcare Nursery in a solar passive glasshouse, constructed using NHT grant funding at Braidwood Central School's agricultural plot 1995. Species selection and location of seed trees on different habitats was a process that spanned several years.

1.4.1 Provenance Variation and Previous Research

All provenances and sites for seed collection were found in the 100 km radius around Braidwood, mainly in the Upper Shoalhaven Catchment. A large diversity of species and habitats were found across a variety of ecosystems in the Upper Shoalhaven Catchment, with some vegetation communities adjacent to developing saltpans (Storey and Gray, 1996). Communities ranged from tall mountainous forests of *Eucalyptus seiberi* (Mountain Ash) and *E. fraxinoides* (Alpine Ash) between Braidwood, Cooma and Captains Flat; to *E. fastigata* (Brown Barrel) - forest canopy for rainforest shrubs and trees in areas such as Tallaganda State Forest and Monga, now part of the Monga National Park (on the escarpment 25 km south-east of Braidwood); to *E. pauciflora* (Snowgum) woodlands with sub-alpine meadows at higher elevations; to upper and lowland heathlands, open woodlands, coastal heathlands, littoral rainforest and riparian communities (Boland *et al*, 1986).

Variation within species inhabiting such a diverse region is to be expected (Blake, 1981; Boland *et al*, 1984; Gibson and Bachelard, 1987; Khurana and Singh, 2001, Atwell *et al*, 2010). In this thesis, "adaptation" is defined as a "trait (i) that enhances the fitness of an organism, and (ii) whose current beneficial characteristics reflect the selective advantage of the trait at its time of origin" (Hochachka and Somero, 2002). Adaptation to rainfall gradients (Prior *et al*, 2005) and to the amount of shade in a plant's habitat has been found in previous work (Shanker and Venkateswarlu, 2011). The amount of shade a seedling is exposed to may relate to canopy cover and forest species sourced from around Braidwood for use in Landcare Nursery projects were found to have poor ability to withstand frost in denuded areas, where no canopy cover was offered. Forest species in the Upper Shoalhaven were deemed attractive for revegetation projects because potentially, they had the dual purpose of lowering the watertable and also providing timber. Seedling sensitivity to frost may be as a result of the protection offered by the forest canopy during germination and early seedling growth and seedling adaptation, as per the work by Shanker and Venkateswarlu (2011).

This finding of forest sensitivity was supported by the work undertaken by Holly *et al*, (1994) and Ball *et al* (1997), which found that the effect of frost was related to a seedling's microclimate; and work by Battaglia and Reid (1993) on the forest species *E. delagatensis* which showed that canopy protected microsites resulted in higher germination and seedling survival and previous research suggested that frost tolerance may also have a link to salt tolerance (Battaglia and Reid, 1993, Marcar, 1989).

The forest species sourced from around Braidwood for use in Landcare projects were also found to have poor ability to withstand frost on saline, waterlogged and acid soils and previous research suggested that frost tolerance may also have a link to salt tolerance (Battaglia and Reid, 1993, Marcar, 1989).

A review of previous research into salt tolerant species revealed that there are conflicting results in glasshouse and field trials for at least 13 *Eucalyptus* species. and several *Acacia*, *Casuarina* and *Melaleuca* species (Niknam and McComb, 2000). It is possible that such inconsistencies were due to provenance variation within the species and, adaptation to local habitats. Variation in salt tolerance has also been found within highly salt tolerant Eucalypt species, such as *Eucalyptus camaldulensis* (Sun *et al*, 1994; Benyon *et al*, 1999; Stirzaker *et al* 2002, Marcar *et al*, 2002, Mahmood *et al*, 2009). Some previous work as mentioned above, has investigated the link between frost resistance and salt tolerance (Marcar, 1989); other work has simply selected seed sources from saline habitats and still found variation within species (*e.g.* Craig and Atkins, 1990). These findings suggest that variation could be one of the strategies for a species' survival and colonisation of regions where climate is extreme and unpredictable; and also suggests that multiple genes govern salt tolerance (Emms and Arnold, 1997, Kawecki and Ebert, 2004, Shanker and Venkateswarlu, 2011).

Previous research identified the need for investigating biodiversity within non-halophyte species and the possible relationship with adaptation to the environmental stresses as particularly important for sourcing seed for reclamation of saline soils though revegetation (Marcar, 1989; Bari and Schofield, 1991; Dunn *et al,* 1994; Greenwood *et al*, 1995; McJannet and Vertessy, 2000; Niknam and McComb, 2000, Mahmood *et al*, 2009). Work quoted in Atwell, *et al,* 2010 suggests that heritability of salt tolerance is partly due to evolutionary pressures from environmental conditions (*e.g.* for salt tolerance differences between rice and wheat), but also that salt tolerance involves separate genes, as mentioned above. Plants with both genes are more salt tolerant. Although some findings suggest that saline environments are likely to produce a higher degree of salt tolerance than less challenging environments for non-halophytic plants (Osmond *et al*, 1987; Niknam and McComb, 2000, Atwell, *et al,* 2010), there are exceptions. These exceptions may be due to factors such as cross pollination, resulting in only one of the genes being passed on; or both genes appearing in progeny from parent trees that only had one gene (Atwell, *et al,* 2010). It is also possible that tolerance to one environmental factor is linked to others, such as in the case of tolerance to one heavy metal conferring tolerance to many (Kawecki and Ebert, 2004), so acid or frost tolerant plants may also contain the salt tolerance gene.

Using methods that isolate single environmental factors was found successful for differentiating between whole species tolerance and habitat adaptation of individual populations (Kawecki and Ebert, 2004, Shanker and Venkateswarlu, 2011). For this thesis the linkage between habitat adaptation and salt tolerance was investigated via daily observations and targeted analysis of germination under conditions of low and medium salinity, waterlogging only and salinity with waterlogging treatments.

1.5. Project Overview in Context with Literature

The effect on germination was investigated using the following hypothesis:

Null Hypothesis

Variation in the germination response to salinity and waterlogging for selected native species found within a 100 km radius of Braidwood, NSW, is not due to a difference in seed tree habitat type.

1.5.1 Rationale for Research

Genetic variation within Australian native species has been recorded, particularly for *Eucalyptus* spp., which hybridise readily and occupy large ranges of habitat types (Boland *et al*, 1984; Gibson and Bachelard, 1987; Bell, 1999, Niknam and McComb, 2000). Previous research has shown that provenance variation exists for seedling growth under environmental gradients, such as rainfall/water stress, salinity, frost and, acidity (Blake, 1981, Boland *et al*, 1984, Gibson and Bachelard, 1987, Bell, 1999, Khurana and Singh, 2001; Standish *et al*, 2007, Atwell *et al,* 2010). Other research also suggested the possibility that seed source habitat could be a contributing factor to provenance variation, particularly for salt and waterlogging tolerances (Bell, 1999, Niknam and McComb, 2000). The overall health and morphology of seed trees had previously been established as important when selecting trees for seed collection (Greening Australia, 1992).

This research project investigated whether or not intraspecies variation and adaptation to various habitat characteristics resulted in seeds that retained these adaptations, allowing them to germinate under conditions of salinity and waterlogging. 'Provenance' has been defined in previous work as 'geographically separate in origin' (Boland *et al*, 1984; Stirzaker *et al*, 2002). Such separation over time, according to Darwin's theory of evolution, can lead to a genetically distinct population within a species, particularly where environmental conditions vary widely from one location to another and adaptation can occur in response (Suzuki *et al*, 1986, Kawecki and Ebert, 2004, Standish *et al*, 2007). In this project, each seed collection site was regarded as geographically separate, with a different habitat and therefore, a different provenance. Each collection site was characterised by a relatively simple definition of habitat type. Previous research demonstrated that provenance variation occurs in germination under water stress; and under differing conditions of waterlogging, salinity; and waterlogging with salinity (Gibson and Bachelard, 1987, Bell, 1999). Prior research also showed variation can be linked to environmental extremes in their native habitat, such as frost tolerance, drought, waterlogging and salinity tolerance (Blake, 1981; Sun and Dickinson, 1995, Bell, 1999).

Experiments were set up to mimic conditions observed in the field, presumed to result from a rising watertable, with conditions of low to moderate dryland salinity and waterlogging. The forms of land degradation dealt with specifically in the germination trials were exposure of seeds to moderate and low saline and waterlogged conditions.

All seed trees for this project were within a 100 km radius, so the geographical separation was actually small and hence, it is likely most of the different stands were from common ancestral stock (Kawecki and Ebert, 2004). If differences were found to occur at this scale, it will differ from Bell (1999), who found that provenances which showed marked differences in salinity and waterlogging tolerances were geographically separated by several thousand kilometres. Some previous work has also found changes in germination under differing conditions of salinity and waterlogging, but no correlation between seed tolerances and seedling tolerances in the field (Ladiges et al 1981; Clemens *et al*, 1983; Pearce-Pinto *et al*, 1990, Bell, 1999) or adult plants (Morris 1984).

1.5.2 Measuring Salinity and Waterlogging Tolerances

Previous experiments have noted provenance variation to salinity tolerance for a range of growth factors, including seed germination and leaf morphology, showing some possible linkages to seed tree habitat (Sands, 1981; James and Bell, 2001; Niknam and McComb, 2000). Much of this previous work has focused on investigating the salinity tolerances of Australian native tree species in glasshouse trials, rather than field trials (Blake, 1981; Aswathappa *et al*, 1987; Marcar, 1989; van der Moezel *et al*, 1991), which is an acceptable method, as it limits the factors being tested, allowing focus on a particular trait (Kawecki and Ebert, 2004). Glasshouse trials were the method chosen for investigating germination trials discussed in this thesis.

Glasshouse trials that isolate each factor (salinity, waterlogging and salinity with waterlogging) may help to reveal the underlying genotype and whether adaptation is occurring at the level of local habitat in native tree species, that results in higher tolerances to salinity and waterlogging (Kawecki and Ebert, 2004). Seed germination and early seedling growth are physiological processes affected by soil salinity and to a lesser extent, waterlogging so, if measured over a six week period, germination and early seedling growth may help to assess potential salt and waterlogging tolerances and whether variation within local species is a result of adaptation to habitat (Kawecki and Ebert, 2004, Atwell, *et al,* 2010). Germination and measurements of seed viability – as reported in this document followed methods of previous work (Australian Tree Seed Centre; Clemens *et al,* 1983), particularly methods used by Clemens *et al* (1983).

The exploration of broad categories of habitat used for this experiment are described below, in the context of species selection and the literature.

1.5.3 Saline Habitats

A saline habitat was defined as a site hosting a group of trees or shrubs with topsoil ECe of >1.0 dS/m and several species of interest were present (making repeat visits more cost effective). The first saline habitat to be investigated was the coastal littoral rainforest, fringing the Pacific Ocean, near Kioloa and Bateman's Bay. In the early days of project development, possible frost sensitivity due to its coastal provenance was deemed to be of less importance for glasshouse germination trials than actual salt exposure of the seed tree. The sea winds carry large volumes of salt spray several kilometres inland and species fronting the ocean are subjected to high levels of both airborne and soil salt (Malloch, 1971; Moser, 1979). Several species of interest were located directly fronting the ocean and exposed to constant salt spray both in the air and on the ground. Some nearby specimens of interest were located growing under a canopy of *E. maculata and E. botryoides.* For rainforest specimens, soil testing beneath and around the *Eucalyptus* canopy occurred, to ensure plants were actually exposed to soil salt. It was deemed possible that specimens growing directly beneath the canopy were not exposed to high levels of salt and thus, not sufficient environmental pressure for the plants to develop adaptation to salt tolerance (Kawecki and Ebert, 2004). In some types of undisturbed coastal vegetation, salt loads can drop off rapidly in the first 500 m (Malloch, 1971; Moser, 1979). Any form of vegetation may act as a screen; even a bare twig can capture airborne salt and prevent it from travelling further inland (Boyce, 1954). So, despite the orographic uplift of the Great Dividing Range within 60 km of the coastline drawing in large volumes of sea mist over the lower slopes, only the first 250 metres of vegetation inland from the beach were surveyed for potentially salt tolerant species. It is possible that highly salt tolerant specimens may be found growing further inland, but the barrier for this project was set at 250 m. This was clearly the zone of high salt deposition, made visible by asymmetrical growth of tree and shrub canopies (described in coastal studies by Parsons, 1966, Parsons and Gill, 1968), leaf tip necrosis (described in studies by Boyce, 1954; Moser, 1979; Barbour, 1978) and the stunting of rainforest tree species into shrubs. Soil results, as shown in Table 6, for Kioloa, support this.

Potential salt tolerant species on the coastal sites identified for possible inclusion were: *E. maculata, E. botryoides Leptospermum flavescens, Casuarina stricta* and *C. littoralis.* Previous work testing various *Casuarina* species showed that many exhibited salt tolerance (Aswathappa, 1987; Sun and Dickinson, 1995). *E. maculata* was found to be salt tolerant in field trials (Biddiscombe *et al*, 1981) south east of Perth, where it was one of the tallest species on both mid and upslope. *E. botryoides* was found to be salt tolerant in field trials, but not in liquid culture trials leading up to the field trials. It was suggested that genetic variation within the species was the reason for this anomaly (Blake, 1981). Despite previous work suggesting the salt tolerance potential of the two *Eucalyptus* species species, *E. maculata* and *E. botryoides*. These were discarded from the trial, due to a lack of frost tolerance (evidenced by frost kill of seedlings within the propagation houses at the Braidwood Landcare Nursery). Viable seed from *C. stricta, C. littoralis* and *L. flavescens* were sourced from relatively healthy trees, on coastal saline sites and seedlings grown from it were found to survive the frosts that killed the Eucalypts.

Eucalyptus species were sought on saline habitats on the Southern Tablelands. Severe salt scalding recorded on "Millended Springs" (C.S.G. 1992) lead to field surveys of remnant stands and their topsoil. Several other scalds were observed on nearby properties along the Nerriga and Mayfield Roads, North-east of Braidwood and these were also investigated for identification of remnant species and topsoil sampling. These sites were denuded and eroded landscapes, with stunted trees and large areas of bare ground, sliced open by erosion gullies.

Vegetation and soil surveys on and around the scalds enabled the sourcing of seed from several species of *Eucalyptus* and *C. littoralis*. Seed capsules were collected from the healthiest specimens found near scalds or on the adjacent erosion gullies.

1.5.4 Waterlogged Habitats

Waterlogged soils were defined as being perennially wet, due to natural springs, wetlands and/or riparian zones. Remnant vegetation stands within 5metres of a waterbody were assessed over several years for potential species. Trials on a saline site in the Northern Tablelands conducted by Sun and Dickinson (1995) showed that some of the most salt tolerant native species were from seed sourced in riparian zones. Several waterbodies surveyed had a wide diversity of species. In some cases there were >10 species. /m^2, with

>10 specimens within a 50 m^2 area. Seed was collected for a range of *Eucalyptus* and *Acacia* species, *C. littoralis, L. flavescens* and *Callistemon seiberi.*

1.5.5 Acid Habitats

The Upper Shoalhaven district has widespread, natural soil acidity (Jenkins, 1996), so it was relatively easy to source seed from the selected species from remnants growing on acidic habitats. An acid habitat was defined as having a topsoil which had a pH< 5.0. It was not expected during the discussion of experimental design that differences in salinity and waterlogging tolerances would be measurably influenced by an acid habitat, so topsoil measurements were kept low-cost and simple. This was definitely an oversight and is discussed further in the chapters of results and discussion.

1.5.6 Dry Habitats

A dry habitat was defined as having a soil pH of 5 or more and an ECe of <1.0dS/m and, not adjacent to a waterbody or other type of waterbody. Sites which contained remnant vegetation that were perennially dry, not acid and not saline were rare in the project area. This is indicative of the widespread soil degradation in the Upper Shoalhaven Catchment.

1.6 Effects of Salinity and Mechanisms for Tolerance

Soil salinity has two types of stress. The first stress is adverse water relations, due to the high salt levels disrupting osmosis at the root tip and the second stress is the toxicity of the ions present in the soil (Munns, 1980). The ions disrupt the cation exchange mechanisms at the root tips and the osmotic potential of guard cells in the stomata that facilitate the absorption of water (Bell, 1999). The cell walls of soil microbes and biota are also affected by the high concentration of salts in the surrounding soils. A reduction in microbial biomass and loss of soil biota has been recorded with increasing soil salinity (Chowdhury *et al*, 2011). Osmotic stress is experienced because the root hair or the microbial cells have insufficient dissolved salts (osmolytes) to draw water from the surrounding saline topsoil via osmosis (Atwell *et al*, 2010, Chowdhury *et al*, 2011). High levels of dissolved salts in the watertable often contain a high percentage of the chloride ion (Cl^-), which has the ability to become a free radical that can break molecular bonds and interfere with mechanisms necessary for biological processes. Sodium cations (Na^+) are also an issue and may cause sodium toxicity, called sodicity (Atwell *et al,* 2010). Salt tolerance in non-halophyte plants is mainly due to mechanisms at the root hair for excluding salts, particularly sodium and chloride ions; and mechanisms in the leaves for locking up the salts which enter the plant from both the soil and the air (Atwell *et al,* 2010).

Salt tolerance has been studied in more detail in commercial, non-halophyte plants and found to be based on separate genes which govern mechanisms for exclusion of both Cl^- and Na^+ ions at the root zone and compartmentalisation in the leaf or shoot zone (Atwell *et al,* 2010). The genes which govern each exclusion mechanism can occur singly or together and this in itself leads to genetic variation within a species (Kawecki and Ebert, 2004, Atwell *et al,* 2010). If only one gene is present in a population, this may lead to less salt tolerance when compared to the same species from a different population which contains all genes because genetic recombination during meiosis may result in the loss of a single gene, but with more than one gene present possibly on different alleles, it is less likely to occur. It is possible that other genes are also connected to these salt tolerant genes in some species, leading to multiple tolerances (Kawecki and Ebert, 2004). Screening of possible salt tolerant species, measuring growth rates, biomass and transpiration rates (which affect watertable levels) have been the emphasis of much of the previous scientific research into trees for successful rehabilitation (*e.g.* Sands, 1981; Dunn *et al*, 1994; Sun *et al*, 1994; Sun and Dickinson, 1995; Benyon *et al*, 2001), with little work specifically on variation within species and its possible link to localised adaptation to habitat. If adaptation to the local habitat is occurring, it is suggested that this could be passed on to the progeny. For discrete, discontinuous populations across diverse habitats, such as found in the Upper Shoalhaven, this localised adaptation may be measurable. This assumption formed the basis for this project's testing of the hypothesis.

1.7 Effects of Waterlogging and Mechanisms for Tolerance

Waterlogging occurs naturally in wetlands, marshes, near underground springs and beside streams, but also with a rising watertable as the first stage of dryland salinity (Taylor, 1991). Waterlogging causes anaerobic conditions in the soil which induces changes in root structure. Tolerant plants respond with the formation of adventitious roots and aerenchyma tissue at the root membrane and exclusion of toxic ions or other anoxic compounds (Kozlowski, 1984, Bell, 1999). Cellular changes to the guard cells and stomatal opening have been noted, which can cause changes in water intake, transpiration rates and levels of plant hormones, particularly abscissic acid, cytokinins and gibberellins (Davison and Tay, 1985, Kozlowski 1984, Bell, 1999). When waterlogging is combined with soil salinity, the mechanisms needed for tolerance increase. Waterlogging tolerant Eucalypts exposed to low levels of salinity were found unable to form adventitious roots (van der Moezel *et al,* 1989, Marcar, 1993). Other adaptations to the anaerobic conditions of waterlogging that increase oxygen levels in root tissues are also disrupted by soil salt. Salinity with waterlogging tolerant plants must have multiple mechanisms of tolerance that include the ability to draw in water at the root hairs in the absence of oxygen and in the presence of competitive cations and destructive anions (Bell, 1999).

1.8 Effects and Extent of Acidity in Australia

Many Australian soils are millions of years old and hence, highly leached. The result is natural acidification (Jenkins, 1996). Many Australian native species are adapted to soil acidity and low availability of phosphorus (Rahman, 1991). Agricultural enterprises which rely on introduced species for animal production or broad scale cropping, often require additional application of phosphate and nitrogen to be economically viable. Research has shown there can be < 50% absorption of nitrogen and potassium, and 10% absorption of phosphorus in applied fertiliser (Baligar *et al*, 2001). The low absorption rate of phosphorus requires high level applications to achieve desired plant growth, often resulting in run-off and accumulation of superphosphate. Anthropogenic acceleration of soil acidification through long term use of superphosphate - a preparation of rock phosphate and sulphuric acid, is occurring in Australia's agricultural zones and soil acidity has been identified as a substantial problem (Scott *et al*, 2000; Stirzaker *et al*, 2002, SoE, 2011). Across Australia, it is estimated approximately 50 million ha of agricultural land are affected by soil acidity (SoE, 2011). In 2001, the estimated cost due to lost agricultural production as a direct result of soil acidity was $1.585 billion, which incidentally, is approximately eight times the estimated cost of soil salinity at that time (SoE, 2011).

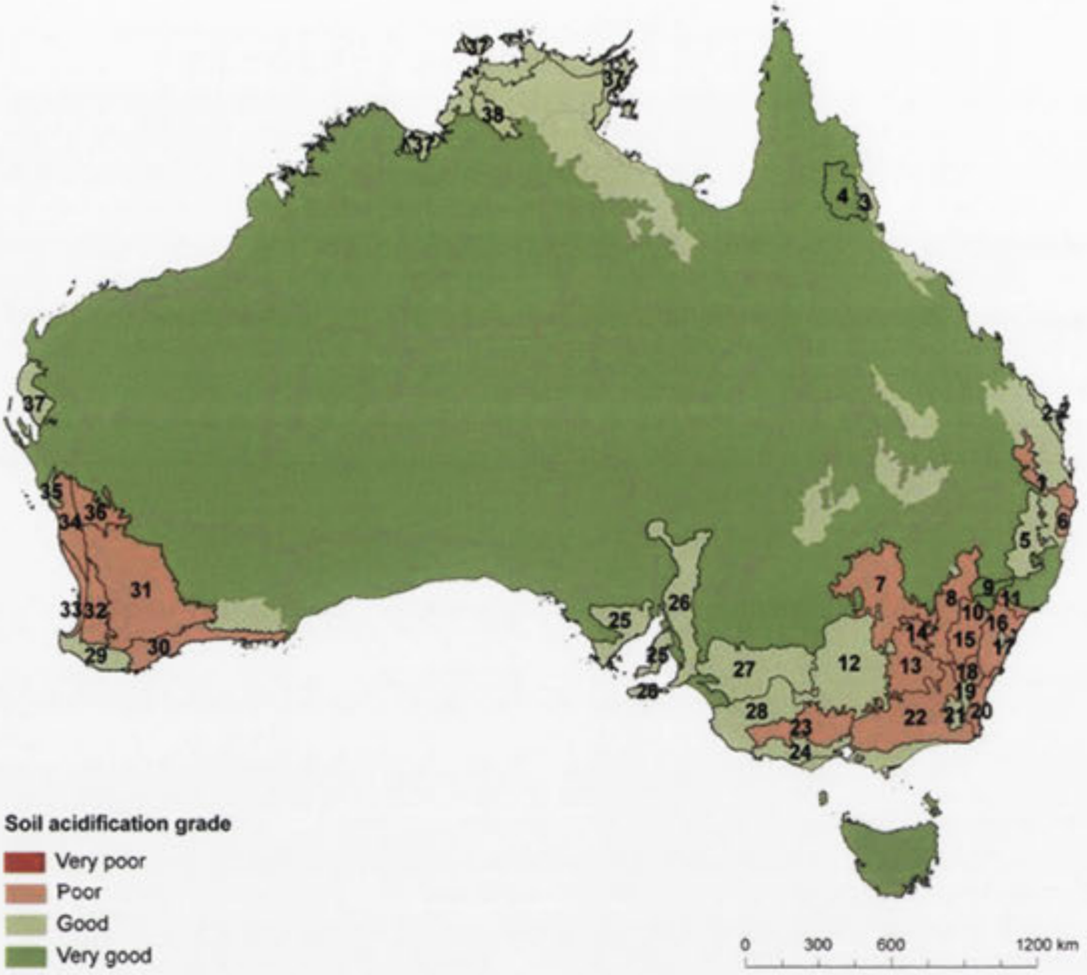


Figure 2. Soil Acidity in Australia (from SoE, 2011)

The process of soil acidification (natural or anthropogenic) is measured as a decrease of soil pH. This can lead to changes in soil chemistry resulting in the breakdown of compounds which are chelated with Aluminium (Al), Iron (Fe) and Manganese (Mn). These three elements can then be released in ionic form into the soil solution environment, leading to toxicity, which have been shown to correlate with a decrease in the availability of phosphorus and limiting to plant production (Kochian *et al*, 2004). When the soil is pH<5.0 Aluminium is released in the form of Al^{3+} into the soil solution, which is a rhizotoxic ion, restricting root hair growth almost immediately and an lead to long-term structural root damage, particularly at the apex, the outer cell walls and can even penetrate into the cell nucleus, so that cell division and root elongation are also disrupted (Kochian *et al,* 2004). Plants rely on root hair absorption of minerals from the surrounding soil and also, microbial activity and cation exchange mechanisms (Cheng *et al*, 2002, Dakora and Phillips, 2002). All these processes are disrupted by high levels of aluminium - associated with a drop in pH (Kidd and Proctor, 2000; Dakora and Phillips, 2002, Kochian, *et al,* 2004). This disruption, coupled with a decrease in the availability of the essential nutrient phosphorus and other micro-nutrients, due to its fixation with Al and Fe oxides on the surface of clay minerals, severely limits plant growth on acid soils (Kidd and Proctor, 2000; Scott *et al*, 2000; Tyler and Olsson, 2001; Kochian *et al,* 2004). Economically viable agriculture is difficult on acidic soils and one of the options is to revegetate degraded soils in the region with tree species (Rahman 1991; Pakeman *et al*, 1998, Scott *et al*, 2000).

Research has shown that plant nutrient absorption and good fertiliser uptake are critical to farming enterprises remaining viable and environmentally sustainable in Australia (Scott *et al*, 2000; Kochian, *et al,* 2004). Nutrient uptake is a characteristic determined by genetics, as well as environment. Plant species that have high nutrient use efficiency (NUE) and intra-specific variation for plant growth are the subject of investigation for use in acid soils and in breeding programs that focus on developing cultivars with high NUE (Baligar, *et al*, 2001, Kochian *et al*, 2004). In the Upper Shoalhaven Catchment around Braidwood, many of the soils have been identified as naturally acidic (Jenkins, 1996), so it is likely that species local to this area are well adapted to soil acidity may have enhanced nutrient absorption capabilities, a high NUE and perhaps be tolerant of multiple environmental factors, such as frost, salinity, waterlogging plus acidity. In the research reviewed by Kochian *et al* (2004), tolerance to Aluminium toxicity was found coupled with multiple internal processes including translocation, as well as exclusion at the root apex; and were sometimes linked to additional tolerance mechanisms, suggesting that Al tolerance is a product of multiple gene loci. Exclusion and translocation are also mechanisms of salt tolerance (Atwell *et al,* 2010).

1.9 Adaptation and Effects of Multiple Environmental Stressors

Plants are able to adapt readily to a single environmental factor, which in some cases may predispose them to be tolerant of multiple factors, but when two or more detrimental environmental factors combine within a short time span, adaptation can be more difficult because the process of local adaptation to extremes of habitat is generally based on natural selection favouring plants with tolerance to a single factor (Kawecki and Ebert, 2004). However successive generations may then adapt to a new factor, with the resultant population tolerant of multiple factors.

1.9.1 Adaptation

Adaptation to environmental gradients and intraspecies variation at the level of local habitat is widely accepted as occurring for a number of species and biota (Wright and Westoby, 1999, Kawecki and Ebert, 2004, Prior *et al,* 2005). Adaptation to local habitats has not been fully investigated as a source for variation in salinity and waterlogging tolerances of Australian native trees and shrubs, although genetic or provenance variation within species is well documented (Ladiges and Kelso, 1977, Gibson and Bachelard, 1986, Marcar, 2002, Mahmood *et al,* 2009). Salt tolerance, acidity tolerance and waterlogging tolerance are complex issues and much depends on the genes governing the tolerances, whether they are (a) a single locus or multiple loci; or (b) whether they are present in the whole population or across the whole species, or (c) whether they are naturally selected for at the local level in response to an adverse environment *i.e.*, adaptation; and whether the adaptation is a product of hybridisation or multiple genetic recombinations (Emms and Arnold, 1997, Kawecki and Ebert, 2004, Shanker and Venkateswarlu, 2011). Multiple tolerances, such as adaptation to both frost and salinity, or salinity and waterlogging also add to the complexity of localised adaptation and variation within species (Marcar, 1989).

Previous research has found that when detrimental environmental factors are combined, plant growth and survival can be affected much more than with each factor separately (Marcar 1993; Hoy *et al,* 1994*;* Walsh *et al,* 1995). Trials on wheat seedlings showed the combined effect of waterlogging and salinity was more detrimental to plant growth than either factor alone, leading to rapid chlorosis (yellowing of leaves), defoliation and mortality (Barrett-Lennard, 1986). Research projects conducted on Australian *Eucalyptus* species and *Casuarina* species supported these findings also showing the adverse effect on seedling growth was greater for a dual effect than for each factor singularly. It was found that Australian native seedlings survived the stress combination better than wheat, but *E. camaldulensis* and *C. cunninghamiana*, despite good initial survival, eventually succumbed to salt and waterlogging stress over time (Marcar 1993; Hoy *et al*, 1994; Walsh *et al,* 1995).

Other research also comments on the pressures from a multiplicity of environmental variables and how combinations of detrimental factors, such as salinity with waterlogging, might also produce more salt tolerant seedlings (Barrett-Lennard, 1990; Marcar, 1993; Stirzaker *et al*, 2002, Kawecki and Ebert, 2004).

Marcar (1993) imposed a waterlogging pre-treatment on seedlings in an experiment of dual stress. His work found that waterlogging pre-treatment of seedlings significantly improved the growth response to the subsequent combination of salinity and waterlogging stress. Many Australian soils exhibiting soil salinity often have accompanying waterlogging problems, regardless of whether it is dryland or irrigated salinity (Taylor, 1999). In the case of dryland salinity, there is a slow rise in the watertable until it is at the surface. The initial problem is often waterlogging followed by low levels of salinity (Marcar, 1993). Soil salinity can then slowly become moderately high, after many years of evaporation at the soil surface (Peck, 1978, Taylor, 1991, Marcar, 1993).

Previous research into salt tolerance incorporating field trials that screened large numbers of species sometimes documented seed source habitat, particularly when it was found in riparian zones (Bari and Schofield, 1992, Sun *et al*, 1994, Marcar *et al*, 2002), but few trials have noted its importance. A riparian zone, riverbank or streambank, may include subsurface water flow within several meters either side of the midpoint of the surface flow, depending on the soil type and streambed strata and a sandy riverbed can hold subsurface water for more than five meters either side of the main flow channel, in coastal waterways of south-eastern Australia (Corbett, *Pers. Comm.*, 2000). Results of previous research (*e.g.* Bari and Schofield, 1992, Sun *et al*, 1994, Marcar *et al*, 2002) show a possible relationship between seedling success on saline soils and the seed sourced from riparian zones. However, the distance from nearest water course was not noted in the research, simply that the collection site was riparian (Sun *et al*, 1994, Marcar *et al*, 2002). Field trials on a saline site in the Northern Tablelands of Queensland, undertaken by Sun and Dickinson (1995), show that of the species screened, most of the salt tolerant natives found were grown from seed populations sourced in riparian zones. It is possible that the seed sourced from riparian zones trials have localised adaptation to a waterlogged habitat, if seed trees were sourced within a few metres of the waterbody. Riparian zones are generally less than 20m wide in NSW so with 5m either side subject to waterlogging, it is likely that riparian seed sources equate to waterlogged habitats (Corbett, *Pers. Comm.*, 2000).

Research work by van der Moezel *et al*, (1989, 1991) as well as the work by Marcar (1993, 2002) investigating salt tolerance in seedlings, suggest it is possible that a riparian, waterlogged habitat may predispose the progeny to be more salt tolerant than seed sourced from other habitat types (van der Moezel *et al*, 1989, Marcar, 1993). The work by Marcar (1993, 2002) found a positive correlation between waterlogging tolerance and tolerance to the combined conditions of salinity and waterlogging in glasshouse trials, as did van der Moezel *et al*, (1989 and 1991). Niknam and McComb (2000) suggested in their review of salinity research in Australia that seed sourced from saline provenances performed better than seed from non-saline habitats. However, there have been enough exceptions for Niknam and McComb (2000) to recommend including other types of provenances when seeking salt tolerant natives, particularly species which have shown a capacity for "biodiversity", *i.e.* those found on a large range of soils and habitat types.

1.10 Effect of Salinity and Waterlogging on Germination

In most salinised sites in South-Eastern Australia, salt mostly occurs as sodium chloride which causes disruption to cation exchange at the root hair and overabundance of chloride ions, resulting in free radicals forming that destroy plant tissues, including membranes and retard cellular processes, including germination (Stirzaker *et al*, 2002, Atwell *et al*, 2010). Root hairs and microbes need oxygen. Salinity kills off the oxygen producing, aerobic bacteria found naturally in the soil (Cheng *et al* 2002). Salinity also reduces earthworm activity that oxygenates the soil and modifies nutrients for microbial break down (Murphy, 1993). Waterlogging has a similar effect, with salinised and waterlogged soils becoming anaerobic and toxic. Plants that are waterlogging tolerant and ale to germinate and survive in anaerobic soils, may have specialised root structures, such as aerenchyma and modified root absorption processes, such as exclusion of toxic cations (Ladiges and Kelso, 1977; van der Moezel *et al*, 1991; Stirzaker *et al*, 2002). Salinity disrupts the root absorption processes making the germination process difficult (Sands, 1981; Sun and Dickinson, 1995; Marcar, 2000). Oxygen diffuses ~10,000 times slower through water-filled pores than through gas-filled pores. Soil respiration continues after soils become waterlogged but with a decrease in oxygen supply, sols become oxygen deficient after 1-2 days (Barrett-Lennard, 1986)

By affecting the environment of germinating seeds and their delicate root hairs, soil salinity, acidity and waterlogging directly affect the function, growth and development of the emerging plant (Ladiges and Kelso, 1977; Munns and Termaat, 1986; van der Moezel *et al*, 1991; Kochian *et al*, 2004). For plants to survive and grow in acidic, saline and waterlogged soils, such as found in the project's study area, species need to have a range of adaptive mechanisms and, multiple tolerances.

It is possible that natural selection may cause physiological change in response to the local environment and result in adaptation to the localised conditions (Kawecki and Ebert, 2004). Whether these adaptive mechanisms are genetically inherent within a whole species, or gained via locally adopted accessions to extremes of habitat, forms the basis for this project's investigation.

1.10.1 Effect of Salinity on Root Associations

Disruptions at the root hair may also affect root associations (Thrall *et al*, 2000, Cheng *et al*, 2002). The loss of native vegetation, acidity and increasing soil salinity detrimentally affect the abundance and diversity of native rhizobia and soil biota (Thrall *et al*, 2000, Cheng *et al*, 2002). Use of a biologically active fertiliser derived from worm castings for reclamation of degraded land may be enhance establishment of native legumes; especially in agricultural areas "where appropriate hosts can be completely absent and soil biota have disappeared from the soil profile" (Thrall *et al*, 2000).

The two main groups of symbiotic root bacteria and fungi that assist plant root uptake in Australian natives have been identified as mycorrhizae and rhizobia. Root exudations feed these microbes in exchange for essential nutrients (Albrecht *et al*, 1994; Cheng *et al*, 2002; Dakora and Phillips, 2002). The fungal hyphae of mycorrhizae have been shown to be crucial to plant uptake of phosphorus (Dakora and Phillips, 2002) and rhizobia nodules are involved in nitrogen fixation, particularly in legumes (Cheng *et al*, 2002). The absorption processes of root hairs, nodules and fungal hyphae are driven by osmotic pressure, plus the concentration of anions and cations both within the root and the surrounding soil. Soil salinity adversely affects the action of these microbes and may even cause death (Bell, 1999, Chowdhury *et al*, 2011). Use of the 'PuraCast', a biologically active liquid fertiliser derived from worm castings, with microbial activity (approximately 2 million cells/ml), was proposed as one of the factors in this research to assess whether the addition of microbes assisted with germination under conditions of low salinity.

1.11 Frost Tolerance

Frost is a factor which greatly limits seed germination, seedling and plant growth, even on fertile soils and can be extremely detrimental in combination with degraded soils (Stirzaker *et al*, 2002). However, although it is a limiting factor to seedling growth, frost can also assist with revegetation as shown by research work investigating a correlation between frost resistance and salinity tolerance for forest species of *Eucalyptus* (Marcar, 1989).

Frost is an environmental factor due to a region's elevation and climate and is generally not regarded as an anthropogenic effect – although reduction of forest canopy via clearing can increase the localised effect of frost due to changes in the microclimate (Battaglia and Reid, 1993). The Upper Shoalhaven Catchment is subject to regular frosts from May to November, which vary in severity and frequency according to temperature, aspect and elevation (Hosking, 2003). Tolerance to frost was the first factor to be discovered in Landcare field plantings as an indicator of local habitat adaptation. Variation in frost resistance was found for seedlings of the same species grown from seed collected approximately 40 km apart from each other in work undertaken through the Landcare Nursery. Mongarlowe and Ballalaba are two localities 25 km east and west of Braidwood respectively that show a gradient in frost severity and frequency. Ballalaba has a higher frost frequency and severity than Mongarlowe (Hosking, 2003). Seedlings grown from seed collected at Ballalaba showed a higher frost resistance than seedlings of the same species grown from seed sourced near Mongarlowe (unpublished work, S.E. Gray, Landcare Nursery, Braidwood, 1994-99).

When intra-species variation exists in one environmental gradient, *e.g.* frost resistance, previous research has postulated that variations in response to other environmental gradients may also exist *e.g.* salinity (Marcar 1989; Prior *et al*, 2005). Competition for moisture is a critical factor for tolerating frost, as water freezes inside plant cells, dehydration occurs within the plant and competition for soil water reserves increases. The work of Ball *et al* (1997) investigating the effect of frost on *E. pauciflora* in Bungendore (near Braidwood) showed that competition from nearby grass cover can inhibit seedling growth in frosty conditions. This raises the question of whether competition for moisture may be similar to the effect of salinity, impacting water availability via restriction of osmosis at the root hair. The work by Marcar (1989) showing a possible correlation between salinity tolerance and frost tolerance supports this idea.

This thesis aims to provide some insight into the importance of considering seed source habitat when collecting seed for rehabilitation of degraded soils and the potential for adaptation within local species to soil degradation or climatic extremes leading to intraspecies variation. Investigating salinity and habitat adaptation at the level of genotype, via identification of individual genes or isolation of genetic markers or loci and investigation of "hitchhikers" (Kawecki and Ebert, 2004) has been left for the other research projects.

CHAPTER 2. PROJECT STUDY AREA

Some of the soils around Braidwood are from 300 million year old sediments (Jenkins, 1996) and have become naturally acidic in the A and B horizons as a result of incident rainfall leaching out water soluble cations over time, carrying them down through the soil profile and eventually, into the watertable (Jenkins, 1996). A large portion of land in the Upper Shoalhaven Catchment is regarded as highly acid, *i.e.* pH 4.3-4.7 (Jenkins, 1996), which hosts vegetation remnants with possible multiple tolerances as listed above. Soil surveys conducted by the local Soil Conservation Service and Lands Department (now called the Local Lands Service; unpublished data held within the Braidwood office). Studies of topsoil clods taken across the Upper Shoalhaven Catchment as part of this project's work, showed that most topsoils had a $pH_{1:5} < 5.5$ for the region within 100 km of Braidwood, which is supported by Jenkins' findings (1996).

Dryland soil salinity in the Upper Shoalhaven Catchment occurs north and north-east of Braidwood in the Little Bombay area, Tarago and Lake Bathurst, Windellema and Mayfield districts. Salt affected areas range from 10-50 ha areas of waterlogging with little or no salinity, to waterlogging with low salinity (EC < 1 dS/m), to small $5m^2$ ephemeral salt scalds of EC 1-2 dS/m, to large areas 100-300 ha of salinised, highly eroded land with hard salt pans spanning several hectares of EC > 20 dS/m. The air photo in Figure 2-below shows salt affected land in the Upper Shoalhaven Catchment along the Cooma Road, from Ballalaba to Braidwood, near seed collection sites and field trial sites. Salt scalds can be seen as white or light grey areas circled in Figure 2 below.

Figure 3. Air Photo showing major salt scalds near Shoalhaven River in 1994.

Comparison of Figure 2 with Figure 3 below shows development of a scald in previously vegetated land north of the sharp right-hand bend in the river. The salt-affected land shows fairly well-vegetated areas near the scald, which illustrates that dryland salinity is not necessarily due to the clearing of land in the immediate area, but as a result of clearing in the recharge area (Barrett-Lennard, 1986, Taylor, 1991; Lefroy and Stirzaker, 2002).

Figure 4. Air Photo showing major salt scalds near Shoalhaven River in 2012

The scalded area in the bottom of the image is roughly the same size as in 1994, but other areas have improved, whilst new areas have been cleared and show scalding as mentioned above.

2.1 Spatial Spread of Project Study Area

The township of Braidwood is centrally placed in the Upper Shoalhaven Catchment. The catchment extends from the slopes of Mt Badja, which is in the Deua National Park, south-east of Braidwood to Boro, Bungonia and Tarago, including the locality of Windellema and north-east to Nerriga, on the western edge of Morton National park.

Figure 5. Map showing Locality of Braidwood, NSW.

Seed was collected from sites within a 100 km radius of Braidwood. The furthermost collection site was at Mt Majura near Canberra. Northern sites extended from Windellema, Goulburn and north-east along Nerriga Road, Mongarlowe to the east, then south-west to Ballalaba, the slopes of Mt Gillamatong and south-south-east to Majors Creek and Araluen near the edge of escarpment; south-east to Murramarang National Park near Kioloa and

Batemans Bay. Appropriate permits for seed collection were obtained from the various land-holders including NSW National Parks and Wildlife.

Almost 75% of seed tree sites were within 25 km of Braidwood and 37 out of the 48 seed collections were less than 50 km from each other. Geology and soil type varied across this fairly short geographical distance, as per Jenkins (1996). Riparian zones on waterways which hosted "waterlogged" habitats for sourcing seed were variable in soil type, from sandy-loams, to acidic clays.

2.2 Climate

In the Upper Shoalhaven Catchment, annual rainfall is 600-800 mm for most of the region, with some areas (*e.g.* Mt. Badja) receive up to 1100 mm per annum (Adomeit *et al*, 1987). Frosts occur regularly across the Upper Shoalhaven Catchment from April to September. The first frost is usually in March and the last frost is often in late November (Jenkins, 1996). The mean period of heavy frost risk is 189 days (Braidwood Technical Manual, Soil Conservation Service NSW, unpublished, as quoted in Jenkins, 1996), with records showing an average of 95.4 days annually below 2°C since 1985 (Weatherzone, 2008). Annual temperatures range from 40^{+} °C in summer with occasional drops to minus 15 °C in winter. Soil moisture deficits are prohibitive to seedling growth for approximately 50% of the calendar year, based on Soil Dryness Index (SDI) calculations for the period 1986-1997 (Hosking, 2003), the decade prior to this research project. The tables 1 and 2 below provide further detail of rainfall and temperature.

Table 1. Braidwood Long-term Averages for Rainfall

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Ann
Mean Max °C	26.2	25.6	23.2	19.6	15.8	12.8	11.8	13.6	16.4	18.8	21.4	24.7	19.1
Mean Min °C	11.9	11.9	9.8	5.6	3.3	0.8	0.2	0.4	3.5	5.5	7.9	10.1	5.9
Mean Rain mm	60.6	52.6	40.9	49.0	50.7	47.1	34.7	52.4	55.4	60.9	75.3	55.2	641.5
Median Rain mm	40.8	36.4	33.0	33.2	29.8	29.8	21.7	33.4	52.0	61.0	73.6	48.9	584.8
Mean Rain Days	11.7	9.7	10.6	10.9	11.9	12.5	12.9	11.0	10.8	11.5	12.8	9.8	135.7

Tables 1 and 2: © The Weather Co. 2008 Information supplied based on data from the Bureau of Meteorology. Collection site: Braidwood Racecourse AWS South Coast, New South Wales/ACT: 35.4253°S 149.7835°E 665m AMSL Site No. 069132

Table 2. Braidwood Average Number of Days With Monthly Temperature Averages

Month/ Temp °C	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Ann
≥ 40°C	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
≥ 35°C	2.3	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	4.0
≥ 30°C	8.2	6.8	2.3	0.1	0.0	0.0	0.0	0.0	0.0	0.3	2.2	5.0	24.6
≤ 2°C	0.2	0.1	0.4	6.2	12.3	16.8	18.9	18.7	11.7	6.9	2.6	0.6	95.4
≤ 0°C	0.0	0.0	0.2	2.8	8.2	12.3	13.8	14.0	7.1	3.1	0.8	0.1	62.2

The Braidwood annual monthly mean temperature is below zero for June-August. The 'per Week' values show sub-zero temperatures from May-September.

Table 3 below shows that frost and low temperatures are severely limiting to plant growth for five months of the year.

Table 3. Braidwood 'per Week' Values (14th/86th Percentiles)

Month / Temp °C	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Ann
High Max	33.3	31.7	28.4	24.4	19.0	15.8	14.1	16.3	20.9	23.1	27.1	30.1	26.0
Low Max	20.2	19.4	18.1	15.1	13.0	10.3	9.8	10.7	12.6	14.7	15.0	19.2	12.2
High Min	15.2	15.6	13.5	10.5	8.1	5.4	4.8	5.3	8.6	10.1	12.3	13.9	12.3
Low Min	6.9	7.7	5.8	1.4	-2.3	-4.3	-4.6	-4.5	-1.2	0.9	3.1	6.4	-0.9

2.3 Soils in the Upper Shoalhaven

The Upper Shoalhaven Catchment on the NSW Southern Tablelands lies on the Lachlan Fold Belt. Soils originating from Ordovician metasediments are the oldest and most extensive. Soils also occur on alluvial Silurian volcanics and Tertiary sediments. Lithologies include siltstones, quartzites, rhyolites and tuffs and granitic intrusions are also common. The Shoalhaven River riverbeds and tributary streambeds are comprised of fluvial sands and gravels. The geology and lengthy time period for leaching has given rise to extensive areas of sodic and acid soils over a moderately saline (EC_w1.0 -2.0 dS/m) watertable (Jenkins, 1996, Jenkins and Morand, 2004). The watertable was measured in this project via installation of piezometers and shown to be 1.0 - 1.5 m below the surface in low-lying areas adjacent to or on salt pans and shown to be of low to moderate salinity of EC_w1.0 – 2.0 dS/m (Taylor, 1999).

2.4 Salinity, Acidity and Alkalinity in Project's Study Area

On some degraded sites in the Upper Shoalhaven, loss of topsoil through vegetation dieback and subsequent soil erosion has revealed calcareous soils. Calcium carbonate clumps form in the C horizon and acidic topsoils can overlay alkaline calcareous soils around Braidwood (Jenkins, 1996). When saline sols erode and combine with exposed patches of alkaline subsoil this can lead to the formation of sodium carbonate which can make a site highly alkaline, denuded of vegetation and difficult to revegetate. There are eroded calcareous soils showing calcium carbonate clumps in saline areas and acidic soils in the Upper Shoalhaven Catchment, measured during the project (reported on in this thesis), that had an alkaline pH of around 8.5-9.0, adjacent to acidic topsoils, with pH 4.0-5.5. Figure 6 below shows a denuded area of degraded soil prepared for planting via ripping and mounding. The large white lumps of calcium carbonate are clearly visible at the soil surface in this saline, acidic soil.

Figure 6. Revegetation of salinised land upslope and in the scald

The Upper Shoalhaven Catchment acidic soils are generally found on older sediments, not in the valleys, but in the foothills and lower slopes, ranging from a pH of 3.75 to 4.9. Soil acidity in the region is regarded as natural, due to the weathering and leaching of 300 million year old soils – not due to overuse of fertilisers such as superphosphate (Jenkins, 1996). This project found occurrences of acid soils adjacent to erosion gullies with exposed alkaline subsoils of pH 9-10. This duplex soil is very hard to rehabilitate and establish tree cover from seedlings (Jenkins and Morand, 2004), is highly degradable and in the SoE

(2011) soil acidity is described as a costly, more widespread problem than salinity in rural landscapes. In 2004, 47% of NSW soils were deemed sodic, with 4% of these also acidic, pH <5.5 (Jenkins and Morand, 2004).

Or the purposes of this thesis, acid soils are defined as having a pH in the 1:5 extract as <5 to define acid soils. Sodicity was not measured and this can affect the levels of acidity measured, so perhaps was an oversight (Jenkins and Morand, 2004). Chapter 3 provides more details on the measurement of soil acidity and salinity in this project.

Figure 7. Highly acid soil with trial plantings of project species

The image above shows one of the rehabilitation sites that this habitat study has assisted. Plantings shown were seedlings grown from seed trialled in this project, for establishment on a highly acidic and slightly saline soil that was completely denuded, as it still is in the foreground. Two of the seed collection sites listed below, Sandholes Road and Jillamatong Creek are in close proximity to the rehabilitation site shown in Figure 7 above.

CHAPTER 3. METHODS

The following chapter describes soil analysis methods, habitat definitions, seed collection, seed sorting and the experimental design.

3.1 Topsoil Analysis

Topsoil clods were analysed for EC and pH using the methods described by Loveday, (1986), to define habitat type at each of the seed collection sites. The final measurement of EC incorporated soil texture, which defines amount of clay in the soil sample, as described by Taylor (1991 and 1999). Soil Analysis followed standard methods used in the department of Ecosystem Dynamics, Research School of Biological Sciences, A.N.U. and as described by Loveday, (1986). John Loveday (retired CSIRO Soil Scientist) also provided personal guidance in soil sampling and analysis techniques. The EC and pH of the top 20-30 cm of soil was measured over a five year period 1995-2000, when seeds were collected.

Soil sampling involved clod sampling in a zigzag transect beneath the canopy and adjacent to the remnant stands of interest. Clods were approximately 20-30 cm x 20-30 cm. Where soil was not able to be collected in a clod, a volume of approximately 500 cm^3 was collected. Very few soils in the Upper Shoalhaven Catchment have topsoil to 30 cm deep (Jenkins, 1996), so most of the clods included some subsoil, but no attempt was made to classify or quantify soil layers. Soil clods sampled have been called topsoil, although some may have included subsoil, especially on degraded sites where topsoil was lost due to erosion.

Clods were dissected and the central core of soil was sieved, air-dried for five days, then samples from each site were bulked together (between 2 and 6 clods generally) and 200 g of soil was analysed in a 1:5 soil: water mix, at room temperature after being shaken for an hour in a rotating shaker located in the Research School of Biological Sciences, A.N.U.. Soil solutions were shaken and EC and pH measured using handheld Hanna pH and EC meters with probes placed into in the solution. These methods follow standard methods used in the late 1980's and 1990's (Greenberg *et al*, 1981, Loveday 1986; Rhoades and Loveday, 1990; Taylor, 1991). The EC and pH meters were freshly calibrated before each measuring session. Standard solutions made up from laboratory grade sodium chloride were used for calibration of EC meters; commercially available EC and pH standards were also used. Preliminary soil analysis was done at room temperature, in a field laboratory in Braidwood, using a CSIRO kit for soil pH as well as the Hanna handheld meter after shaking for one hour. Soils from every collection site also underwent analysis in a laboratory at the Research School of Biological Science, ANU. Preliminary analysis was also done at PuraCast Industries laboratory, Braidwood. The "ribbon" test (Taylor, 1991) used for estimating soil texture was done at the PuraCast laboratory and a smaller shaker machine was used for the preliminary analysis of $EC_{1:5}$ and $pH_{1:5}$.

The $EC_{1:5}$ values were averaged from topsoil clods collected from saline sites across the region and soil texture values estimated to get the ECe averages for low, medium and high salinity. These were: 1.09 dS/m; 6.3 dS/m; 28.3 dS/m; respectively. The classes of low, medium and high were set according to Taylor, 1999 (Department of Land and water Conservation, now Local Lands Service). This classification also fits within the definitions currently found in the literature (Barrett-Lennard, 1986, Stirzaker *et al*, 2002; Atwell *et al*, 2010).

Two seed collection sites had slightly different soil pH results, beneath the tree canopy and away from the tree canopy. It was noted on saline sites that the canopy of *Eucalyptus* species appeared to have a mitigating effect and topsoil showed a lower soil salinity compared to the surrounds, possibly due to decomposing leaf litter and root action, also known as the 'mulching effect' (*Pers. comm*. Gitay, 2000). It was assumed this was also the case with pH, with the action of root associations, microbial and fungal hyphae, modifying soil pH beneath the canopy (Malloch, 1971; *Pers. comm*. Gitay, 2000). See Tables 5 and 6 below for a list of all seed collection sites, their habitat definitions, the species found there and topsoil analysis results for EC and pH.

Salinised soils at most sites in the Upper Shoalhaven Catchment measured an ECe of < 6.0 dS/m and were generally around 1.0 -2.0 dS/m, which is usual for salinised soils in south-east Australia (Marcar, 1993). There were some areas in excess of this (16.3 -28.5 dS/m) but for catchment-wide revegetation plans and successful rehabilitation, it was thought that very few tree species would tolerate these higher levels of salinity. For germination trials, the levels imposed were equivalent to 1.0 -2.0 dS/m in the field (Marcar, 1993), which is appropriate for up to 50% of sites requiring rehabilitation in the Upper Shoalhaven Catchment.

3.2 Species Selection, Seed Collection and Habitats

Soil analysis for EC and pH were used to define a broad seed tree habitat of acid or saline, waterlogged or dry. The final 10 species selected for the project's germination trials were those with known occurrences on a saline habitat; and ideally, at least two other habitat types in the local area. Habitats were targeted for diversity of species present, potential seed trees and soils were analysed.

The project's germination trial seedbank comprised 48 collections in total, from species found occurring in open woodlands or heathlands in the Upper Shoalhaven Catchment and surrounding areas. The ten chosen species were found on degraded sites in the Upper Shoalhaven on soils of $EC_{1:5}$ in the range of 1.0 -15.0 dS/m and a $pH_{1:5}$ range of 4-7.

Vegetation communities were surveyed for desirable species and seed collection sites were identified within the designated 100 km radius of Braidwood. Most collection sites were isolated remnant stands (as defined in Ozolins, 1999), rather than being part of an open woodland or forest. Some collection sites however, were in riparian zones that had vegetation corridors which ran continuously for several kilometres along a watercourse. Although narrow, these sites may not be regarded as isolated. The more extreme environments of eroding saltpans covering several hectares had poor biodiversity (*e.g.* the Catchment Services Group owned property - "Woodlands" in the locality of Mayfield) and these sites hosted only two or three plant species in total for seed collection. Some collection sites, particularly remnants along waterways, were highly diverse environments.

3.2.1 Habitat Definitions for Trial

The classification of habitat type for each seed collection site used in this study was based on the simple and inexpensive tool of topsoil analysis for EC and pH (Loveday, 1986; Taylor, 1991), rather than more complex methods used elsewhere (*e.g.* Bennett and George, 1995). Perennial waterbodies were visited regularly 1994-1999 to establish they were in fact, perennial, even during periods of drought. Climate and elevation differences were not dealt with, nor were sites sampled in a complex fashion - which may have been another oversight (Blair and Lefroy, 1993). The complexities of multiple interacting environmental factors, *e.g.* variation in the regularity and severity of frost in the project's study area and incident rainfall were not measured at individual sites.

The category of saline seed habitat was refined further, due to observations in the field and when harvesting and sorting seed under the microscope. There were visible differences at the field sites, with shrubs and trees stunted in growth at the higher salinity sites, with fewer leaves, some with leaf tip necrosis and, fewer capsules available for collection. There were noticeable differences in harvesting volume/gram of seed capsules and irregularities in the shape and size of seed collected from high salinity habitats, when compared to seed sourced from low salinity sites. It was thought these differences would be lost if collections were not differentiated in the experimental design and the overall result for the seed sourced from saline habitat types may have been adversely affected. Critical values of low, medium and high salinity were drawn from Taylor's Salinity Extension Notes (1991) and, the saline habitat was broken up into three categories as follows:

Table 4. Saline Seed Habitat Classes

Saline Seed Habitat (and abbreviation)	**ECe**
Low salinity (LS)	<2 dS/m
Medium salinity (MS)	2-5 dS/m
High salinity (HS)	>5 dS/m

The habitats of collection sites were further refined due to concerns that waterlogging in combination with other detrimental conditions (*i.e.* soil acidity) may also lead to differences in seedling tolerances (e.g. Marcar, 1993); giving additional habitats of Dry and Acid (DA) or Waterlogged and Acid (WA). In total, there were seven categories of habitat type, with at least 2 or more seed sources of each, available for the germination trial. Table 5 below shows the habitats and their seed sources of the ten species collected.

3.2.2 Final species selection

A key element of initial species selection in this project, was based on Australian native species screened in previous work (e.g. Sun *et al,* 1994; Hoy *et al,* 1994; Sun and Dickinson, 1995; Marcar 1993, 2000, Niknam and McCoomb, 2000,). Salt tolerant species found in the literature and, occurring locally on multiple habitat types, went into the list of possible candidates for trial. From 1992-1999, a range of habitat types were surveyed (Gray, unpublished data; Storey and Gray, 1996) and this data was used in conjunction with previous research (*e.g.* Sun *et al,* 1994; Hoy *et al,* 1994; Sun and Dickinson, 1995; Marcar, 1989) to compile the final list of desirable species for trial. For a species and the hypothesis to be trialled, viable seed was required. Seed was collected from 5-10 healthy trees within each location and stored in sterile glass jars after dehiscing. Seed from these collections was sorted and underwent preliminary germination trials before being used for germination trials.

Table 5. Species and Seed Collections by Habitat Type

Species/Collections	**Total**	**Saline (low)**	**Saline (med)**	**Saline (high)**	**Wet**	**Dry**	**Dry Acid**	**W**
Abbreviations		**LS**	**MS**	**HS**	**W**	**D**	**DA**	
Eucalyptus pauciflora	10		1	1	3	2	2	1
Acacia melanoxylon	6		1		3	1		1
Casuarina littoralis	9	1	1	1	2	1	2	1
E. ovata	8	1	1		3	2	1	
E. aggregata	1			1				
E. blakelyi	1			1				
Leptospermum flavescens	4		1				2	1
C. stricta	2		1			1		
A. decurrens	3	1		1	1			
E. dives	4	1	1		1		1	
TOTALS:	48	4	7	5	13	7	8	4

Table 5 above shows the ten species trialled, the number of seed sources and categories of habitat type. Seed was sourced from 32 different geographical locations across the region. Two species selected were found on more than one habitat type, but sufficient viable seed for use in the trial was sourced from only the saline habitat. In the table below, the "Waterlogged" habitat has been shortened to "Wet" to fit into the available space. Seed tree habitats are shown with their species and numbers of seed collections and their abbreviations.

3.2.3 Collection Sites, Species and Habitat Soils

The broad definitions of habitat: acid, waterlogged, saline and dry were further refined, using three classes of salinity and sites which had multiple factors, as per Table 5. The soil results for each of the collection sites and their associated species are shown in Table 6, below.

Table 6. Collection Sites

Habitat	Collection Site	Species & Seed Source No.	EC dS/m	pH
D	Halls Lane	*E.pauciflora{34}, E.ovata{27}*	0.47	6.4
D	Kings Hwy (Highway) - Shire boundary	*A.melanoxylon{3}*	0.47	5.5
D	Long Flat	*E.pauciflora{30}, E.ovata{24}*	0.47	5.5
D	Mt Majura	*Casuarina stricta{8}*	0.47	6.0
D	Windellema @M.Royd's	*C.littoralis{11}*	0.31	5.0
DA	20.3km Mongarlowe (along Bourke's Rd from Mongarlowe PO)	*Leptospermum flavescens{28}*	0.16	4.8
DA	Little Bombay – Parking spot	*E.dives {6}*	0.47	3.8
DA	Mongarlowe Swamp	*C.littoralis {13}*	0.94	4.8
DA	Northangera Rd	*E.pauciflora {21}, E.radiata{63}*	0.16	4.5
DA	Sandholes Rd Jillamatong	*E.pauciflora{19}, E.ovata{39}*	0.31	4.0
DA	Stuarts Crossing Rd	*L.flavescens {43}*	0.31	4.0
DA	Wallace's Gap	*C.littoralis {4}*	0.31	4.9
DA	200m past Brick Kiln X'g (Crossing)	*Acacia melanoxylon{29}*	0.63	4.9-5.5
HS	"Woodlands" Saline Site # 2	*A.decurrens{46}, C.littoralis{9}, E.pauciflora{20}*	21.25	5.8
LS	"Warragunia" Mayfield Rd.	*A.decurrens{45}, E.rubida {62}*	1.38	6.2
LS	Braidwood-Goulburn Rd	*E.ovata{40}, A.melanoxylon{2}*	1.09	5.9
LS	"Woodlands" Saline Site # 1	*E.dives{42}, E.blakelyi{15}, C.littoralis{26}, E.mannifera{60}*	1.03	5.7
MS	"Bobar" - Kings Hwy	*E.dives{5}, E.mannifera{65}*	2.34	6.1
MS	Kings Hwy, Goulburn X'g	*E.pauciflora{32}, E.ovata {23}*	4.38	5.8
MS	Kioloa – Pretty Beach NP	*C.littoralis{12}, C.stricta{7}, L.flavescens{35}*	2.97	6.3
MS	Bombay saltpan@Lan's	*E.aggregata{22}*	5.31	7.4
W	Jillamatong creek	*E.pauciflora{16}*	0.31	6.3
W	Jillamatong creek - old	*E.pauciflora{18}*	0.31	6.3
W	"Roscommon" creekside	*C.littoralis{10}*	0.47	6.0
W	Currawan Creek waterfall	*A.melanoxylon{1}*	0.31	5.5
W	Mongarlowe swamp	*E.ovata{38}*	0.47	5.5
W	"Mornington" creekside	*A.melanoxylon{48}, C.littoralis{14}, Callistemon seiberi{56}*	0.31	5.5
W	Kings Hwy Mulloon Creek	*E.ovata {25}*	0.63	7.4
W	Mongarlowe Rd Tantulean Creek X'g	*E.pauciflora{33}, E.ovata{37}, E.dives{41}, A.melanoxylon{31}*	0.31	5.3
W	Warri Bridge Shoalhaven R	*A.decurrens{36}*	0.63	6.0
WA	"Nithdale" Shoalhhaven R	*E.pauciflora{17}*	0.31	4.9-5.5
WA	20.7km Mongarlowe	*C.littoralis{47}, L.flavescens{44}*	0.16	4.5

3.4 Seed Harvesting and Preparation

The experimental methods used for seed harvesting were based on techniques used by the Tree Seed Centre, C.S.I.R.O. Canberra, A.C.T. as described via personal communications with the manager and seed harvesters. Greening Australia (1992) and Clemens (1983) also provided methods and guidelines for seed collection used in this experiment.

3.4.1 Seed Harvesting

Seed was harvested from multiple trees to allow the assessment of a potential "population", rather than investigating the genetics of a single tree, as has been done in previous research (Gibson and Bachelard, 1987; Ball *et al*, 1997). A group of trees was identified down to the level of species, or subspecies in the case of *E.pauciflora*; and mature seed capsules were placed in paper bags, stored in a warm, dry place and allowed to dehisce. Seed was sifted out and stored in airtight containers, placed in a dark cupboard and held at < 25°C until required for experimental work. There were 48 seed collections in all, including an older seed source of one collection (to test *E. pauciflora* seed longevity).

3.4.2 Seed Preparation

Seed was sorted under a dissecting microscope at 2-10x magnification. The methods of seed sorting followed the standard methods used by the Australian Tree Seed Centre, C.S.I.R.O., Canberra, A.C.T. (*Pers Comm*, Australian Tree Seed Centre 1999, Clemens *et al,* 1983). The healthiest and most intact seeds were chosen. Seed characteristics were viewed under the microscope and recorded for all provenances of the 10 species used in the glasshouse trials, plus other collections of interest. Once the viable seeds had been sorted out from the chaff, they were placed in sterile containers.

For the germination trials, 120 intact and healthy seeds were required. Several hundred seeds were generally sorted to yield 120 viable seeds. Not all of the seed collections were able to yield the necessary number of viable seeds for the trial, so this further limited the selection of seed sources for trial. Each seed source was prepared for trial by placing the 120 sorted seeds into six vials – 20 seeds per vial. Stratification (cold treatment) of the *Eucalyptus* species' and scarification (boiling water treatment) of the *Acacia* species' was carried out prior to the seeds being placed in petri dishes, 10 seeds per dish.

3.5 Experimental Design

The petri dishes were laid out on five tables in the glasshouse, as per the layout shown in Figures 8 and 9, below and the overall statistical design in shown in Table 7 below.

The statistical design took into account the differences in species per habitat replicates and, seed numbers for the second and third replicates of habitat that were placed into pots. The experiment was set up as an unbalanced, split-plot design. Experimental units were nested: the structure was habitat.replicate/block/dish. The dishes within plots were randomly assigned to one of the six treatments, via computer-generated randomisation. There were 10 species, from 32 collection sites, with 7 habitat types. It was proposed that six treatments be imposed during glasshouse seed germination trials. The statistical analysis used was the WALD test, using Genstat. Section 3.5 details the statistical analysis.

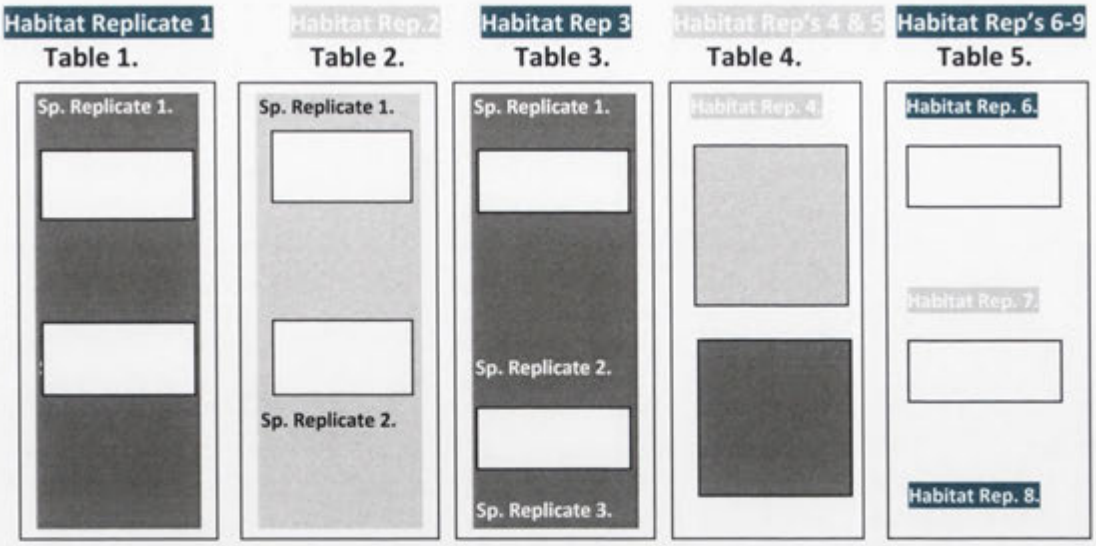
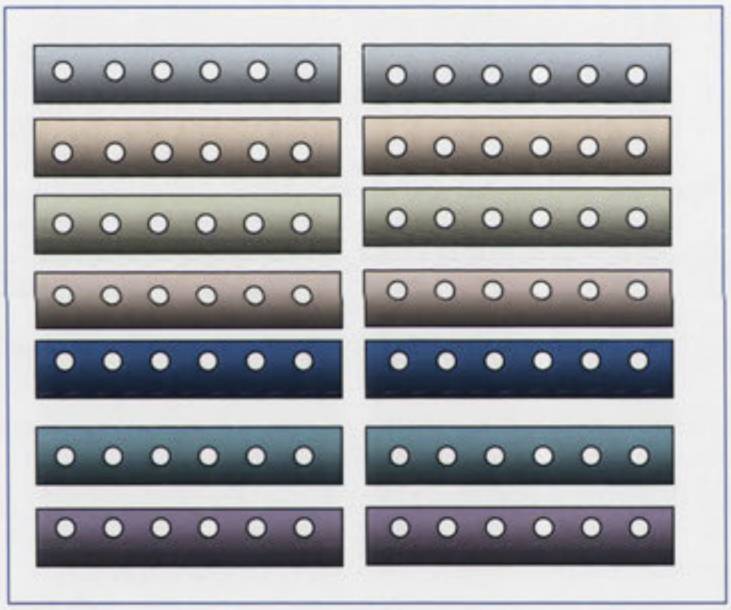


Figure 8. Experimental Block Design& Table Layout



Legend

7 Plots – trays (corresponding to seven habitat types) => ▭

6 Subplots – 6 round petri dishes (corresponding to six treatments) => ○

Figure 9. Petri Dish Treatment Replicate & Habitat Layout

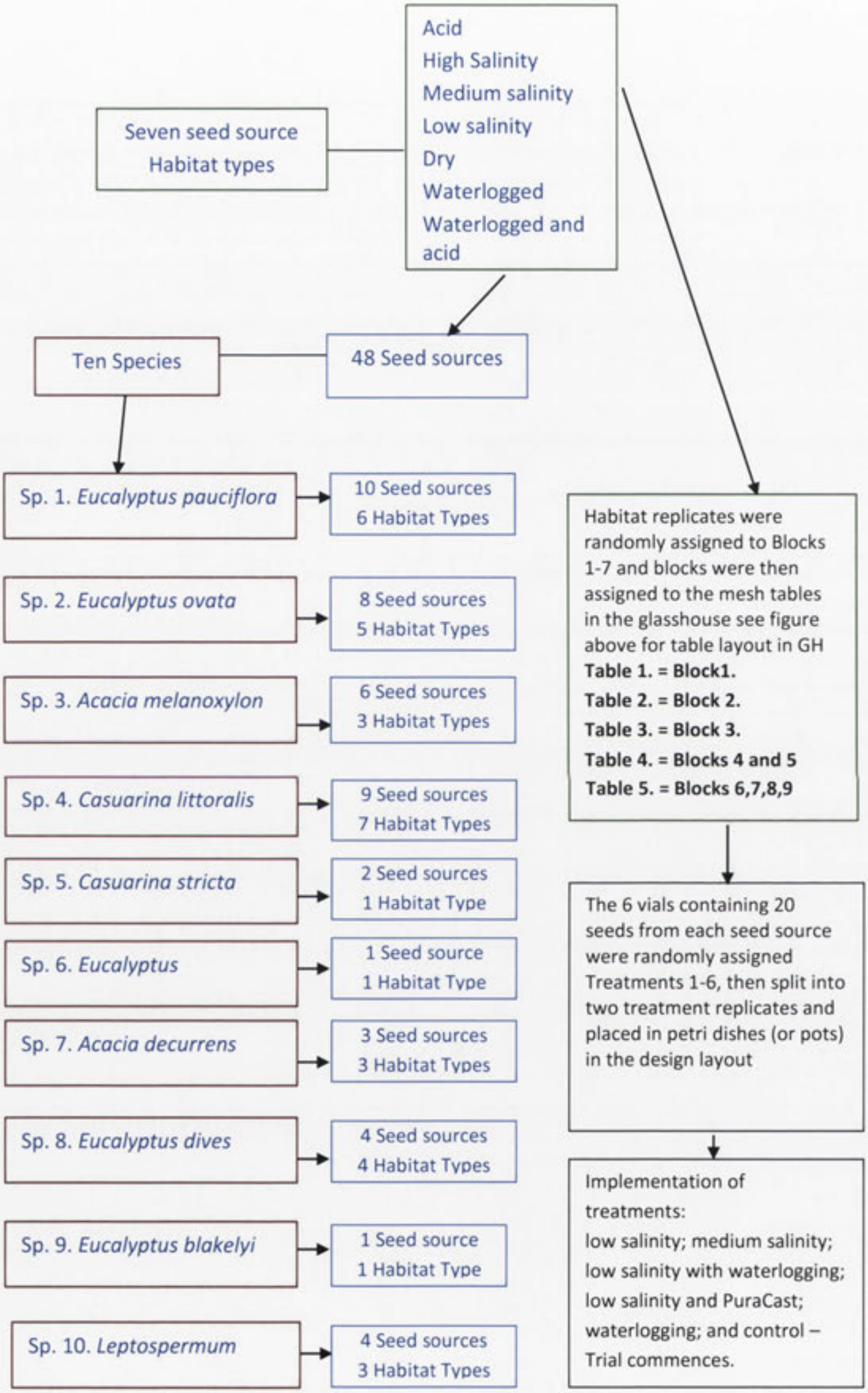
The Figure 8 above shows the petri dishes laid out in their six treatment replicate plots side-by-side. Each habitat type/collection site is an individual row in the block. The block above is showing all of the seven habitat types. The layout of habitat replicates across the five tables in the glasshouse (which technically are duplicates as there are species differences) is shown in Table 7 below and their actual physical placement is shown in Figure 7 above. Figure 9 shows a flowchart of how the seven habitat types with their species and seed collections were assigned to the habitat replicates and the six treatments. There were unequal replicates of habitat type, due to the fact that not all species were found on all seven types of habitat. Collections sites were grouped into their habitat classification (see Table 7) and one site was selected randomly for assignation to the seven plots of different habitat types. The species found at the collection site were designated as the species replicates (also see Table 7) and placed in adjacent blocks of 2 x replicate treatment plots of petri dishes, as shown above in Figure 8. The full list of collection sites, their designation to replicates of habitat (Blocks 1-9), their species replicates (1-3) are shown in Table 7 below and placements across the five glasshouse tables in Figure 7 above. Table 7 shows each of the nine replicates of habitat in alternate shading. Figure 7 shows the spatial spread of these replicates across the 5 tables in the glasshouse.

3.6 Experimental Methods

The standard method of germinating seeds on filter paper in a closed petri dish was used for assessing radicle protrusion and cotyledon emergence (Australian Tree Seed Centre, CSIRO, *Pers. Comm.,* 1995). Germination on filter paper in a glass petri dish allowed viewing of all stages of germination, from radicle protrusion, to cotyledon emergence and full expansion of the first leaves. Alternative growing media, such as vermiculite (Australian Tree Seed Centre, CSIRO, *Pers. Comm.,* 1995), or beds of sandy loam (Ball *et al*, 1997) would only reveal cotyledon emergence above the growing medium.

Table 7. Germination Trial Experimental Design

Collection Sites & Habitat		Replicate	Species Replicates 1, 2, 3,4 and Collection No.			
			1	2	3	4
Halls Lane	D	1	*E. pauciflora 34*	*E. ovata 27*		
Sandholes Rd Jillamatong	DA	1	*E. pauciflora 16*	*E. ovata 39*		
"Woodlands" # 2	HS	1	*E. pauciflora 20*	*C littoralis 9*	*A. decurrens 46*	
Woodlands # 1	LS	1	*E. dives 42*	*E. blakelyi (15)*	*C. littoralis (26)*	
Kings Hwy, Goulburn (Glbn) T	MS	1	*E. pauciflora (32)*	*E. ovata (23)*		
Jillamatong Creek	W	1	*E. pauciflora (17)*			
20.7km Mongarlowe	WA	1	*C. littoralis (47)*	*L flavescens (44)*		
Long Flat	D	2	*E. pauciflora (30)*	*E. ovata (24)*		
Northangera Rd	DA	2	*E. pauciflora (21)*			
"Warragunia" Mayfield Rd.	LS	2	*A. decurrens (45)*			
Kioloa	MS	2	*L. flavescens (35)*	*C. littoralis (12)*	*C. stricta (7)*	
Jillamatong Creek – older seed	W	2	*E. pauciflora (19)*			
"Nithdale" Shoalhaven River	WA	2	*E. pauciflora (18)*			
Kings Hwy – Shire boundary	D	3	*A. melanoxylon (3)*			
Wallace's Gap	DA	3	*C. littoralis (4)*			
Braidwood-Glbn Rd	LS	3	*E. ovata (40)*	*A. melanoxylon (2)*		
"Bobar" - Kings Hwy	MS	3	*E. dives (5)*			
Tantulean Creek	W	3	*E. pauciflora (33)*	*E. ovata (37)*	*A. melanoxylon (31)*	*E. dives (41)*
Brick Kiln	D	4	*A. melanoxylon (29)*			
Mongarlowe	DA	4	*C. littoralis (13)*			
Little Bombay saltpan	MS	4	*E. aggregata (22)*			
Currawan Creek	W	4	*A. melanoxylon (1)*			
Windellema	D	5	*C. littoralis (11)*			
Little Bombay	DA	5	*E. dives (6)*			
"Roscommon"	W	5	*C. littoralis (10)*			
Mt Majura	D	6	*C. stricta (8)*			
20.3km Mongarlowe	DA	6	*L. flavescens (28)*			
Mornington Creek Ballalaba	W	6	*C. littoralis (14)*	*A. melanoxylon (48)*		
Stuarts Crossing Rd	DA	7	*L. flavescens (43)*			
Warri bridge Shoalhaven River	W	7	*A. decurrens (36)*			
Mulloon Creek	W	8	*E. ovata (25)*			
Mongarlowe swamp	W	9	*E. ovata (38)*			



Habitat replicates were randomly assigned to Blocks 1-7 and blocks were then assigned to the mesh tables in the glasshouse see figure above for table layout in GH

Table 1. = Block1.

Table 2. = Block 2.

Table 3. = Block 3.

Table 4. = Blocks 4 and 5

Table 5. = Blocks 6,7,8,9

The 6 vials containing 20 seeds from each seed source were randomly assigned Treatments 1-6, then split into two treatment replicates and placed in petri dishes (or pots) in the design layout

Implementation of treatments: low salinity; medium salinity; low salinity with waterlogging; low salinity and PuraCast; waterlogging; and control – Trial commences.

Figure 10. Species, seed sources and habitats flowchart

3.6.1 Germination Trial Measurement

The germination trials assessed overall germination percentages (% of final count/total seed no.) and germination rates via daily counts of the number of germinants. Germination was measured in three stages: number of days until emergence of the radicle 0.1mm out of seed coat; number of days until 50% protrusion of the cotyledons from the seed coat; numbers of days until full expansion of cotyledons. Counts were made twice daily, morning and evening for the first 10 days, then once per day as germination progressed. High temperatures after Day 25 resulted in some mortality and the number of injured and dead seedlings was recorded. To further test the effect of the glass petri dish, replicates two and three of seed tree habitat, had half of the vial's seeds placed in a heat treated sandy loam, in 5cm sterilized black plastic pots as well as in the petri dishes. The spatial design and setup of the pot trial was identical to the petri dish trial, but the 5cm pots were positioned on tables 0.6 m south of the dishes. Pots were filled with heat treated sandy loam.

3.6.2 Data Analysis

The fixed effects (habitat, treatment and species) were analysed using Genstat (2003) and the WALD statistic to generate chi-squared probabilities for radicle protrusion on three strategic days– Day 10, Day 25 and the final count, on Day 39. The analysis used linear mixed models with species, habitat, treatment and their interactions as fixed effects and habitat.replicate/block/dish as random effects. Wald tests were chosen to test the significance of fixed effects at the 5% ($P<0.05$) and 1% ($P<0.001$) levels of significance. The chi-square distribution for Wald tests is an asymptotic approximation (*i.e.* for large samples) and underestimates the probabilities in other cases. There were over 5000 seeds in the germination trial, so this can be regarded as a large sample and the Wald test, an appropriate choice. Data plots were used to confirm the analyses and the distributions clearly showed they were valid. Values displayed are probable means and not expected values.

The germination trial was run in a solar passive glasshouse at the Landcare Nursery, Braidwood Central School. December in Braidwood is usually mild (see Chapter 2) but ambient air temperatures rose above 40°C from Day 27 to Day 29. The Glasshouse was not temperature controlled and this affected germination.

3.7 Treatments

Seeds were placed in 4.5cm petri dishes on ashless filter paper (Watman No. 42). 1 mL of treatment solution was chosen as the volume to add to each dish because the volume of the petri dish was ¼ of that used by Clemens *et al* (1983), where 4mL of solution was added to the dish. For waterlogging, dishes were filled up to a mark. Implementation of each of the six treatments is detailed below:

(i) CONTROL (C): seeds germinating on filter paper in closed glass petri dishes, watered with deionised water to maintain damp filter paper

(ii) WATERLOGGING (W): seeds placed on filter paper in a (closed) dish filled up to a mark with deionised water; and topped up regularly to maintain the designated water level

(iii) LOW SALINITY (LS): one mL of the 0.01M NaCl standard saline solution - in distilled water - was added to dampen filter paper and the seeds. This level of salinity mimics those found in the field at low salinity sites in the Upper Shoalhaven Catchment. After the initial increment of saline solution, deionised water was sprayed to dampen the paper. Evaporation occurred between waterings, leading to a slight oscillation in EC, but this mimics nature

(iv) MEDIUM SALINITY (MS): one mL of the 0.1M NaCl standard saline solution (*i.e.* 10X low salinity solution) - in distilled water - was added to dampen filter paper and the seeds. This level of salinity is found at medium salinity sites in the Upper Shoalhaven Catchment. After the initial increment of saline solution, a deionised water spray dampened the paper

(v) SALINITY and WATERLOGGING (WS): as for waterlogging, but a saline solution of 0.01M NaCl was added up to a mark. The solution was topped up with deionised water thereafter, to maintain levels at the designated watermark without adding further salt

(vi) SALINITY and LIQUID WORM CASTS (PS): 1.0mL of 0.01M NaCl and 1.0ml of 1:10 dilution of liquid fertiliser, watered with deionised water thereafter to dampen filter paper.

CHAPTER 4. RESULTS

The experimental findings from the glasshouse germination petri dish experiment are set out in sections below. Section 4.1 presents the effects of temperature fluctuations on germination results and the rationale behind the presentation of results having a focus on radicle protrusion. Section 4.2 details the microscopic analysis of seed sources, seed measurements and qualitative observations of seed morphology. Section 4.3 presents the results of radicle protrusion and the final germination expressed as a proportion of 1.00.

There was statistically significant variation in germination due to habitat type under the different treatments; as well as differences between species. The trial design was set up to measure differences due to habitat type as the primary factor, across all species. Differences between species are considered secondary factors.

These results show significant variation exists between different provenances of the same species (*P<0.001*) and there was significant variation between habitat types across all species, with an interaction between treatment and habitat type (*P<0.05*). Analysis of probable means for germination within and between species are presented in relation to the six treatments imposed, with a brief discussion of statistical weighting of the different numbers of collections from habitat types. The probable means are presented unweighted with outliers removed, for analysis of germination for the 7 different habitat types across all species. The means are also presented weighted, with outliers included, which is more statistically correct (*Pers. comm.* Ann Cowling, 2005), but gives a result which is unexpected and not thoroughly supported by the data. Strategic days were chosen for discrete analysis of the data: Day 10, Day 25 and Day 39, regarded as the final day.

4.1 Temperature

Results after Day 21 are confounded by high temperatures particularly Day 27-29 where it rose above 40°C. The petri dishes and pots completely dried out in between twice daily watering sessions.

Table 8. Germination Trial Daily Temperatures

Day No.	Minimum Temperature (°C)	Maximum Temperature (°C)
1	1.0	30
2	1.0	30.5
3	1.0	34.5
4	2.0	23.5
5	3.5	24
6	3.5	26
7	2.0	28
8	2.0	30.5
9	2.0	30.5
10	**2.0**	**30.5**
11	6.0	28.5
12	6.0	28
13	6.0	28
14	7.0	28.5
15	**8.0**	**29**
16	8.0	29
17	10.0	29
19	12.0	29.5
19	7.0	32
20	7.0	32
21	10.0	37
22	10.0	37
23	11.0	28
24	11.0	37
25	**5.0**	**35**
26	9.0	38
27	11.0	40
28	10.0	43
29	10.0	41
30	14.0	32
31	14.5	37
32	**11.5**	**36.5**
33	12.0	37
34	12.0	37
35	16.0	35
36	12.5	33
37	10.5	32.5
38	10.5	32.5
39	**5.0**	**35**
40	12.5	34

Ambient air temperature maximums and minimums within the glasshouse for the trial duration are found in Table 8 above, with days chosen for data analysis shown in bold type. Inside the glasshouse, temperatures rose above 40°C for a 3 day period, on Days 27-29. Seedling death resulted. Temperatures inside the glass petri dishes could not be measured accurately (as it was not possible to insert a thermometer into the small dish and keep the lid on), but some of the smaller dishes were above 45°C, which is prohibitive to plant growth. Data collected after Day 25 is influenced by these high temperatures particularly, the results for cotyledon emergence, where the emerging cotyledons were killed by the higher temperatures. Results for radicle protrusion were less affected by the high temperatures, as most seeds had germinated by Day 27. The radicle protrusion results form the basis of the data analysis and presentations in this chapter, with cotyledon emergence providing supporting evidence. Waterlogging treatment (W) dishes and waterlogging with salinity treatment (WS) dishes were particularly affected by high temperatures because the water heated up and retained the heat for longer than filter paper, the figure below shows *L. flavescens*. One hardy specimen is still alive.

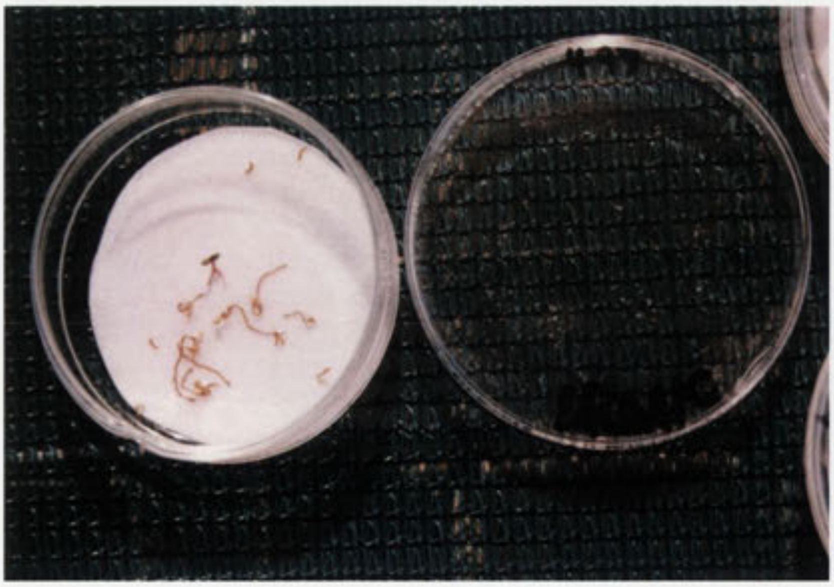


Figure 11. *L. flavescens* showing broiling effect

4.2 Seed Morphology under the Microscope

The seed characteristics, as seen under the dissecting microscope are presented below in Table 9. The Collection numbers correspond to those shown in Table 7 and can be correlated with the sites and soil results. Table 9 below shows the seed characteristics of each species and each collection in minute detail, as expected via microscopic sorting.

There were marked differences in seed morphology for different genera, but definite similarities in size, shape and colour for species within the same genus. Seed within the same genus were alike in terms of relative shape, but there were obvious species differences in terms of size and colour. Within each species, the shape was consistent between provenances, but there were differences in average seed weight, length and/or width and sometimes in colour. The differences in length and width between seed sources of the same species were generally quite minor, but some species had greater variation. It is not totally clear from this dataset whether these differences can be directly related to seed tree habitat, but it was noted that collections from highly degraded habitats produced less viable seed per gram of seed and chaff; and seeds were often more irregular in shape. The high salinity habitats produced comparatively less seed, often smaller and very irregular in shape for the *Eucalyptus* species.

Please see Table 9 below for further detail.

Table 9. Seed Characteristics

No.	Species	Habitat	Weight (g)	Length (mm)	Width (mm)	Seed characteristics via visual assessment 2-10x
36	*Acacia decurrens*	W	0.2422	2.6 - 3.0	1.6 - 1.9	Brown-black, plump ovoid seed, regular in shape; creamy scutellum, occasional horseshoe crack.
45	*Acacia decurrens*	LS	0.2407	2.2 - 3.1	1.2 - 1.7	Shiny black-dark grey; slightly pimpled surface; occasional horseshoe crack of dark brown. Plump, ovoid, regular in shape. Prominent creamy coloured scutellum.
46	*Acacia decurrens*	HS	0.2218	2.2 - 3.1	1.2 - 1.7	Black, ovoid seed, regular in shape; with creamy scutellum, a bit shrivelled and sometimes missing
1	*Acacia melanoxylon*	W	0.2838	2.1 - 2.8	1.3 - 1.8	Shiny black-brown seed; a bit muddy; annulus light apricot to dark apricot, with muddy spots and often wrinkled; plump seed case; with faint horseshoe crack
2	*Acacia melanoxylon*	LS	0.2235	2.3 - 3.0	1.4 - 2.0	Dark brown in colour with horseshoe crack very distinct; salt crystals on seed coat that appear to be exudations; cream annulus not as wrinkly as in other collections.
3	*Acacia melanoxylon*	D	0.3544	2.5 - 3.3	1.5 - 2.0	Shiny black-brown in colour; faint horseshoe crack; annulus slightly wrinkled; light apricot; occasionally dark grey; plump, well rounded seeds; insect predation.
29	*Acacia melanoxylon*	D	0.3485	1.9 - 2.8	1.4 - 1.8	Shiny, black, plump ovoid seed; slightly tapered at one end; horseshoe crack is very faint; annulus plump, apricot colour and very convoluted.
31	*Acacia melanoxylon*	W	0.2945	1.8 - 2.6	1.4 - 1.7	Similar to Brick Kiln collection but smaller and brown-black, plump ovoid seed; horseshoe crack is very faint; annulus apricot in colour and very convoluted.
48	*Acacia melanoxylon*	W	0.2364	2.0 - 3.2	1.4 - 1.7	Dark shiny brown, ovoid, seed with horseshoe crack not very deep; apricot coloured, convoluted annulus, very occasionally turning to a cream colour

No.	Species	Habitat	Weight (g)	Length (mm)	Width (mm)	Seed characteristics via visual assessment 2-10x
4	*Casuarina littoralis*	DA	0.045	2.5 - 7.0	0.8 - 2.5	Shiny brown with cream fringe and translucent wing; highly variable size; plump, aerodynamic seeds with large dimples on base of seeds
9	*Casuarina littoralis*	HS	0.0346	3.3 - 5.8	1.0 - 1.3	Shiny dark brown plump seed with creamy flecks but no cracks.
10	*Casuarina littoralis*	W	0.0651	4.0 - 6.6	1.1 - 2.4	Shiny dark brown plump seed with light coloured midvein and creamy flecks more than #9. Mid vein dark in colour near seed, then light at the end of wing.
11	*Casuarina littoralis*	D	0.0329	3.0 - 4.5	1.0 - 1.5	Shiny dark brown solid colour, flecks are faint and light brown, rather than cream. Midline is light. Seed is plump, dimpled; with faint cracks; wings damaged.
12	*Casuarina littoralis*	MS	0.0742	4.2 - 5.8	1.3 - 1.7	Light brown colour, with creamy flecks and dark brown tip; very wide cream band; plump, more pointed than others. Midline orange and blunt ended. Seed shrivelled.
13	*Casuarina littoralis*	DA	0.0487	3.7 - 5.7	1.0 - 1.6	Shiny, dark to medium brown in colour; plump seed, faintly flecked, with wide, creamy band before wing; medium brown midline very distinct.
14	*Casuarina littoralis*	W	0.0736	3.5 - 5.0	1.3 - 1.8	Shiny, dark brown tip; flecked and wide creamy band; like Kioloa seed source; but much rounder shape; including a rounder shorter wing, that is flared.
26	*Casuarina littoralis*	LS	0.0545	3.2 - 5.5	0.8 - 1.5	Shiny, dark to medium brown in colour; plump seed, faintly flecked, with wide, creamy band before wing; medium brown midline very distinct.
47	*Casuarina littoralis*	WA	0.0513	NA	NA	Shiny, dark to medium brown in colour; seed faintly flecked, with wide, creamy band before wing; medium brown midline
7	*Casuarina stricta*	MS	0.1051	6.0 - 6.5	2.0 - 2.4	Dark brown, flat, very cracked seed, with slight dimples and distinct midline (in wing) with dark brown tip; curled slightly.
8	*Casuarina stricta*	D	0.0994	4.3 - 7.9	1.2 - 2.3	Dark brown, flat, slightly cracked; midline as above. Greater variety in seed size and colour.

No.	Species	Habitat	Weight (g)	Length (mm)	Width (mm)	Seed characteristics via visual assessment 2-10x
16	*Eucalyptus pauciflora*	DA	0.0401	0.9 - 1.8	0.9 - 1.2	Dark shiny brown-black in colour; plump, slightly pocked segmented surface with 3-4 faces; one large, curved side meeting at a flattened apex.
17	*Eucalyptus pauciflora*	W	0.0294	0.9 - 1.6	0.7 - 1.2	Shiny medium brown in colour; plump, slightly pocked surface; shape as above.
18	*Eucalyptus pauciflora*	WA	0.031	1.1 - 1.8	0.8 - 1.2	Shiny dark brown-black in colour; plump, slightly pocked surface; shape as above.
19	*Eucalyptus pauciflora*	W	0.0338	0.9 - 1.6	0.8 - 1.2	Old collection - different to newer collection (17) in colour, these older seeds were much darker in colour, almost black, very shiny and plump.
20	*Eucalyptus pauciflora*	HS	0.0245	1.0 - 1.6	0.7 - 1.2	Shiny dark-medium brown; plump; slightly pocked surface; shape irregular, similar to above, but twisted or narrow sunken faces in ripe seeds.
21	*Eucalyptus pauciflora*	DA	0.0259	1.1 - 1.5	0.7 - 1.1	Seed similar to #20, but brown-black in colour and slightly smaller.
30	*Eucalyptus pauciflora*	D	0.0246	1.1 - 1.5	0.7 - 1.2	Very shiny, plump, medium to dark brown colour with slightly pocked surface; standard pauciflora shape. Large infertile seeds, collection slightly unripe.
32	*Eucalyptus pauciflora*	MS	0.0329	NA	NA	Very shiny dark brown with light brown edges; slightly pocked surface; large size variation in sample; fairly uniform shape; some less plump.
33	*Eucalyptus pauciflora*	W	0.0281	0.9 - 1.4	0.7 - 1.4	Shiny medium brown in colour; plump, slightly pocked surface; shape as above.
34	*Eucalyptus pauciflora*	D	0.033	NA	NA	NA
5	*Eucalyptus dives*	MS	0.0196	1.0 - 1.6	0.7 - 1.1	Light golden brown; plump, faceted seeds with one large rounded side; flattish bottom, then faceted up to an apex.
6	*Eucalyptus dives*	DA	0.0109	1.0 - 1.3	0.6 - 0.8	Dark golden brown plump and shiny; smaller and darker than above; same shape.
41	*Eucalyptus dives*	W	0.0175	0.9 - 1.3	0.6 - 0.8	Shiny, very dark brown, plump seed; similar shape to *Eucalyptus pauciflora*; slightly pocked surface; regular shaped.
42	*Eucalyptus dives*	LS	0.0119	0.9 - 1.3	0.6 - 0.8	Shiny, light-dark brown, plump seed; similar shape to above but more irregular; slightly pocked surface.

No.	Species	Habitat	Weight (g)	Length (mm)	Width (mm)	Seed characteristics via visual assessment 2-10x
23	*Eucalyptus ovata*	MS	0.006	0.6 - 1.6	0.5 - 0.6	Grey-brown colour, prune shaped seeds, shrivelled and dimpled irregular rounded shape; sometimes pointy at one end; standard selected for trials - round.
24	*Eucalyptus ovata*	D	0.0086	0.8 - 1.5	0.4 - 0.6	Grey-brown in colour; shrivelled irregular shape; often prune like; sometimes rhomboidal. Pocked surface with central oval or tear shaped brown spot
25	*Eucalyptus ovata*	W	0.0054	0.7 - 1.5	0.4 - 0.7	Light grey-brown colour, prune shaped seeds, shrivelled and dimpled irregular rounded shape; sometimes pointy at one end; standard selected for trials - round.
27	*Eucalyptus ovata*	D	0.0099	0.8 - 1.5	0.5 - 0.6	Light brown-grey; very shrivelled and irregularly shaped, mainly ovoid. Slightly pocked surface with glistening flat spots. The pale central oval spot is faint.
37	*Eucalyptus ovata*	W	0.0087	0.7 - 1.5	0.5 - 0.7	Dark grey-black colour, ovoid shape mostly, irregular and prune like, with central creamy oval spot.
38	*Eucalyptus ovata*	W	0.0049	0.7 - 1.4	0.4 - 0.7	Dark grey-black; very shrivelled, prune like mostly, but more irregularly shaped than No. 37. 10% triangular all with creamy oval central spot.
39	*Eucalyptus ovata*	DA	0.0099	0.8 - 1.5	0.4 - 0.8	Dark grey-brown with creamy central spot on one side; prune like ovoid shape, occasionally tear shaped; flattened. Pocked surface that glistens under light.
40	*Eucalyptus ovata*	LS	0.0084	0.7 - 1.4	0.6 - 0.8	Dark grey – brown, shrivelled ovoid seed, irregular shape; with creamy central spot. Pocked, glistening surface. Tear shaped, triangulated, and rhomboidal shapes.
15	*Eucalyptus blakelyi*	LS	0.0032	0.5 - 0.8	0.4 - 0.6	Brown to black in colour; almost triangular pyramid shape; slightly shrivelled.
22	*Eucalyptus aggregata*	MS	0.0026	0.7 - 1.0	0.4 - 0.6	Light brown fairly matt colour; prune shaped seed with oval dimples; shrivelled rounded, irregular shape.

No.	Species	Habitat	Weight (g)	Length (mm)	Width (mm)	Seed characteristics via visual assessment 2-10x
28	*Leptospermum flavescens*	DA	0.0024	1.2 - 1.7	0.1 - 0.2	Amber-light brown colour; curved shape; with very fine hairs at both tips; widest in middle, tapering to ends; hairs 4mm long.
35	*Leptospermum flavescens*	MS	0.0041	3.5 - 5.3	0.4 - 0.5	Yellow-amber to creamy coloured, striated, long squarish to cylindrical seed with slight "S" curve; one end tapered, the other end with small 'foot' at one end.
43	*Leptospermum flavescens*	DA	0.0024	1.1 - 1.5	0.1 - 0.3	Honey-amber to brown in colour; long striated, "S" shaped seed mainly, occasional "C" shape, very curved; with shaped fine hairs at tip striations; foot more a stalk.
44	*Leptospermum flavescens*	WA	0.0006	1.1 - 1.5	0.1 - 0.3	Honey-amber to brown in colour; long striated, "S" shaped seed mainly, occasional "C" shape, much curved; with shaped fine hairs at tip striations.

4.3 Germination Results – all species

The statistical analysis and its overall findings show there were significant differences in germination that were related to habitat of the seed source ($P<0.001$). The results also show a two-way interaction between treatment and seed source ($P<0.05$). Seed from saline, waterlogged and acid habitats had a significantly higher germination that seed sources from dry habitats ($P<0.001$). Differences in germination were also significant between the high and low salinity habitats and other habitat types ($P<0.001$). There was generally no significant difference in germination between low and high salinity habitats, which suggests that low salinity habitats would be preferred for seed collection as there was a much higher yield per gram of seed, as shown via the seed sorting under the microscope (see Table 9.). Some species however, did show a difference so individual species are looked at in more detail in the sections below. The germination of seed from acid habitats was also significantly different ($P<0.001$). There was some consistency in the way different habitat types reacted to the treatments across all species ($P<0.05$). There were similarities in treatment response between species of the same genus and some similarities between genera ($P<0.001$; $P<0.05$).

4.3.1 Data Analysis and Summary

The negative impacts of glass petri dishes and high temperatures leading to growth of fungi, algae and germinant mortality due to overheating in the dishes, particularly those with water were included in all of the data analyses as confounding factors. The data analysis of these pot trials was used as a comparative tool to assess the detrimental effect of the glass petri dish for analysis of cotyledon emergence results.

7 of the 10 species tested had three or more seed sources from different habitat types. Analysis at Day 10 showed a significant difference in the germination rates of seed from different habitat types for five of the six species that had commenced germination. The significant difference in germination due to the fixed effect of habitat continued for the duration of the trial for nearly all species, to final analysis at Day 39.

When compared to all other species trialled, *E. pauciflora* was slow to germinate. *A. decurrens* showed treatment and habitat differences at Day 25, prior to extreme temperatures, but this difference was not apparent at the final count, however the two-way interaction of treatment and habitat appeared as moderately significant (P<0.05).

Treatment was also a highly significant effect during the trial, mainly due to the effect of the medium salinity treatment which significantly retarded the onset of germination and the final proportion of germinants. The medium salinity treatment was found to be a significant factor in determining germination rates at for all species (P<0.001) apart from *C. stricta,* which showed no significant difference in germination due to treatment at the end of the trial. *E. aggregata* and *E. blakelyi* also showed no significant differences in germination response to treatment at the final count, but the onset of germination was delayed by the medium salinity treatment (P<0.001).

All treatments impacted on germination to some degree, at some point in the trial, with final germination of < 0.08 in most cases. *E. ovata* was a notable exception with germination over 0.9.

Treatment was a significant factor in the final germination for *A. melanoxylon, E. pauciflora, C. littoralis and E. dives* (P<0.001). There were significant differences in radicle protrusion according to habitat type for all species as mentioned above, with a moderately significant interaction between habitat type and treatment for the full model (*P*<0.05).

Table 10 lists all of the significant factors and interactions (*P*<0.05 or *P*<0.001). The results for radicle protrusion at Day 10 show significant differences in germination found within each individual species and, the full model shows significant differences for analysis of all species together. Treatment is a significant factor for germination results of all species except *E. pauciflora*, which had not commenced germination by this date and also not significant for *A. decurrens* at that time between its two saline habitats. Habitat was a significant factor for *A. melanoxylon*, *E. ovata*, E. dives, *L. flavescens* and *C. stricta*.

At Day 10, *A. melanoxylon* showed a two-way interaction between treatment and habitat, meaning that there were consistent significant differences in the germination response to treatment, based on habitat type.

At Day 10, when the full model is analysed, there was also a significant two-way interaction for treatment and habitat. This is maintained throughout the trial, which clearly demonstrates there is a significant difference in germination in response to treatments for salinity and waterlogging, based on seed source habitat.

Please see the results for individual species (section 4.4) and in Table 10 below.

Table 10. Statistically Significant Differences in Germination

Species	Habitat Types	Significant Factors & Interactions
Radicle Protrusion at Day 10		
Acacia melanoxylon	D W	Treatment + Habitat + Treatment.Habitat
Eucalyptus ovate	D DA LS MS W	Treatment + Habitat
Eucalyptus pauciflora	D DA HS MS W WA	no germination
Casuarina littoralis	D DA HS LS MS W WA	Treatment
Acacia decurrens	HS LS	no differences
Eucalyptus dives	DA LS MS W	Treatment + Habitat
Leptospermum flavescens	DA MS WA	Treatment + Habitat
Casuarina stricta	D MS	Habitat
Eucalyptus aggregata	MS	Treatment
Eucalyptus blakelyi	LS	Treatment
All species - full model	**All of the above**	**Treatment.Habitat**
Radicle Protrusion at Day 25		
Acacia melanoxylon	D W	Treatment + Habitat
Eucalyptus ovate	D DA LS MS W	Treatment + Habitat
Eucalyptus pauciflora	D DA HS MS W WA	Treatment + Habitat
Casuarina littoralis	D DA HS LS MS W WA	Treatment + Treatment.Habitat
Acacia decurrens	HS LS	Treatment + Habitat
Eucalyptus dives	DA LS MS W	Treatment
Leptospermum flavescens	DA MS WA	Habitat
Casuarina stricta	D MS	Habitat
Eucalyptus aggregata	MS	no differences
Eucalyptus blakelyi	LS	no differences
All species - full model	**All of the above**	**Treatment.Habitat**
Radicle Protrusion at Day 39		
Acacia melanoxylon	D W	Treatment + Habitat + Treatment.Habitat
Eucalyptus ovate	D DA LS MS W	Habitat
Eucalyptus pauciflora	D DA HS MS W WA	Treatment + Habitat
Casuarina littoralis	D DA HS LS MS W WA	Treatment + Habitat
Acacia decurrens	HS LS	Treatment.Habitat
Eucalyptus dives	DA LS MS W	Treatment + Habitat
Leptospermum flavescens	DA MS WA	Habitat
Casuarina stricta	D MS	Habitat
Eucalyptus aggregata	MS	no differences
Eucalyptus blakelyi	LS	no differences
All species - full model	**All of the above**	**Treatment.Habitat**

4.3.2 Treatment and Habitat Interaction Summary

Table 11 below shows probable means for each habitat type, using the radicle protrusion results, across all species at Day 39. Probable means are shown unweighted, averaged over all species except *E. pauciflora*, which germinated poorly and could be removed from the calculations, as an outlier. The overall means show the medium salinity treatment (MS) was the most detrimental to germination. When the means are unweighted, the statistical analysis shows the Low Salinity (LS) seed source habitat had the highest average germination across all treatments and species (line 1 and line2), with the dry habitat seed sources showing the lowest germination across all treatments. The LS habitat also showed the highest germination of all tests, in the PuraCast and low salinity treatment (0.96). For statistical correctness, the probable means with weighting across the whole dataset are the most likely true, showing Waterlogged and Acid (WA) habitat with a significantly high mean germination, across all treatments ($P<0.001$).

Table 11. Average Germination by Treatment and Habitat Type

Habitat type	D	DA	HS	LS	MS	W	WA	Mean
Probable Means – all species – Unweighted	0.57	0.69	0.62	0.72	0.59	0.59	0.59	
Probable means unweighted excluding *E. pauciflora outliers*	0.66	0.77	0.80	0.82	0.74	0.79	0.78	
C	0.73	0.78	0.85	0.88	0.80	0.86	0.88	0.79
LS	0.77	0.80	0.85	0.86	0.79	0.89	0.80	0.79
MS	0.51	0.57	0.58	0.62	0.56	0.64	0.63	0.54
PS	0.76	0.80	0.73	0.96	0.71	0.81	0.78	0.75
W	0.55	0.81	0.85	0.78	0.76	0.76	0.78	0.74
WS	0.65	0.85	0.95	0.80	0.79	0.77	0.80	0.79
Habitat type	**D**	**DA**	**HS**	**LS**	**MS**	**W**	**WA**	
Probable means incl. outlier & weighting	**0.56**	**0.66**	**0.64**	**0.65**	**0.61**	**0.56**	**0.74**	**SED = 0.04**

Table 11 provides useful information when viewing the responses to individual treatments the low salinity (LS) and high salinity habitats (HS) produced seed with the highest or equal highest germination in 4 out of 6 treatments.

The LS habitat seed sources had the highest germination in all treatments without waterlogging. In the combined treatment of salinity with waterlogging, the seed sourced from HS habitats had a significantly higher germination than all other seed source habitats and also, in the waterlogging only treatment.

HS seed was equal highest in the control treatment (C) with seed sourced from waterlogged and acid habitats (WA), waterlogged habitats (W) and LS habitats. In the medium salinity treatment, the most detrimental treatment, the seed sourced from waterlogged and acid habitats was not significantly higher than germination of seed from LS and W habitat types. The seed sourced from dry acid habitats (DA) had the second highest germination or equal second highest germination in 5 out of 6 treatments. The seed from dry habitats (D) had equal lowest germination in the medium and low salinity treatments and the lowest germination in the Control; Waterlogging; and Waterlogging with Salinity treatments.

4.3.3 Radicle Protrusion Final Results - by Habitat Type

The germination trial had unequal replicates of habitat type and minor confounding factors due to the position in the glasshouse and dish differences (511 petri dishes of the same type were needed for the experiment but were simply not available in the same size and glass thickness). Once outliers were removed from the data, *i.e.* data from *E. pauciflora*, the number of seed source replicates ranged from 3 replicates of high salinity habitats, to 5 replicates of waterlogged habitats and 5 for medium salinity habitats. The tables below show probable means for each species, grouped according to habitat type. This information is also shown in the appendices, but tables are by species. The aim of the thesis is to discuss differences due to habitat type, so data is focused on that. Outliers have been defined as seed sources of < 40% overall germination and their data is excluded from the calculations of probable means. For calculation of significant differences, add or subtract the average Standard Error of Difference (SED) of 0.04.

Table 12. Dry Habitat (D) Final Germination

Treatment	Ep	Eov	Amel	Clitt	Cs	Mean
C	0.21	0.90	0.54	0.66	0.80	0.73
LS	0.20	0.95	0.64	0.79	0.70	0.77
MS	0.06	0.85	0.52	0.15	0.50	0.51
PS	0.08	0.90	0.72	0.66	0.75	0.76
W	0.35	0.95	0.24	0.40	0.60	0.55
WS	0.29	0.90	0.28	0.70	0.70	0.65
Average over all treatments	**0.20**	**0.91**	**0.49**	**0.56**	**0.68**	**0.66**

SED=0.04

Table 13. Dry and Acid Habitat (DA) Final Germination

Treatment	Ep	Eov	Clitt	Ed	Lf	Mean
C	0.44	1.00	0.50	0.63	1.00	0.78
LS	0.43	1.00	0.61	0.67	0.90	0.80
MS	0.15	0.90	0.50	0.10	0.79	0.57
PS	0.21	1.00	0.75	0.51	0.95	0.80
W	0.61	1.00	0.47	0.76	1.00	0.81
WS	0.55	1.00	0.71	0.78	0.89	0.85
Average over all treatments	**0.40**	**0.98**	**0.59**	**0.575**	**0.92**	**0.77**

SED=0.04

Table 14. High Salinity Habitat (HS) Final Germination

Treatment	Ep	Am	Clitt	Mean
C	0.29	0.9	0.80	0.85
LS	0.28	0.95	0.75	0.85
MS	0.08	0.75	0.40	0.58
PS	0.12	0.8	0.65	0.73
W	0.45	0.95	0.75	0.85
WS	0.38	1.00	0.90	0.95
Average over all treatments	**0.27**	**0.89**	**0.71**	**0.80**

SED=0.04

Table 15. Low Salinity Habitat (LS) Final Germination

Treatment	Eov	Ad	Clitt	Ed	Eb	Mean
C	0.80	1.00	0.90	0.41	0.81	0.88
LS	0.90	0.80	0.75	0.45	1.00	0.86
MS	1.00	0.50	0.15	0.04	0.81	0.62
PS	1.00	0.90	0.95	0.29	1.00	0.96
W	0.85	0.70	0.55	0.56	1.00	0.78
WS	0.90	0.60	0.85	0.58	0.86	0.80
Average over all treatments	**0.91**	**0.75**	**0.69**	**0.39**	**0.91**	**0.82**

SED=0.04

Table 16. Medium Salinity Habitat (MS) Final Germination

Treatment	Ep	Eov	Clitt	Ed	Ea	Cs	Lf	Means
C	0.36	0.80	0.80	0.74	0.65	1.00	0.10	0.80
LS	0.36	0.90	0.60	0.78	0.68	1.00	0.20	0.79
MS	0.11	0.85	0.40	0.16	0.40	1.00	0.20	0.56
PS	0.17	0.90	0.20	0.63	0.80	1.00	0.10	0.71
W	0.54	0.80	0.40	0.84	0.75	1.00	0.20	0.76
WS	0.47	0.90	0.80	0.85	0.60	0.80	0.20	0.79
Average over all treatments	**0.33**	**0.86**	**0.53**	**0.67**	**0.65**	**0.97**	**0.17**	**0.73**

SED=0.04

Table 17. Waterlogged Habitat (W) Final Germination

Treatment	Ep	Eov	Am	Clitt	Ed	Means
C	0.26	1.00	0.86	0.71	0.37	0.86
LS	0.26	0.95	0.91	0.81	0.41	0.89
MS	0.07	1.00	0.86	0.07	0.04	0.64
PS	0.11	0.90	0.93	0.60	0.26	0.81
W	0.42	1.00	0.63	0.66	0.52	0.76
WS	0.35	0.92	0.69	0.71	0.54	0.77
Average over all treatments	**0.25**	**0.96**	**0.81**	**0.59**	**0.36**	**0.79**

SED=0.04

Table 18. Waterlogged and Acid Habitat (WA) Final Germination

Treatment	Ep	Clitt	Lf	Means
C	0.22	0.80	0.95	0.88
LS	0.21	0.75	0.85	0.80
MS	0.06	0.40	0.85	0.63
PS	0.09	0.60	0.95	0.78
W	0.36	0.65	0.90	0.78
WS	0.30	0.85	0.75	0.80
Average over all treatments	**0.21**	**0.68**	**0.88**	**0.78**

SED=0.04

4.4 Radicle Protrusion - Intraspecies Variation

When variation within each species is looked at in relation to their different seed source habitats, and the genera are looked at together, their responses to treatments and the patterns pertaining to each genus can be seen in more detail. This is perhaps more meaningful than analysing all species together. The radicle protrusion results are presented for *Acacia*, *Eucalyptus, and Casuarina*, showing only the species that had multiple habitats tested.

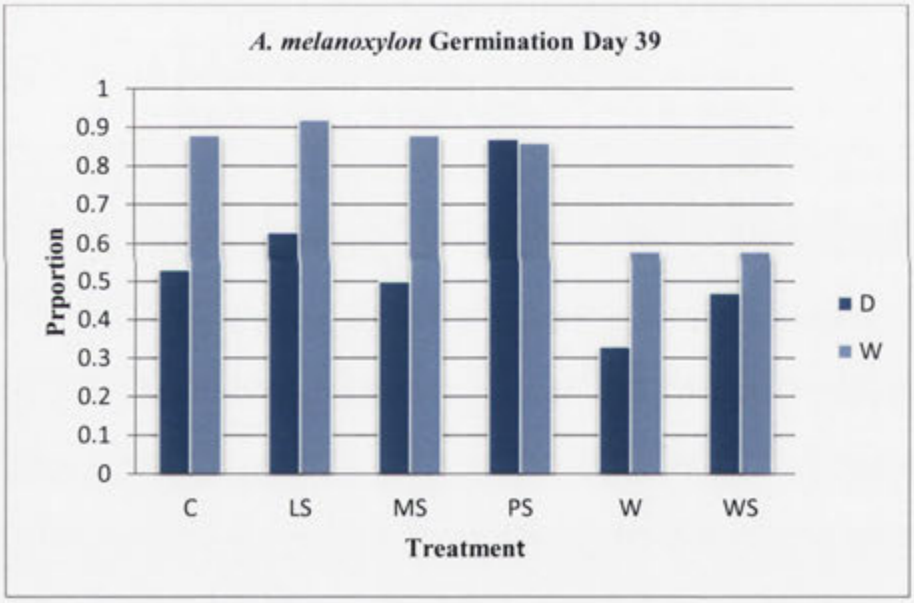
4.4.1 Results for *Acacia* species

***Acacia melanoxylon* – Blackwood or Hickory Wattle**

A. melanoxylon had statistically significant differences in germination rates and final total proportion of germinants due to seed source habitat type ($P<0.001$), treatment ($P<0.001$) and showed moderately significant differences for the interaction of treatment by habitat ($P<0.05$). The seed sourced from low salinity habitats (LS) germinated successfully in viability tests the year before, but was unviable in this trial. The seed was more than 2 years old and although it was stored in a purpose built cabinet, it was not fully temperature-controlled storage and must have deteriorated. *A. melanoxylon* in the project region sets seed every year, so it is likely the seed is short-lived. Seed sourced from other habitats of *A. melanoxylon* were of similar age, so longevity was not an issue.

There were significantly lower germination rates and proportions due to treatments of waterlogging and waterlogging with salinity for both habitat types tested compared to the controls ($P<0.001$). Dry habitat seed (D) had the highest germination in the *PuraCast* and low salinity (PS) treatment. This suggests that *A. melanoxylon* seed sourced from less challenging habitats may respond to the use of a biologically active fertiliser. The second highest germination was in the low salinity treatment, then the control; and medium salinity treatment, with little or no significant differences. This suggests a degree of salt tolerance, where low and medium salinity treatments show little variation in germination when compared with germination under the control treatment.

In Figure 12 below, seed sourced from waterlogged habitats had higher overall germination that seed sourced from dry habitats in 5 out of 6 treatments ($P<0.001$). The highest germination rate for W was in the low salinity treatment; the second highest was in the control and MS treatments, closely followed by the *PuraCast* with LS treatment. This suggests a degree of salt tolerance and, a moderate response to biologically active fertiliser.



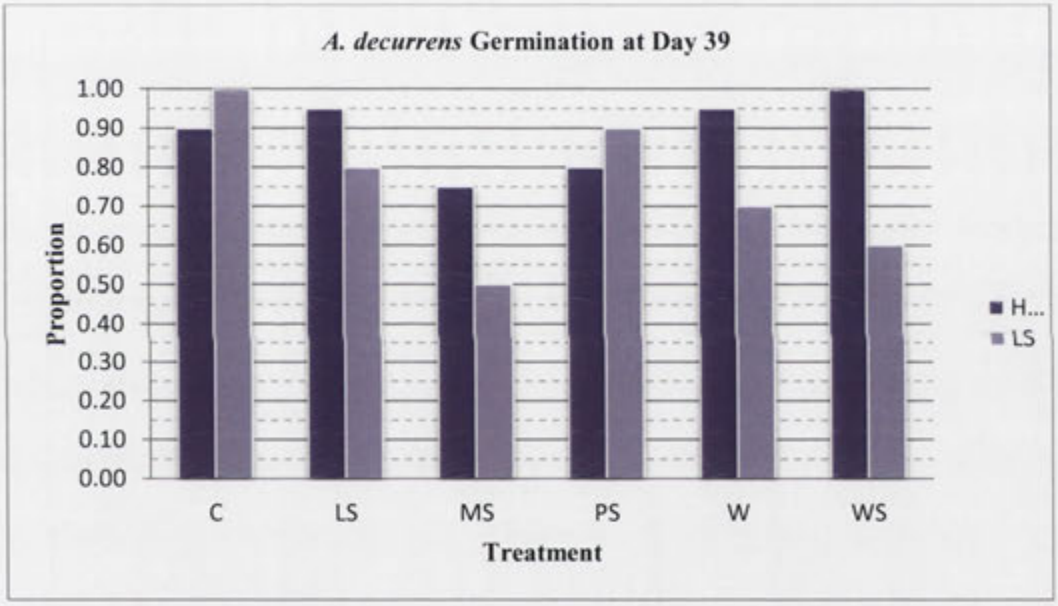
SED= 0.04

Figure 12. *A. melanoxylon* Final Germination - showing each habitat type

Acacia decurrens* – *Silver Wattle

A. decurrens seed sourced from highly saline habitats had the highest germination in low salinity with waterlogging combination treatment and the lowest germination in the medium salinity treatment. *A. decurrens* seed sources from low salinity habitats had the low germination in the medium salinity treatment, with highest germination in the control.

The germination patterns, as seen in Figure 13 below, are not regular with one habitat type performing better across all treatments. The seed from highly saline habitats showed less variation in germination across all treatments when compared to the low salinity habitat, which suggests a higher degree of salinity tolerance. There was a significant difference in final germination according to seed source habitat type (*P*<0.001), with the seed sourced from high salinity habitats performing better under all saline and waterlogged treatments (SED=0.04). HS seed sources also showed a significantly higher overall germination under the most stressful treatments of medium salinity and waterlogging with salinity.



SED= 0.04.

Figure 13. *A. decurrens* Final Germination

Seed from low salinity habitats (LS) had a higher germination in the control. This has been commented on elsewhere, that seed sourced from low salinity habitats had a higher viability than seed sourced from highly saline habitats. The LS seed also showed a stronger response to *PuraCast* with low salinity treatment, producing a higher germination than under the low salinity treatment. The high salinity habitat seed germination was lower in the *PuraCast* with low salinity treatment than it was in the low salinity treatment. This suggests that seed from less stressful habitats is able to respond more readily to fertilizer applications, which supports the findings for *A. melanoxylon*.

4.4.2 Results for *Eucalyptus* species

The highest germination was found for seed sourced from Dry Acid parent tree habitats followed by the seed from saline and waterlogged habitats. Seed sourced from less challenging habitats generally showed the lowest germination. Figure 14 below shows this. See subsections below for more detail on each species.

<u>Eucalyptus pauciflora – Snowgum</u>

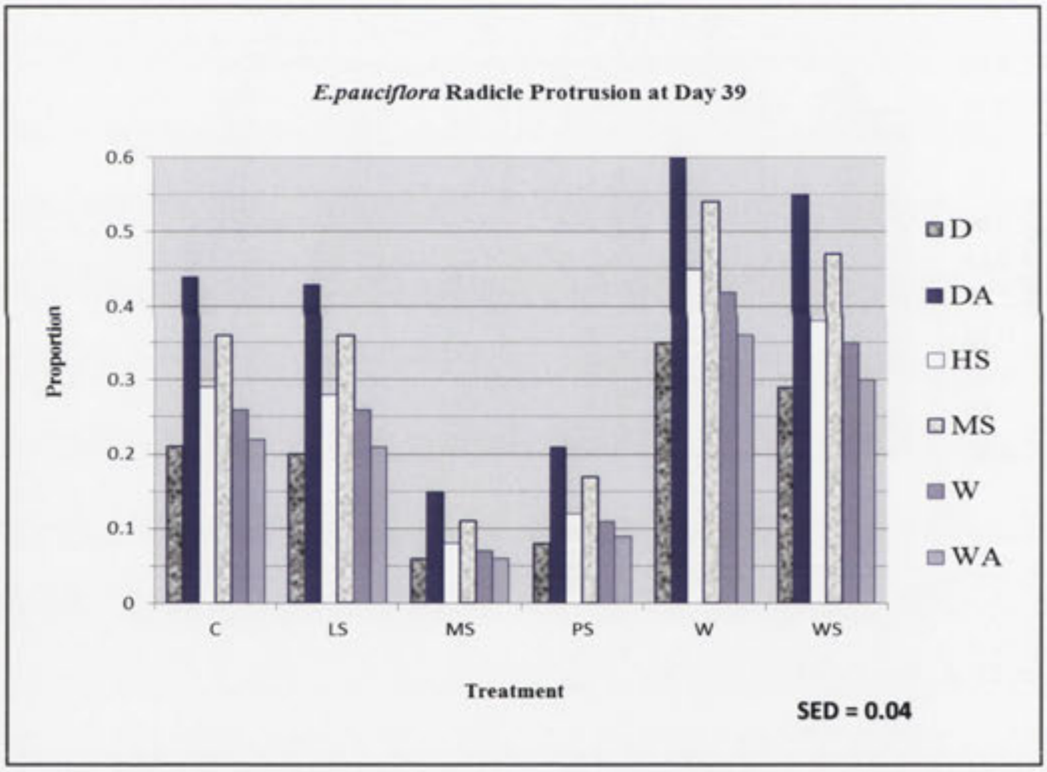


Figure 14. *E. pauciflora* Final Germination

All seed sources of the *E.pauciflora* had pre-trial germination rates greater than 0.75 (75%). Seed sources used in the trial showed final germination 60% or less. Onset of germination was also delayed, with < 20% germination average at Day 10. The graph above shows significant differences between treatments (P<0.001) and habitats (P=0.006). Seed sourced from dry and acid habitats (DA) had the highest germination across all treatments, with seed from dry habitats showing the lowest germination. The seed from medium salinity habitats (MS) had higher germination rates than seed from high salinity habitats (HS). Other differences of note were the higher germination of all habitats under both waterlogging treatments; and the relative lower germination of the waterlogged and acid habitat (WA) seed compared to seed from waterlogged (W) habitats.

Eucalyptus ovata – Swamp Gum

The germination of *Eucalyptus ovata* was significantly different between treatments and also, between habitats (P<0.001 and P=0.001 respectively). *E.ovata* had the highest germination compared to the other Eucalypts with multiple habitat seed sources. The dry and acid habitat seed sources (DA) had the highest average in each treatment, particularly in the combination treatment of Waterlogging with salinity (WS in graphs below) and the fastest germination rates (see figures below for more detail).

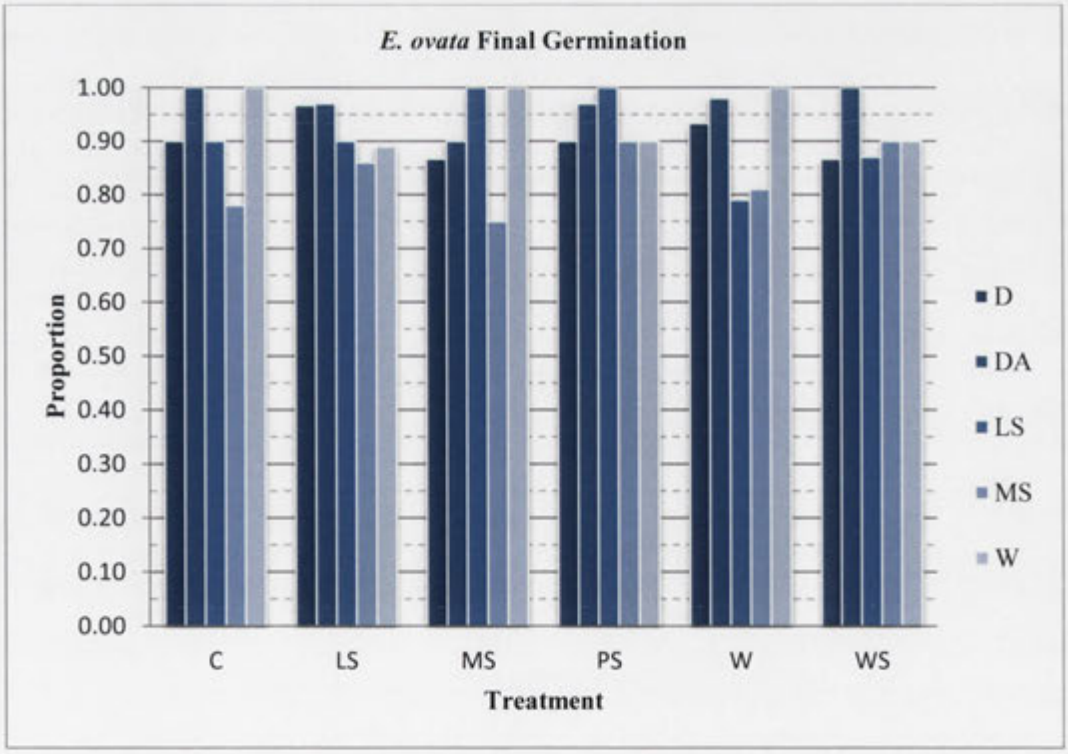
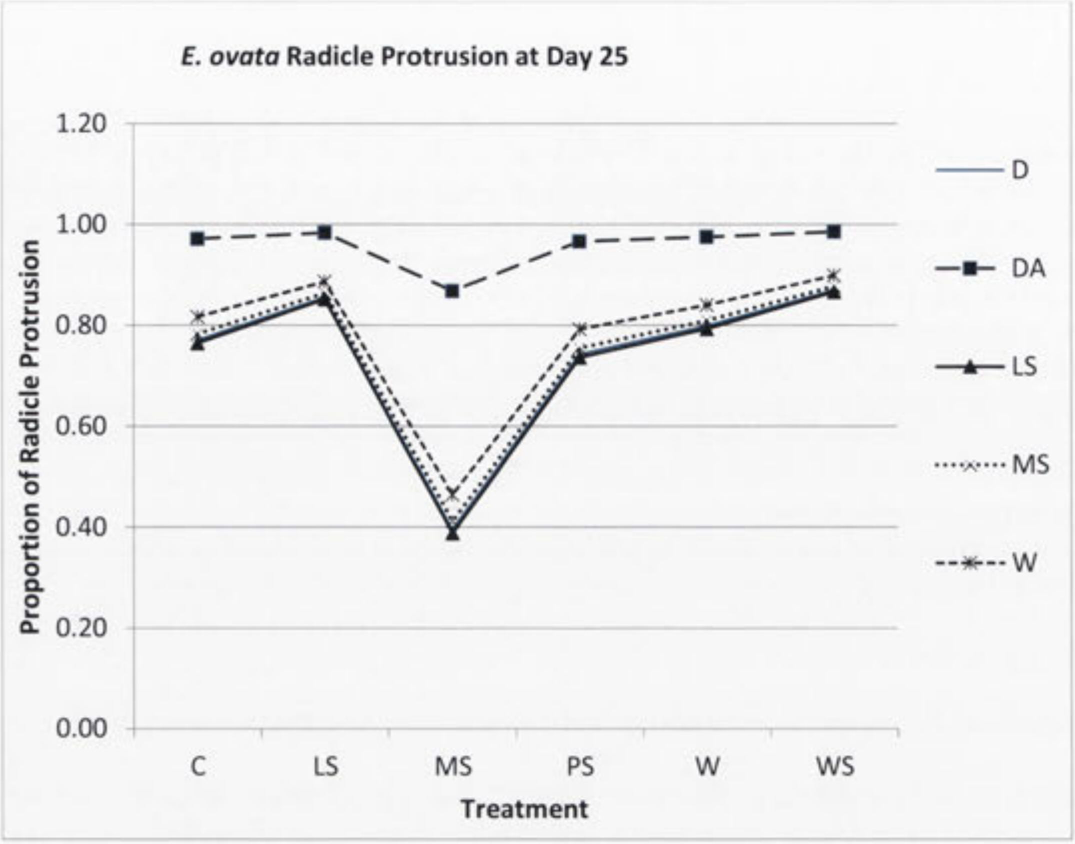


Figure 15. *E. ovata* Final Germination



SED = 0.04

Figure 16. *E. ovata* Germination Rates

The effect of Medium Salinity treatment as shown above is significantly detrimental to germination at Day 25 (in Figure 16 above), showing reduced germination for all habitats. The DA habitat seed was the least affected under the medium salinity treatment at Day 25, although it did not have the highest overall germination at the end of the trial (Day 39 – see Figure 15), this could have been due to the confounding factor of high temperatures. The LS and Dry seed sources had the lowest germination and the lines are almost inseparable in Figure 16, above. The waterlogged habitat seed (W) showed second highest germination. Overall, the MS habitat seed source showed third highest germination for all treatments. The results for the Dry Acid habitat were significantly higher than germination of all other habitat types, across all treatments.

E.dives – Broad-leaf Peppermint Gum

The seed sourced from medium salinity habitats (MS) for *E.dives* had a significantly higher germination in all treatments, notably in the two treatments with waterlogging. This is possibly indicative of the moisture stress experienced through drying out of the petri dishes in the treatments, as *E.pauciflora* seed sources exhibited the same characteristic. The broiling effect was not as severe for the *Eucalyptus* species. The adverse effect of the medium salinity treatment on all seed sources of *E.dives* is significant, with final germination of only 5-15%. The acid habitat seed sources had the second highest germination for all treatments for *E.dives* which follows the pattern found with the other Eucalypts, where the DA seed sources had the highest or second highest germination of all habitat types.

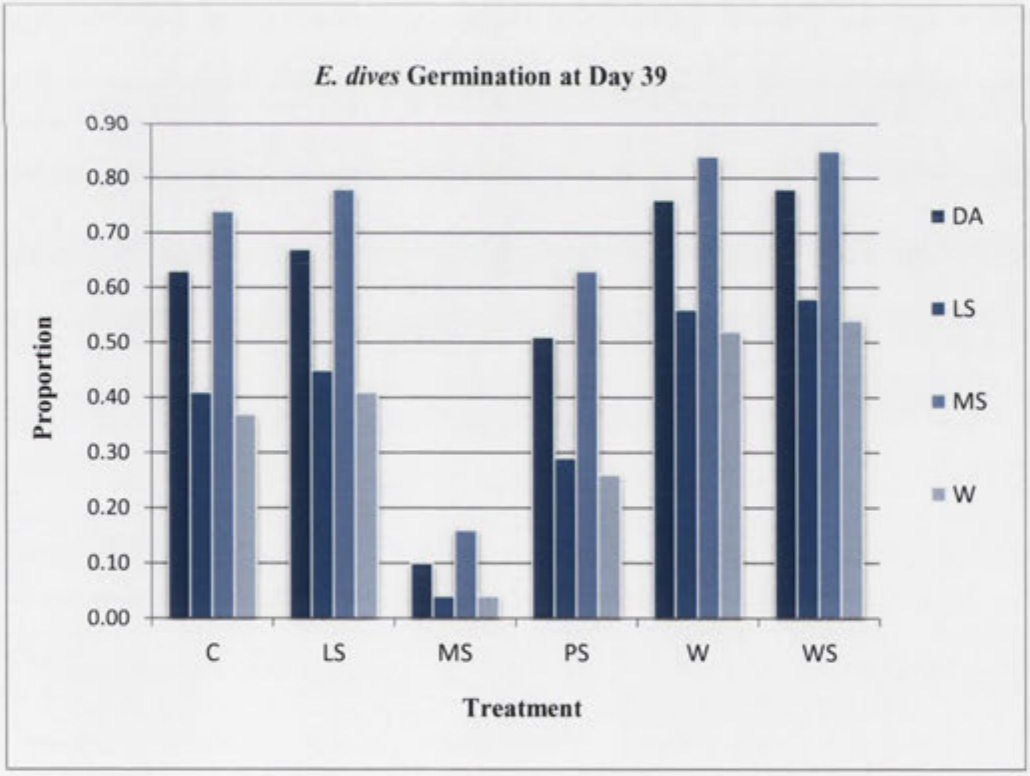


Figure 17. *E. dives* Final Germination SED = 0.04

4.4.3 Results for *Casuarina* species

Casuarina littoralis

C. littoralis had the full range of seed sources, with seed sources from all seven habitat types. They exhibited a varied response to each treatment and these differences were significant ($P<0.001$). None of the habitats had consistently higher germination across all the treatments, but as was found for the other species, the dry acid habitats, high, medium and low salinity habitat seed sources all showed higher germination than seed from other habitats in the more stressful treatments.

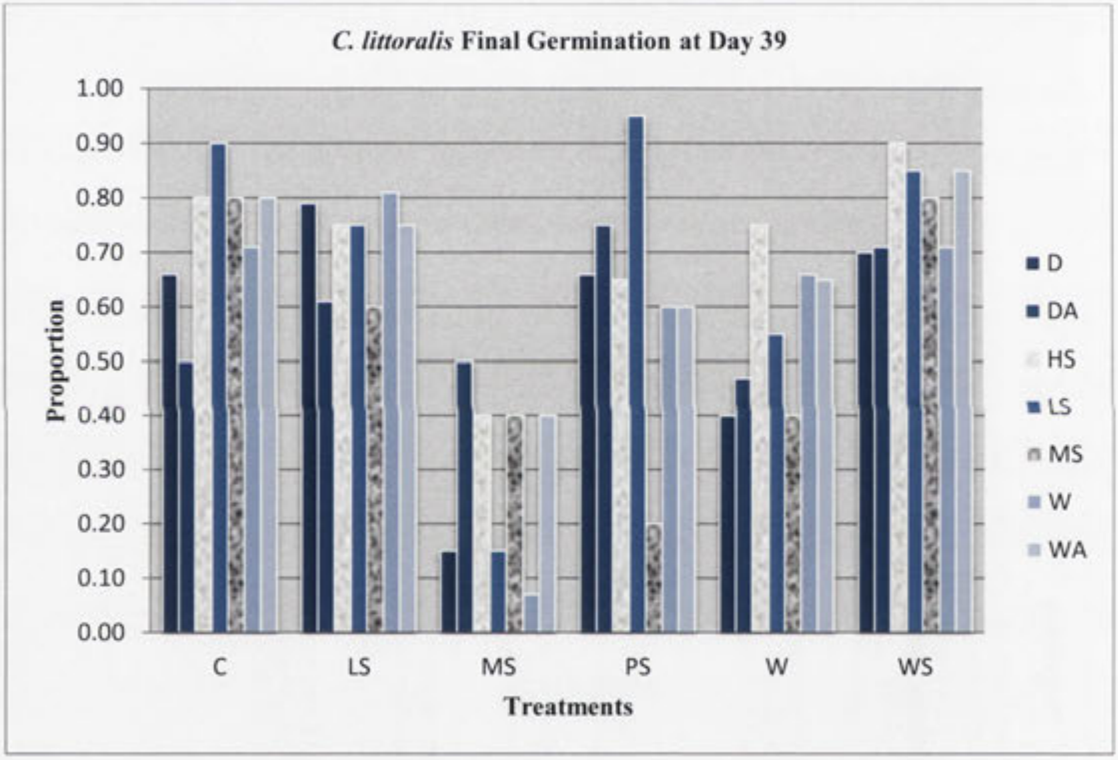


Figure 18. *C. littoralis* Final Germination – All Habitats SED=0.04

In Figure 18 above DA habitat had the highest germination rate of all seed sources in the medium salinity treatments, which supports results found for Eucalypts. The seed from WA, HS and MS habitats had the second highest overall germination under the treatment of medium salinity. The high salinity habitat seed showed the highest germination under the waterlogging with salinity treatment. This is also backs the *Eucalyptus* species findings.

The waterlogged acid and low salinity habitats had equal second, with the medium salinity habitat seed sources, third highest germination.

C. stricta

The medium salinity habitat seed sources showed the highest final germination percentage for *C. stricta* across all treatments. The most noticeable difference in germination in the first 10 days was the response to the medium salinity treatment (MS). The MS treatment delayed onset of germination in nearly all species and habitat types and significantly lowered the final number of germinants, in comparison with the control ($P<0.001$). All species were affected at Day 10 except *C. stricta* seed sourced from the medium salinity habitat. See Table 19 below showing probable means of radicle protrusion at Day 10. The germination in the medium salinity treatment, when germination for all other species and habitats at Day 10 was zero, is a significant result, and may suggest that the MS habitat of *C. stricta* is highly salt tolerant.

Table 19. C. stricta Radicle Protrusion at Day 10

Habitat	Dry	MS
Treatment	Germination	
C	0.25	0.8
LS	0.35	0.8
MS	0	0.3
PS	0.35	0.6
W	0.35	0.6
WS	0.3	0.4

The germination pattern for *C. stricta* was similar to *C. littoralis* in that there was not a regular order of magnitude difference in germination between habitat types from one treatment to the next, as was found for most of the other species (see Figure 19 below).

The Figure 19 below shows the dry habitat (D) seed had comparatively much lower germination under the waterlogging and medium salinity treatments, but was only 20-30% lower for all other treatments. The medium salinity habitat seed had 100% germination for all treatments except the combination of waterlogging with salinity treatment. These results suggest that *C. stricta* is highly salt tolerant, but less adapted to the combined stress of waterlogging with salinity. See Figure 19 below for more detail.

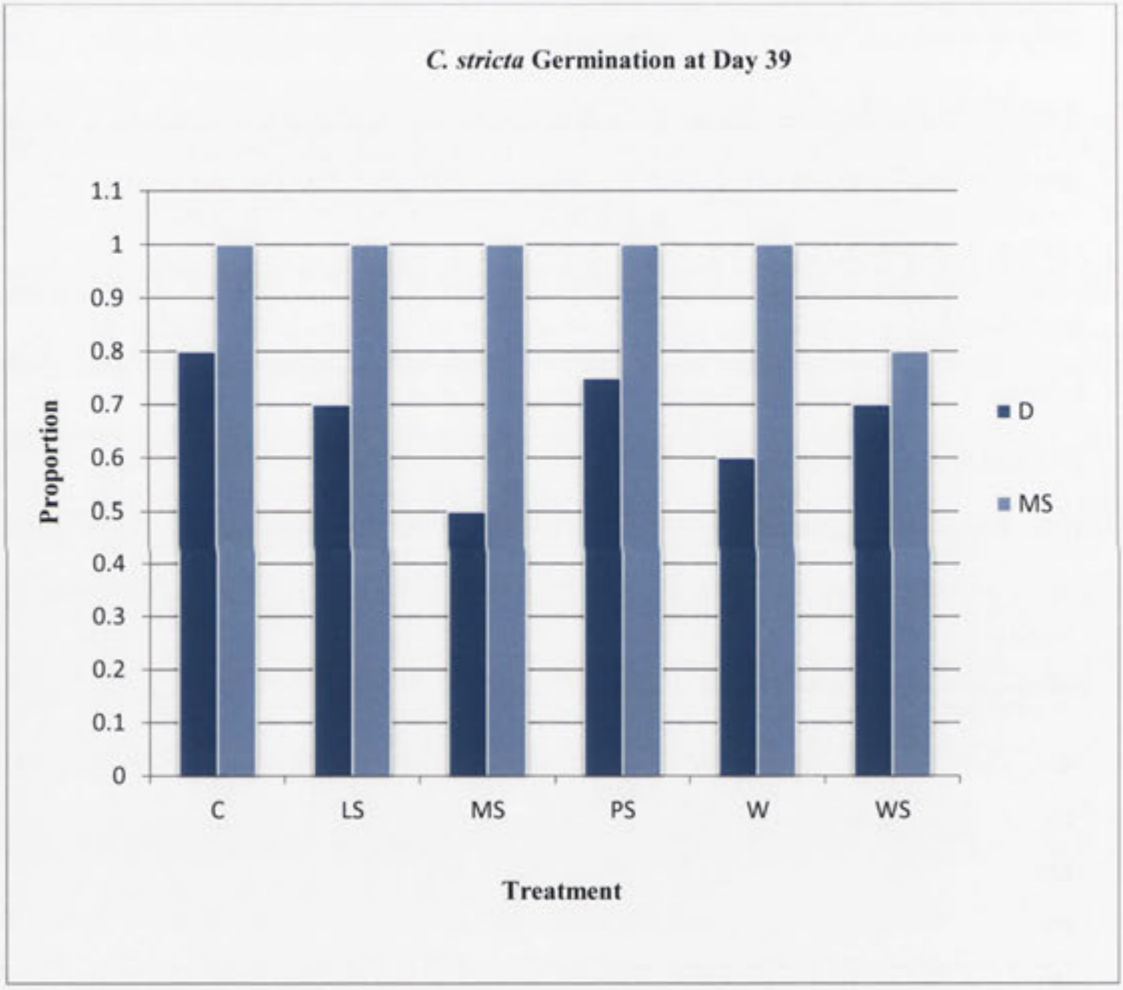


Figure 19. *C. stricta* Germination at Day 39 SED=0.04

Leptospermum flavescens

As with the *Eucalyptus* species, the Dry and Acid habitat had the most viable seed when averaged over all treatments, but also, a slightly lower germination in the medium salinity treatment when compared to seed from waterlogged and acid habitats. The seed from medium salinity habitats had a low viability over all, which may have been due to heat stress during the trial, or perhaps age, as it had germinated well the year before. The highest germination was in the waterlogging treatment. Figure 20 below shows further detail.

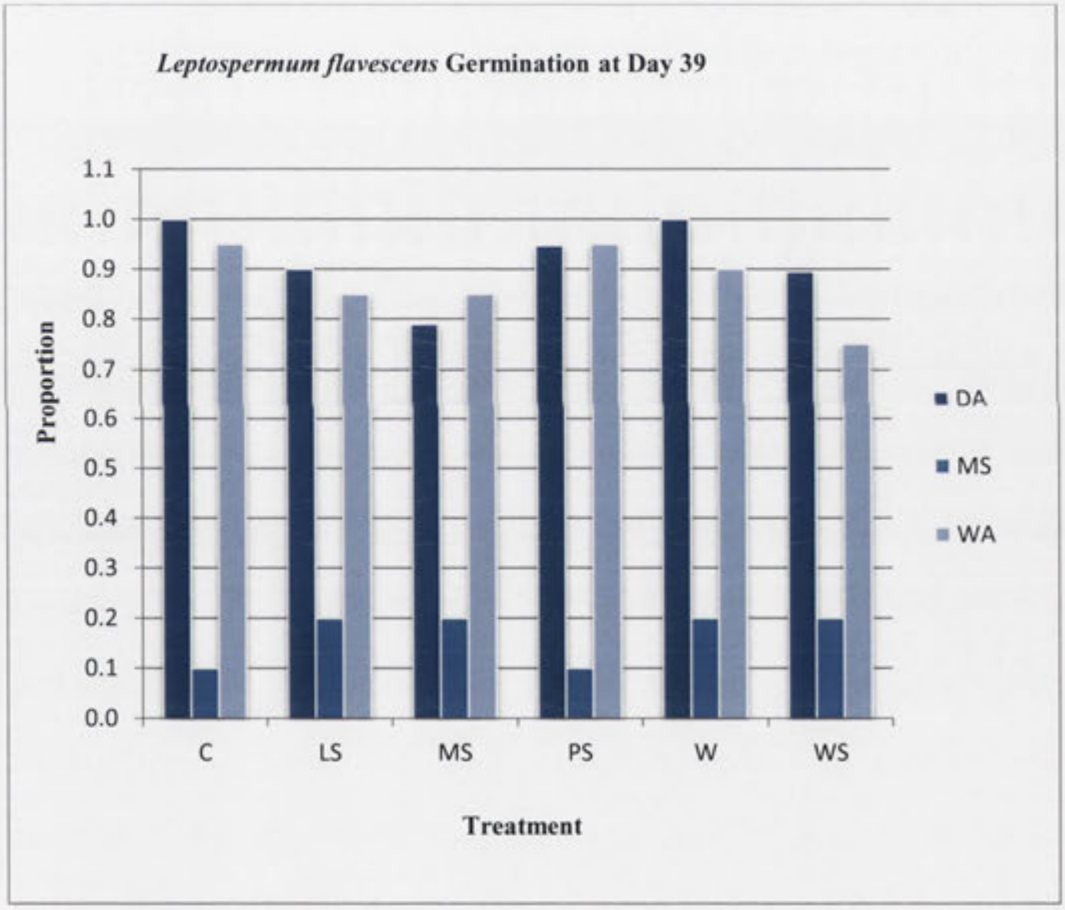


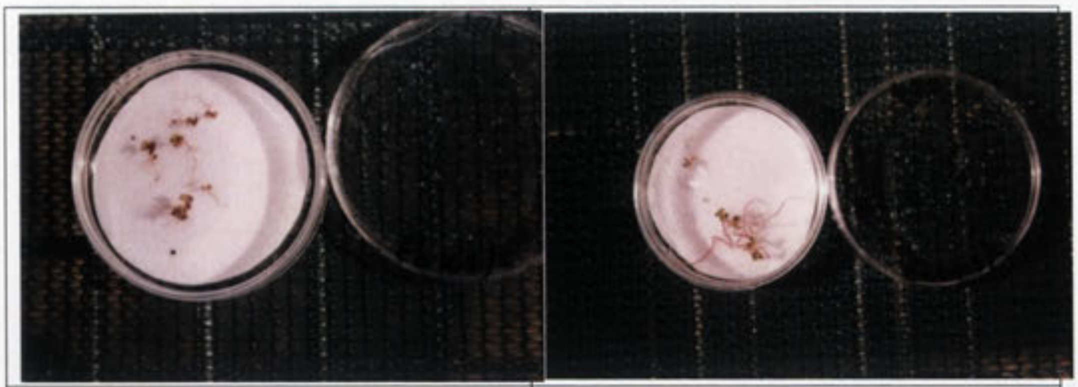
Figure 20. *L. flavescens* Germination at Day 39 SED=0.04

4.5 Summary of Results for Cotyledon Emergence

At Day 25, cotyledon emergence was significantly affected by treatment for 7 out of 10 species. Three of the Eucalypts showed an interaction between treatment and petri dish, suggesting that the effect of the dish compared to the sister experiment conducted in small pots of sandy loam was influencing the treatments. Seed source habitat was a significant factor for determining cotyledon emergence for Day 25 and Day 32, except for *A. decurrens* and *C. littoralis*. *A.decurrens* showed a two-way interaction between treatment and habitat. Table 20 below shows further detail.

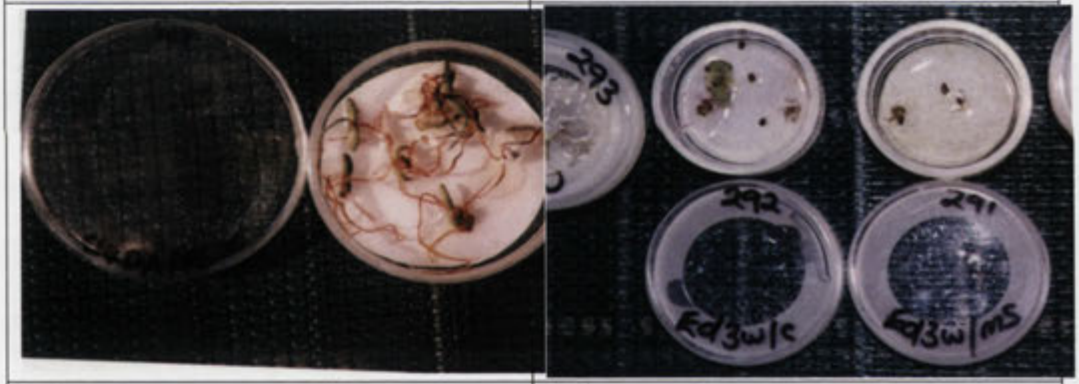
Table 20. Significant Factors ($P<0.001$) affecting Cotyledon Emergence

Species	Habitat Types	Day 25	Day 32
A. melanoxylon	D W	Treatment Habitat	Treatment Habitat
E. ovata	D DA LS MS W	Treatment, Habitat, Dish, Treatment.Habitat Treatment.Dish	Treatment, Habitat, Dish, Treatment.Habitat, Treatment.Dish, Habitat.Dish
E. pauciflora	D DA HS MS W WA	Dish	Treatment, Habitat, Dish
C. littoralis	D DA HS LS MS W WA	Treatment	Treatment
A. decurrens	HS LS	Treatment	Treatment Treatment.Habitat
E. dives	DA LS MS W	Treatment.Dish	Treatment.Dish, Habitat.Dish
L. flavescens	DA MS DA	Habitat	Habitat
C. stricta	D MS	Habitat	Habitat
E. aggregata	MS	Treatment	No differences
E. blakelyi	LS	Treatment	Treatment

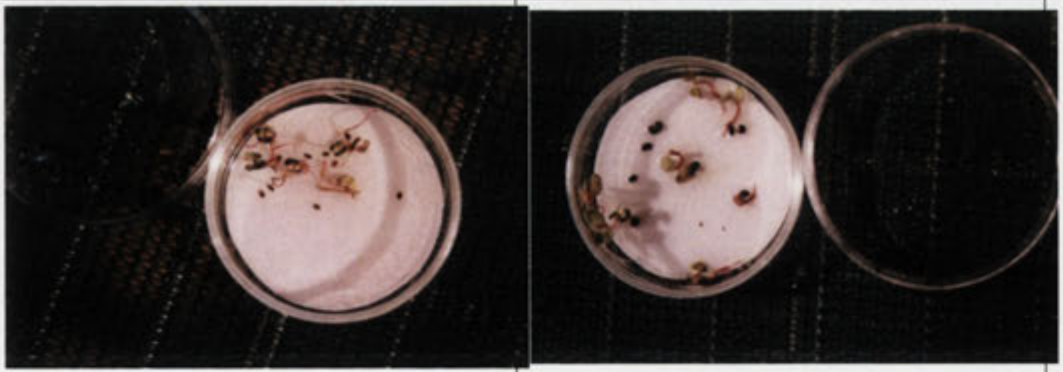


E. blakelyi germinants | *E. aggregata germinants*

L. flavescens germinants | *A. melanoxylon germinants*



C. littoralis germinants | *E. dives germinants*



E. ovata germinants | *E. pauciflora germinants*

Figure 21. Dishes in the Trial showing germinating seeds and cotyledons

CHAPTER 5. DISCUSSION

The results are discussed and the project's experimental methods and suggestions for ways of improving them are reviewed in this chapter. Future work is discussed and proposed. The importance of seed tree habitat and its potentially advantageous provenance selection for each species was shown in the context of germination results as a single factor, was highly significance (*P*<0.001 in most cases). The two-way interaction between habitat and treatment (P<0.05) was moderately significant at Day 10, Day 25 and Day 39. This has ramifications for rehabilitation of degraded soils in the Upper Shoalhaven Catchment and potentially, for other regions where frost and soil degradation are limiting factors to seedling establishment. The research detailed in this thesis had its origins in Landcare. Planting of seedlings and direct seeding into soils adjacent and upslope of scalded areas occurred in the Upper Shoalhaven Catchment 1995-2000 to address environmental degradation, via Landcare and Salt Action projects. Whether the adopted approach of community and youth groups collecting and distributing seed and planting trees across the landscape is a successful method for addressing the problem of land degradation is yet to be fully established and must be kept as a separate issue from revegetation of the landscape as a means of addressing the problem and investigating suitable provenances (Austin, 1980, Christiansen *et al,* 1994; Stirzaker *et al*, 2002; Standish *et al*, 2007).

5.1 New Findings

The most notable new finding as a result of this project was the higher germination under medium salinity conditions of seed collected from an acid habitat, suggesting salt tolerance and seed sourced from high and medium salinity habitats had higher germination in the combined treatments of salinity and waterlogging, also suggesting salt tolerance with multiple gene loci (Kawecki and Ebert, 2004). This was found for all of the *Eucalyptus* species tested and *L. flavescens* (averaged over all treatments).

The literature supported the concept of testing seed sources from saline and waterlogged habitats, with the expectation that the more saline environments would produce more tolerant seed (Bell, 1999, Niknam and McCoomb, 2000). This project found that to be the case for some species, but not all. The low salinity habitats in some species produced a larger volume of viable seed that showed higher or equal germination to seed from medium and high salinity habitats. This is a notable finding for rehabilitation purposes as seed collection is a time consuming and labour intensive process, so collecting from trees that are salt tolerant but also have a higher yield is much more cost-effective.

Other research also found that adaptation to one factor can often accompany adaptation to multiple factors, *e.g.* heavy metal toxicity (Kawecki and Ebert, 2004), so this is a possible explanation for the higher germination of waterlogged habitat and high salinity habitat seed under the treatment of salinity with waterlogging. For highly significant differences ($P<0.001$) to be occurring within multiple species based on seed source habitat differences from a gene pool within a 100 km radius in a frost-prone region, strongly suggests adaptation is occurring from one generation to the next and that seed trees on detrimental habitats may confer multiple tolerances on their progeny.

The high salinity habitats appear to have affected seedset, particularly for *E. pauciflora*. High environmental stress for less salt tolerant species may result in less viable progeny. This may also explain why the *E. pauciflora* and *E. ovata* seed sourced from acid soils was found to be more salt tolerant than seed from the medium and high salinity habitats, with a significantly higher germination percentage under the medium salinity treatment than all other habitat types. The low salinity and medium salinity habitats of *E. ovata* and *E. blakelyi* exhibited seed germination of over 0.09 across all treatments. *E. ovata* showed 1.0 (100%) germination in the medium salinity treatment, which was found to be the most detrimental treatment in this trial, suggesting that *E. ovata* seed sourced from low to medium salinity habitats has a high degree of salt tolerance.

The high salinity habitat seed sources for *A. melanoxylon* showed 1.0 (100%) germination in the waterlogging with salinity combination treatment. *C. littoralis* sourced from the highly saline habitats also showed the highest germination across all treatments and 0.9 (90%) germination in the waterlogging with salinity treatment.

The waterlogged habitat also provided salt tolerant seed sources for *E. ovata* and *A. melanoxylon*, showing germination at over 85% in the medium salinity treatment.

Intra-species variation in tolerances to a combination of environmental stresses, for Australian native trees and shrubs has previously been documented (*e.g.* Bell, 1999). Soil acidity and waterlogging, accompanied by fertilisation and seedset is likely to produce a seed source more tolerant of the conditions found in the parent habitat, should viable seed actually be produced (Kawecki and Ebert, 2004). But how does this explain why these seed sources were more salt tolerant? This is question is discussed in more detail below, after discussion of the salt levels chosen for treatments.

5.2 Overall Experimental Findings

The difference in germination found under six treatments of differing conditions of salinity and waterlogging was strongly related to seed source habitat type ($P<0.001$). The moderately significant two-way interaction between treatment and seed source habitat ($P<0.05$) was demonstrated by results that showed Dry Acid (DA), Medium salinity (MS) and Waterlogged (W) habitats produced seed more tolerant of medium salinity (MS) and the combination salinity with waterlogging (WS) treatment than seed from other habitat types, especially the Dry (D) habitat. Seed sourced from High Salinity (HS) and Waterlogged Acid (WA) habitats had a higher germination under the combination treatment of salinity with waterlogging (WS) than seed from other habitat types, suggesting seed sourced from these habitats is more tolerant of multiple environment stressors. However, these differences were not consistent amongst all habitats and species, so the three-way interaction of treatment, habitat and species was not found to be significant using the Wald statistic and analysing all data, including outliers ($P=0.06$).

The seed from Dry (D) habitats had the lowest germination across all species and genera, under the most stressful treatments, so this is strongly suggestive that collecting seed from stressed environments is a strategy for success when revegetating saline land.

The most stressful environments generally produced the highest germination rates in the most stressful treatments, suggesting they have more tolerant seed. For example, all 3 *Eucalyptus* species from Dry Acid (DA) habitats or Medium Salinity (MS) habitats had higher germination across all treatments and particularly, the medium salinity treatment (MS) and combination of salinity with waterlogging. Figure 16 at Day 25. *E. ovata* results clearly shows that the Dry Acid (DA) habitat seed had the highest germination across all treatments, suggesting it produces the most tolerant seed for this species and possibly, for the genus.

The results also showed significant differences in germination due to habitat within each species (*P*<0.001); but there was inconsistency in the responses, where one habitat type had a higher germination, on average, in one species than in another species. When seed germination from different habitats are viewed in terms of Genera, the treatment response and these differences are less. Each of the genera showed a consistency in the type of habitat type with seed showing the highest germination under the most stressful treatments of medium salinity (MS) and salinity with waterlogging (WS). Each genus showed inherent tolerances, for example, *Eucalyptus* species responded poorly to the medium salinity treatment, but favourably to waterlogging and waterlogging with salinity treatments. Field observations suggest each genus and many species have a preferred niche in the Upper Shoalhaven, where frost is a limiting factor. For example, *Casuarina* species found growing in coastal zones were generally more frost sensitive as juveniles and could only be found in protected environments on the tablelands, *e.g.* beneath larger tree canopies or rocky outcrops.

Differences in the germination of habitat types shown within genera could be partly due to the unequal number of seed collections for each habitat type but also likely due to inherent differences between species and their preferred niche. For example, *E. ovata*, the Swamp Gum, showed that its adaptation as a whole species to waterlogging, may have better prepared it for salinity tolerance (Marcar, 1993) and the heat stress experienced during the trial, favoured it over *E. pauciflora,* the Snowgum. The waterlogging (W) and dry acid (DA) habitats for *E. ovata* showed significantly higher germination under the medium salinity treatment (0.9) which was similar to the trend in other *Eucalyptus* species with these two habitat type producing the most salt tolerant seed. However, the seed of *E. ovata* from low salinity habitats had 100% germination under the medium salinity treatment, which was highly significant (P<0.001) compared to all other collections for *Eucalyptus*. Slight differences in rankings may be linked to inherent tolerances of each species as a whole, i.e. that waterlogging tolerance of *E. ovata* predisposes it to be salt tolerant (Marcar, 1993), but this can only outweigh the effect of localised adaptation, up to a point. This result supports the concept of an evolutionary process that maintains definition of the species, until localised environmental pressure on a population builds to a point where there is genetic divergence into a new species or sub-species (Kawecki and Ebert, 2004). The morphological differences in seed capsules for the salinity seed sources were not initially, readily identifiable as *E.ovata* (Banks, *Pers. Comm.*, 2000).

5.3 Experimental Design

Several papers in the literature supported an investigation of this project's null hypothesis: that variation in salinity and waterlogging tolerances, if present, has no relation to seed source habitat. Previous work found variation within Australian native species' tolerances had some relation to provenance and there was a suggestion that waterlogged or saline habitats could produce more tolerant seed (*e.g.* Sun and Dickinson, 1995). There was also some mention of salt tolerance linked with environmental stresses such as frost or waterlogging (*e.g.* Marcar, 1989, Ball *et al*, 1997, Niknam and McComb, 2000, Kawecki and Ebert, 2004, Kochian *et al*, 2004). However, none of the papers directly dealt with the question of variation in relation to habitat for Australian native species collected within a small geographical radius and, whether it conclusively affected salinity and waterlogging tolerances.

Previous research into the use of a liquid worm castings as a possible ameliorator of salinity or enhancer of germination in saline conditions was not found in the available scientific literature at that time. It was a relatively new commercial product at the time and research into its efficacy was in its infancy. The only scientific paper found was by Parle (1963), discussing the value of earthworm casts in terms of its microbiological content, with later references discussing its efficacy in terms of mineral content and enzymes, not so much its use as a biologically active (Murphy, 1993). Discussion with local biodynamic farmers who had successfully (but not scientifically) trialled another biologically active (BD500) on saline soils, formed the basis for including *PuraCast* in this germination trial. Industry support for the project was naturally, another key driver. Techniques for applying the biologically active were kept consistent with imposition of salt, using the standard methods of micro-pipetting 1mL of liquid, as for imposing the salt solutions. The lack of scientific research for assessing the use of a worm castings product for seed germination trials under saline conditions makes the work described here fairly unique, but also a possible weakness - as no previous work could be drawn upon for setting the increments of fertiliser used. A smaller amount may have been just as effective, but without the confounding factors of algae and/or fungal growth affecting results.

All tables were fronting the bank of windows facing north and made of aluminium mesh, with shadecloth overlain. Each table had its own thermometer that was checked daily and this showed the surface temperatures were consistently the same across all tables. This suggests that differences were slight for external micro-climate of petri dishes, in terms of spatial placement, in comparison to the microclimate inside the dishes. The minor factor of spatial placement was considered along with the other minor confounding factors within the greenhouse, when analysing the results.

Seed was standardised via sorting under a dissecting microscope, to enable seed selection as close as possible to 100% viability for every seed used in the trial. Preliminary viability tests showed 85-90% germination or higher for the selected seed sources (as stated previously), but *E. pauciflora* seed used in the trial showed low viability across its whole seedbank and two other species (*L. flavescens* and *E.dives*) had seed sources of low viability (<50%). Results must be viewed taking this into account and future work should factor in the changing viability of alpine seed germination during spring-summer and the longevity of seed from species which set seed annually.

Microscopic sorting of seed clearly showed morphological differences between habitat types and this helped to drive the project so that the results summarised above, could be found. The subsections below provide a more detailed discussion of the results and the new findings.

5.4 Seed Morphology

Measurements and observations of seed morphology under magnification showed variation existed between different seed sources of the same species prior to the trial commencing, in relation to habitat. Seed sourced from extreme habitats was visually different. Species identification was checked and confirmed. The obvious morphological differences in seed and capsules helped to define the seven categories of seed source habitat for the germination trial as mentioned previously. The morphological differences observed in seed sourced from highly saline habitats (rising watertables) and acid habitats can be now be related to localised adaptation, evidenced by the results of this project showing significant differences in the saline and acid habitats' progeny response to the medium salinity treatment and to a lesser extent, waterlogging and waterlogging with salinity treatments.

5.5 Salinity levels

It was suggested in the literature that plant growth responses are evident at 100 mM concentrations of NaCl and that experimental work for testing salinity tolerances of Eucalypts should utilise 100 mM to 250 mM solutions (Marcar, 1993). These were the levels selected for this project. Testing of standard solutions gives molar conversion of NaCl 100 mM = 1.70 dS/m at 25 °C which is somewhere between the low and moderate salinity averages found on degraded sites in the Upper Shoalhaven Catchment. The germinants showed very little retardation in growth due to salinity in the low salinity treatment, but germination rate and overall percentages were significantly reduced under the medium salinity treatment. This suggests that the higher concentration of sodium chloride disrupted germination processes by inhibiting water uptake and the germination processes inside the seed (Atwell *et al*, 2010).

5.5.1 Effect of Treatments

Individual species and habitats showed significant differences in response to the treatments of medium salinity, waterlogging and waterlogging with salinity treatments ($P<0.001$).When the effect of treatment was averaged across all species and habitats, the medium salinity (MS) treatment proved to be more detrimental than all other treatments ($P<0.001$). Germination responses were delayed under this treatment, as seen by the lower germination rates at Day 10 and Day 25. Results for radicle protrusion after Day 25 were somewhat influenced by heat and water stress, but most seed sources continued to germinate under this treatment. Cotyledon emergence and expansion were severely affected by heat stress, as has been discussed in the results, but habitat ($P<0.001$) and the two-way interaction of habitat by treatment ($P<0.05$) were still found to be significant for all data sets, for species with multiple habitat types.

The results for the control treatment, supposedly the "ideal" conditions for germination, were generally 75-85% germination, with an overall average of 79%, which is lower than the rates found in preliminary viability tests and equal to the rates found with the treatment of low salinity and, the combination treatment of waterlogging with low salinity. Preliminary germination trials showed that the average viability for sorted seed from each collection was at least 85%. This suggests that the control conditions inside the petri dish were stressful, so in fact, there was no control or "ideal" set of conditions for this trial, but a series of 6 environmentally challenging treatments.

Cotyledon emergence was measured to indicate survival and growth of germinants. *Eucalyptus* species can withstand cycles of wetting and drying, but once imbibition has progressed past a certain level, drying can result in seed mortality (Battaglia, 1993; Battaglia and Reid, 1993), so it was important to establish that radicle protrusion resulted in cotyledon emergence. For the medium salinity treatment and the waterlogging with salinity treatment, the number of germinants and the cotyledon emergence that followed were significantly lower, particularly for *E. pauciflora*, and *E. dives* ($P<0.001$). Five out of the eight species showed the effect of the dish to be significant ($P<0.001$).

The treatment of low salinity combined with PuraCa*st* produced variable results, depending on the species. The *Acacia* species from less challenging habits responded well to the addition of PuraCast, showing higher germination rates than under the low salinity treatment without PuraCast. *Acacia* species form symbiotic root associations (Craig and Atkins, 1991) so this increase in germination is likely due to the presence of microorganisms in the biologically active fertiliser enhancing the processes at the root hair. It is also possible that the low salinity treatment was mitigated by the presence of Potassium which is generally found in relatively high concentrations in worm castings (Murphy 1993), which has been shown in previous work to be a key element for exclusion of salt at the root hair in *Eucalyptus* and *Casuarina* sp. and the less tolerant plants had a deficiency in the root zone (van der Moezel *et al,* 1988). Interestingly, two of the Eucalypts and *C. littoralis* in this project showed a lower germination under the PuraCast with low salinity treatment compared to low salinity, but *E. ovata* showed a significantly higher germination in response to the addition of PuraCast to low salinity (PS) when compared to low salinity (LS). More work is needed to fully understand the processes at work for the combination of salinity with a biologically active fertiliser.

5.6 Mechanisms of Tolerance and Habitat Adaptation

Tolerance of non-halophytes to rising groundwater and increasing soil salinity is based on several physiological processes to exclude and reduce the impacts of salt (both sodium and chloride ions), to allow cells to draw in water and nutrients in unfavourable conditions for ionic transfer and osmosis. For survival in saline environments, growth to maturity, flower production and seed set, a non-halophytic plant needs to have suite of integrated mechanisms working simultaneously (Bell, 1999). These bioprocesses exclude salt at the roots, translocate it to older leaves, maintain moisture levels in the leaves by modifying stomatal aperture; and maintain transpiration and photosynthesis at the optimum rates (Kozlowski 1984, Bell, 1999). Other key processes of tolerance, which are also part of waterlogging tolerance, are the ability to draw water in under anaerobic conditions; to combat metal toxicity and decreasing availability of essential nutrients; and to reduce the transport of salt to shoots and leaves (van der Moezel *et al* 1989, Marcar and Termaat, 1990, Marcar, 1993). The ability to translocate salts to the leaf tip of older leaves protects the newly forming leaves, another key aspect of salt tolerance but it is the adverse water relations at the root tip which is the most detrimental effect of salinity (Greenway and Munns 1980).

5.6.1 Seed source habitat Effects

Salinity tolerance in Australian *Eucalyptus* species has been strongly correlated with the exclusion of ions at the root hair as for other non-halophyte plants, discussed above (Munns and Termaat, 1980, van der Moezel *et al* 1989, Marcar and Termaat 1990, Marcar, 1993). This suggests that some species of Eucalypt may have at least one of the genes needed to exclude sodium. If one gene is present in a population, this may lead to variation in salt tolerance within a species when environmental stress drives a different population to adapt and evolve more than one gene of salt tolerance (Kawecki and Ebert, 2004). It is possible that other genes can also become connected to these salt tolerant genes, leading to multiple tolerances *e.*g. waterlogging and salinity tolerances (Kawecki and Ebert, 2004, Atwell, *et al,* 2010). Seed used in this trial was collected from remnant vegetation communities growing on saline habitats in the frost-prone Southern Tablelands. It is expected that at least one parent was adapted to these localised conditions of soil salinity and frost, for seedset to occur. Moderate to high levels of soil salinity are accompanied by intermittent or permanent waterlogging in the subsoil in the Upper Shoalhaven Catchment. Seed source habitat sites of medium and high salinity may also host waterlogging tolerant trees and specimens with multiple gene combinations of salt and waterlogging tolerances. Some of the seed from the high and medium salinity habitats showed high germination in the combined treatment of salinity with waterlogging as well as the waterlogging only treatment, suggesting multiple genes and tolerances are present (Kawecki and Ebert, 2004, Atwell, *et al,* 2010).

Other genera used in this project were found on coastal saline sites as well as the tablelands, where soil salt was present, but not necessarily waterlogging. These were often the low salinity sites. The results show that low salinity habitats did not generally confer waterlogging tolerance on their seedlings. Seed from low salinity habitats exhibited high germination in treatments without waterlogging, with significantly higher germination in 4 of the 6 treatments, demonstrating salt and heat stress (or drought) tolerance. The coastal provenance of *C. littoralis* was the exception, with seed showing high tolerance to salinity and waterlogging.

Tolerance to anaerobic conditions at the root hair is necessary for germination to progress from radicle protrusion to cotyledon emergence, expansion and survival in waterlogged conditions (van der Moezel *et al* 1989, Marcar and Termaat, 1990, Marcar, 1993). This is not so essential in the low salinity habitats, where germination and seedling growth could occur in the top 20 cm of a soil that was slightly saline but not waterlogged all of the time. High and medium salinity habitats in the project's region were generally where the watertable was within a metre of the surface, much closer than in the low salinity sites. This proximity of groundwater to the soil surface may have conferred waterlogging tolerance as well as salt tolerance on seed from medium to high salinity habitats and predisposed them to tolerate the combination of salinity and waterlogging.

When waterlogging is combined with salinity, the ability to exclude chloride and sodium ions during uptake of water in anaerobic conditions is critical for success in these environments (Morris and Thompson, 1983, Marcar, 1993, Marcar *et al,* 1995). Uptake of ions with water leads to the build-up of salt in plant tissues and can lead to mortality, unless the plant is able to translocate the salts to tips of leaves, preferably older, senescing leaves (Bell, 1999). Combinations of environmental stress require multiple tolerances for viable seed set. The trees from high salinity and medium salinity habitats showed the ability to confer multiple tolerances on their progeny; and demonstrated that adaptation to localised conditions was occurring in species collected for this project.

5.6.2 *Eucalyptus* species

Eucalyptus species showed similar germination under the waterlogging treatment as they did under the control. *E. ovata, E. pauciflora* and *E. dives* exhibited regular germination patterns with an order of magnitude difference for each type of habitat. The highest germination proportions were found in treatments with waterlogging (W and WS). This result suggests that heat and moisture stress were experienced by *Eucalyptus* species in the dry treatments, but they were not so affected by the "broiling" factor in the waterlogging treatments. The seed sourced from dry and acid habitats (DA) had the highest germination in *E. ovata* and *E. pauciflora*, with seed from medium salinity habitats the next highest. For *E. dives* the medium salinity habitat seed germination was higher than the DA habitat seed germination.

Previous studies have shown that some species of *Eucalyptus* are more waterlogging tolerant than others particularly *E. ovata*, commonly known as the swamp gum (Ladiges and Kelso, 1977; Marcar, 1993). *E. pauciflora* has been studied for frost tolerance (Ball *et al*, 1997), but has not been mentioned in recent work on either waterlogging or salinity tolerances in Australian research projects. Previous research has also shown that tolerance to the combination of saline and waterlogged conditions was strongly linked to high tolerance to waterlogged conditions (van der Moezel *et al*, 1988, Marcar, 1993). This was not the case for *E. pauciflora,* but it was for *E. ovata* seed sources, which had the highest germination of all species under the waterlogging with medium salinity treatment.

The seed of *E. pauciflora* and *E. ovata* from dry, non-saline, non-acid habitats had a significantly lower germination result *i.e*. were the least salt tolerant. For all treatments with salinity (including combinations), dry habitat seedlings from *E. pauciflora* had significantly, very poor germination. *E. pauciflora* was the least salt tolerant of all species, according to overall germination rates. However, it was the only Eucalypt sourced from a high salinity habitat, so further work is needed to fully assess this species. Previous research into salt tolerance of *E. camaldulensis* found that seedlings sourced from a benign provenance were significantly less salt tolerant than those from saline or waterlogged provenances (Sands, 1981). *E. camaldulensis* is generally regarded as one of the more salt tolerant Eucalypts (Blake, 1981; van der Moezel *et al*, 1988; Marcar, 1993), so this also supports the view expressed above: that provenance variation is more of a factor in the salt tolerance of *Eucalyptus* than other salt tolerant Australian genera.

To summarise; the dry and acid (DA) and the medium salinity (MS) habitats of *Eucalyptus* species had the highest overall germination rates; with *E. ovata* seed from low salinity habitats (LS) showing the highest tolerances to salinity and the combination of salinity with waterlogging. This suggests that for rehabilitation purposes, seed collections from adverse habitats rather than extreme habitats, is an important aspect when using the *Eucalyptus* species for rehabilitation.

5.6.3 Summary of Differences - Eucalyptus, Acacia and Casuarina

Acacia species tested were limited in their available, viable seed sources on different habitats. In the Upper Shoalhaven Catchment, most species set seed once per year and the seed is available for collection for only a few weeks. So, although they are widespread in their distribution, very few species were collected from more than one habitat type and only two made it to trial. These two species were found growing on saline sites, which was one of the key selection criteria for inclusion in the trial. The results in this trial for *Acacia* species generally follow the principle that the more stressed environments produced seed sources more tolerant of salinity and waterlogging.

All of the *Eucalyptus* species had the highest or second highest germination response in seed sourced from acid habitats. The genus, as a whole exhibited the pattern of medium and low salinity seed sources producing more tolerant seed than the high salinity habitat. Each species had slight differences to the others in their germination rates from different habitat types, but all species showed a pattern in germination response across the different treatments, with all habitat types having an order of magnitude difference. This means the highest germinating seed from one habitat type was consistently highest across all treatments, not just in one treatment.

There were highly significant differences in the germination of seed sources from different species under treatments of salinity ($P<0.001$). *E. blakelyi, E. ovata* and *C. stricta* had the highest average germination under the most stressful treatment of medium salinity and also, in the combined treatment of salinity with waterlogging. Previous studies have shown that inland dry provenances of *C. stricta* were not highly salt tolerant, but suggest that coastal provenances worthy of investigation (Clemens *et al*, 1983). The coastal provenance of *C. stricta* tested in this project's germination had at least 20% higher germination for 5 out of 6 treatments than the inland dry provenance. *C. littoralis* from the coastal zone also germinated well in the combined treatment of salinity with waterlogging.

The trials also showed that *E. blakelyi, E. ovata* and *C. stricta* had the highest average germination under conditions of waterlogging, significantly higher (P<0.001) than the other *Eucalyptus* species, Acacia and *C. littoralis*. Previous work has shown that *E. ovata* is waterlogging tolerant (Ladiges and Kelso, 1977). Other work has shown a strong correlation between waterlogging tolerance and tolerance to the combined conditions (van der Moezel *et al*, 1991, Marcar, 1993), which is backed up by the results of this trial in terms of *E.ovata* as mentioned above, but little information was found on *E. blakelyi*, for either salinity or waterlogging tolerance. *C. stricta* has been shown to be one of the lesser salt tolerant species of the Casuarina species (Clemens *et al*, 1983; Luard and El-lakany, 1984), but its ability to germinate under waterlogged and combined conditions may be worthy of further investigation.

For a comparison of *Eucalyptus* species with *Casuarina* species, little previous work has been done on *C. littoralis* but research has shown that *C. obesa* was more tolerant of salinity and waterlogging combination treatments than 6 *Eucalyptus* tested; and more waterlogging tolerant than 5 of the Eucalypt species. The *Casuarina* species. was less tolerant of waterlogging than *E. ovata*. As mentioned above, *E. ovata* was very tolerant of medium salinity and the combined salinity and waterlogging treatment, exhibiting a higher germination average than *C. littoralis*.

Acacia species have been shown in previous studies to be tolerant of salinity and waterlogging (Craig and Atkins, 1990), but direct comparisons with *Eucalyptus* or *Casuarina* species were not found. *E. pauciflora* was the least salt tolerant of all species and showed the greatest variation, based on seed source habitat. Previous work has also shown that provenance variation is greater for less salt tolerant species (van der Moezel *et al*, 1988). This research backs up previous work.

Previous work research has also shown that a species' natural distribution also impacts on its ability to tolerate salinity, waterlogging and combinations. Most of the species used in this trial, as mentioned already, have a distribution across south-eastern Australia (Boland *et al,* 1986), but each preferentially inhabits certain niches in the Upper Shoalhaven, where climate and soils are very limiting. *C. littoralis* was most common on acidic soils, both dry and moist, but was also found on moderately saline soils, coastal sites and also moist gullies. *C. littoralis* was more of an open woodland species, rather than a remnant or coloniser of highly exposed frost-prone areas.

A. melanoxylon was found to preferentially inhabit the higher rainfall sites in the Upper Shoalhaven, particularly the rainforests where it grows into a large timber tree and riparian zones. It also occurs sporadically on low salinity sites and in drier, more exposed, highly frost-prone sites, with the morphology of a tall shrub rather than a large timber tree. *A. melanoxylon* was absent on most of the acidic sites surveyed, apart from one site which was also quite moist, but sufficient seed was not available for this trial.

E. ovata, preferentially inhabits riverbanks and wet sites, but can also be found on drier more exposed hillsides, or moderately saline and acid soils. It was quite stunted on the saline soils. *E. pauciflora* preferentially inhabits highly exposed, frost-prone areas in the Upper Shoalhaven. It can also be found on riverbanks and acid soils, but is far less common in waterlogged and/or acidic sites than the other three species. It was the only *Eucalyptus* species found in remnant vegetation on the high salinity sites, but its poor germination in the trial suggests the possibility of high salt tolerance is unlikely. However, it cannot be ruled out, as the importance of seed source habitat in conferring tolerances was found to be highly significant across all species and treatments ($P<0.001$).

5.7 The Importance of Habitat

The interaction between habitat and treatment was highly significant ($P<0.001$) for all species with multiple seed sources at Day 10, Day 25 and Day 39. For some species, each provenance in each treatment produced exactly the same shaped line graph (see Figure 16 for *E. ovate* at Day 25), differing only by a changing order of magnitude from the highest to the lowest germination rates across all treatments. For *C littoralis,* each provenance had a variable performance, but the high salinity and waterlogged acid habitat seed sources stood out as being the most tolerant. The importance of seed source habitat and the interaction with treatment is discussed on a species-by-species basis as well as in summary form.

When salinity was combined with waterlogging there was a significant growth reduction compared to waterlogging, for most of the species; and this is backed up by previous research findings of multiple stresses producing more tolerant seed (van der Moezel *et al*, 1988; van der Moezel *et al*, 1991, Marcar, 1993, Barrett-Lennard, 1986, Mahmood *et al*, 2009). In previous work, the problems facing plants in acid soils have been documented, such as lack of oxygen in root zone, aluminium, and manganese toxicity and the locking up of essential nutrients such as calcium which inhibit plant root elongation and overall plant growth (*e.g.* Rahman, 1991; Kidd and Proctor, 2000). But the ability of acid habitats to produce salt tolerant progeny was not found in the literature at the time of writing.

It is likely that acid tolerant plants have developed similar exclusion mechanisms to salt tolerant plants (Kochian *et al*, 2004), to be able to survive and grow in conditions of toxicity; and perhaps this is why the progeny from acid habitats had a high germination comparatively under the medium salinity treatment in these trials. Acid habitat seed also germinated comparatively higher than other habitat types, in the combined conditions of salinity and waterlogging, but not as well as seed sourced from high salinity habitats.

Previous work with *E. ovata* and *E. viminalis* has documented that waterlogging can also cause mineral toxicity in the soils around root zones (*e.g.* Ladiges and Kelso, 1977; Marcar, 1993), which may assist in salt tolerance. This may explain the higher germination result for *Eucalyptus* and *Acacia* seed sourced from waterlogged habitats.

So, based on the findings of this experiment and that of previous research into exclusion mechanisms of acid tolerant plants, the validity of the new findings for frost-prone, acid habitats being able to produce salt tolerant seed, is a strong possibility (Kochian et al, 2004). It seems that mineral toxicity and unavailability of essential nutrients – the conditions found in acid and / or waterlogged soils (Ladiges and Kelso, 1977, Munns and Termaat, 1986, van der Moezel *et al*, 1991, Marcar, 1993, Kochian *et al*, 2004, Mahmood *et al*, 2009) predispose the progeny of these habitats to better cope with the combined conditions of salinity and waterlogging.

The accepted approach to selecting saline habitats for sourcing seed for rehabilitation (*e.g.* van der Moezel *et al*, 1988-1990) may need to be reviewed. The germination trial for this project showed that the high and medium saline habitat seed sources were able to cope with the combination treatment of salinity and waterlogging better than other habitats, but acid seed tree habitats produced more salt tolerant seed in several species, as did some of the low salinity populations. For some of the field trials in the 1990's, salt tolerance was found in seedlings sourced from riparian habitats (*e.g.* Sun and Dickinson, 1995) which may have been a waterlogged habitat (as mentioned in the Introduction). For work on *E. camaldulensis*, seedlings sourced from 10 lakes in Victoria showed a higher salt tolerance than others (Blake, 1981). The germination of waterlogged habitat seedlings in this project was generally equal second highest of salinity or acid habitat types, it may be that for *Eucalyptus* and *Acaci*a species, tolerance to waterlogging confers some degree of salinity tolerance also. This trial used seed, whereas the previous research above was work on seedlings.

For a full assessment of salinity and waterlogging tolerance in relation to seed tree habitat, multiple factors need to be included and analysed. The research work reviewed on assessing salt tolerances of seed selected from saline (Craig and Atkins, 1990) or waterlogged habitats (Blake, 1981) still found variation within species. So, the degree of soil salinity in the seed source habitat (low, medium or high) and whether it is waterlogged intermittently may be a factor in conferring salt tolerance. The germination trials provide a useful assessment of seed source habitats with different levels of soil salinity.

5.8 Summary of Findings

Reviews of previous research (Niknam and McComb, 2000; Stirzaker *et al*, 2002) have pointed out the importance of thoroughly investigating provenance variation; and previous research has also found that the degree of soil salinity at the seed collection site can affect salt tolerance (Craig and Atkins, 1990). The seed sourced from dry, non-saline, non-acid habitats had the lowest or equal lowest germination rate for all treatments, compared to all other habitat types. This result follows the pattern of previous research (Sands, 1981), where the benign provenance seedlings were generally less tolerant of both free-draining saline and combined waterlogging conditions, than seedlings sourced from saline provenances.

The division of the four broad habitat categories into three levels of salinity, plus differentiating between waterlogged and dry acidic soils for the germination trial provided a more detailed view of the effect of habitat. For species with seed sources from three or more habitat types, the differences in tolerance to salinity, waterlogging, and the combination of salinity with waterlogging treatments, were strongly linked to habitat. ($P<0.001$ in most cases). This suggests that seed source habitat type determines a seed's ability to withstand both salinity and waterlogging treatments, plus heat and water stress. It seems that some habitat types produce offspring better suited to germination under extremes of temperatures, combined with dehydration, salinity and waterlogging.

For three widespread species *Eucalyptus ovata*, *Leptospermum flavescens* and *Casuarina stricta*, the only differences in germination were due to seed source habitat type.

The interaction between seed tree habitat and treatment, for the average germination across species distinguishes the high salinity habitat from low and medium salinity habitats as conferring a higher degree of tolerance overall, but particularly, for the low salinity treatment; and the combined treatment of salinity and waterlogging. The high salinity seed source habitat also had one of the highest averages for germination in the control, but the low salinity habitat also conferred a highly significant degree of salt tolerance. The high salinity and dry acid habitat seed sources were equal second under the medium salinity treatment, but had 5% lower germination than the waterlogged and acid seed sources. Dry acid habitat seed sources had the second highest or equal second germination rate for 5 out of 6 treatments, which suggests that this is also a habitat which confers high tolerance to a range of environmental extremes.

Clemens *et al* (1983) found in germination trials for salinity tolerance, that there was a positive correlation between salt resistance in seedlings and high germination rates under control conditions. It was suggested that this is an adaptive mechanism to salt, to allow rapid germination after heavy rains of seedlings which can handle the increase in saline conditions thereafter.

5.9 Future Work

Previous research has shown that germination may not necessarily correlate with the ability of seedlings to survive in the same environment (Clemens *et al*, 1983; Battaglia and Reid, 1993). The germination results need to be viewed in conjunction with seedling trials. When glasshouse trials are backed up by field trials, they become a useful tool for assessing species and provenances for potential use in rehabilitation. A review of the research into salt tolerances of Australian native species, found discrepancies between research for 13 species of Eucalypts and a similar number of Acacias and Melaleucas (Niknam and McComb, 2000). This could be due to provenance variation and/or differences in seed source habitat type, or differences in success in the glasshouse trial followed by failure in field plantings. The glasshouse germination trials in this thesis could be suitably supported by further glasshouse seedling growth trials and field trials.

Investigating the performance of progeny from acid habitat trees as potential rehabilitators of highly acidic soils is another possible offshoot of this research. Further detailed analysis of the 39 days of germination data with other statistical analyses is also worthwhile, if done species by species or as a whole with outliers removed.

5.10 Addressing the Environmental Problem

Seed sourced from different provenances has been shown to exhibit differences not only in germination and stress resistance, but also seedling growth (Khurana and Singh, 2001). Parent tree habitat is a key aspect to consider for seed collection, particularly when selecting widespread and highly adaptable species for use in rehabilitation programs. This project was developed as a means of establishing methods for selecting provenances of species for use in reclamation of saline and waterlogged soils, which is discussed in the paragraphs below. However, an unexpected offshoot of the project was the high germination of seed collected from acidic soils. Soil acidity is becoming an increasing problem in Australia with overuse of superphosphate, particularly on naturally acidic soils and the area of land affected is estimated to be substantially greater than that of salinised land (see Figures 5 and 6, NLWRA, 2001; SoE 2011). The costs of soil acidity to agriculture and the economy could be reduced if acid tolerant tree and shrub provenances with some commercial value (including fodder) were planted. Potential fodder species *Casuarina littoralis* and *Acacia melanoxylon*, using acid-habitat provenances such as found in this project, could be an alternative for planting and reclamation, rather than ongoing cropping or introduced pasture improvement that requires use of superphosphate on soils already at risk. Use of a biologically active fertiliser with NPK could also enhance plantings of Australian native species, particularly *Acacia* and *Casuarina* sp. Fertiliser applications have been found useful in other revegetation work with Eucalypts on degraded soils (Bennett *et al*, 1996).

For rehabilitation of degraded soils, the standard method of revegetation with seed sourced from appropriately researched tree and shrub species upslope of the scalded or degraded area is a more realistic and economical approach than planting into highly degraded soil. Planting directly into the scald, as mimicked by the methods tested in this project, is less effective in terms of germination and early seedling growth. The results showed a significantly lower germination in the medium salinity treatment ($P<0.001$). The establishment success rate (% survival) of a range of tree species tested in previous research (*e.g.* Dunn *et al* 1994) utilising the "upslope" method was significantly higher than projects where plantings were located close to the scalded area (Dunn *et al*, 1994; Greenwood *et al*, 1995; McJannet and Vertessy, 2000). The study undertaken by Dunn *et al* (1994) showed that survival rates dropped as salinity level rose, even for species regarded as salt tolerant.

However, there is also evidence from previous research that successful upslope and midslope (or break-of-slope) plantings do not necessarily coincide with successful lowering of the watertable in the area of the saline seep (McJannet and Vertessy, 2000; Greenwood *et al*, 1995). Bennett and George (1995) found in their studies of *E. globulus* that planting too close to saline seeps resulted in a stressed plantation which would have little effect on reclamation of saline sites, so for reclamation the use of highly salt tolerant and possibly multiple tolerant tree and shrub species are required. To lower the watertable, excess rainfall needs to be captured. Salt and waterlogging trees, some of which are naturally found near wetlands, seeps and scalds (as found in this project and other research) often have the ability to draw upon the groundwater reserves and are useful for reclamation of degraded sites (Bell, 1999). It is useful to establish tree species near to the scald to lower the watertable as it may assist with minimising soil erosion due to salinity (Greenwood *et al,* 1985; Bari and Schofield, 1992; Stirzaker *et al*, 2002). If seedling planting is not cost effective, then despite lower germination rates, direct seeding is an appropriate alternative, particularly for the Upper Shoalhaven and other frost-prone catchments.

Work by Landcare groups in the Windellema area near Braidwood has shown that planting or spreading of suitable seed nearby the scald and upslope of deep rooted tree species such as *E. ovata*, *A. melanoxylon* and *E. pauciflora* is likely to retain soil and offer protection to the site. It has been noted at these sites that herbs and grasses were able to colonise highly scalded sites (provided grazing is excluded), protecting soil from erosion. This has also occurred on abandoned agricultural land in the Western Australian wheat belt, where secondary soil salinity covers several thousand hectares of land (Atwell *et al*, 2010). For full reclamation of degraded land, deep-rooted tree and shrub native species are deemed more suitable than herbs and grasses, but for successful establishment, seed sowing after site preparation to establish "contour planted belts of trees.." as "an effective means of reducing groundwater recharge with minimal tree crop competition for water" (White *et al*,2002).

5.11 Experimental Challenges and possible improvements

The germination trials encountered a number of problems with high temperatures and also, growth of algae and fungus in the dishes. It is possible that inside the glass petri dishes, temperatures were above 50 °C for several hours around midday, for three days after Day 25. This is well above the optimum temperature for germination of most plant species, including *Eucalyptus* (Battaglia and Reid, 1993; Bell, 1994; Bell *et al*, 1999). When temperatures rise, germination is limited. Yates *et al* (1996) found in his germination experiments on *Eucalyptus species*, that temperatures above and below the optimum for germination (25 degrees C), "enforced dormancy occurs and germination is retarded". In these situations, dormancy is overcome when temperatures return to optimum. *E. pauciflora* in this project was obviously affected by the heat, with delayed onset of germination until night time temperatures were below 5°C for consecutive nights. Evidently, seeds can remain moist at sub-optimal temperatures for prolonged periods without losing viability.

For most species, the low salinity treatment produced a higher germination rate than the control and the waterlogged dishes. This is indicative of an increased osmotic potential in the LS treatment, where seeds in slightly saline conditions were better able to retain their moisture and hence, show a faster and higher rate of germination. Battaglia and Reid (1993) suggest that germination for most Eucalypts in South Eastern Australia occurs when water is not a limiting factor. The results from this project support their findings, showing lower germination under water stress caused by drying out between waterings due to extreme temperatures within the dishes.

This project's germination experiment was run at the end of Spring and onset of summer in a solar passive glasshouse, so there was always the possibility that temperatures would rise above the optimum; however based on Yates research mentioned above (1996) this may simply have slowed germination, rather than diminished it. Several species had multiple seed sources with final germination above 75% (averaged across all treatments). These were: *E. ovata, L. flavescens, A. melanoxylon, E. blakelyi, A. decurrens, and C. stricta*. So, although this is lower than earlier viability tests, in the context of the whole project it is relatively minor. The aim was to investigate whether or not there were differences in seed sourced from a range of habitats. Although the treatments imposed were more stressful than intended, the aim of the experiment was still achieved. Irrespective of fungal growth, algae, extreme temperatures, broiling and dehydration, the majority of seed sources tested germinated in all treatments with an average of 65-80%. Despite the limitations of germination in petri dishes during a warm spring, the differences between seed source habitats were still highly significant ($P< 0.001$).

The best way to circumvent this problem in future work and achieve the ultimate germination would be to run germination trials during the milder months of the year, i.e. autumn or early spring. The literature also suggests running trials at a time that mimics each species peak germination time in their native habitat (Battaglia and Reid, 1993; Bell, 1994; Yates, 1996, Bell *et al*, 1999).

CHAPTER 6. CONCLUSIONS

The results from this project show there was a highly significant variation to salinity and waterlogging treatments in relation to seed source habitat for all species trialled ($P<0.001$). There was also a highly significant interaction between habitat and treatment, which suggests that some habitat types are more salinity and waterlogging tolerant than others. The matrix of species and the most tolerant habitat types was complex. Less salinity tolerant species showed evidence of acidic habitats producing the most tolerant seed.

Acid, Waterlogged and High Salinity habitats showed the highest germination rates for the dual treatment of salinity with waterlogging ($P<0.05$), which suggests that these tolerances are located on multiple gene loci and may predispose seedlings to tolerance of multiple environmental stressors.

The null hypothesis is rejected and the new hypothesis presented:

Variation in salinity and waterlogging tolerances within species native to the frost-prone Upper Shoalhaven Catchment can be found by sourcing seed from different seed source habitat types, with more tolerant seed found on saline, acid, and waterlogged habitats i.e. habitats with multiple limiting factors.

The differences within each species, measured by the germination response of seed sources to different conditions of salinity and waterlogging, were clearly related to seed source habitats. These results, along with comparisons to previous research show that seed source habitat is an important factor for determining salt tolerance and that adaptation is occurring within local species, in response to environmental stress, leading to greater salinity and waterlogging tolerances of one provenance over another. Dry, non-acidic, non-waterlogged, non-saline environments produced the least tolerant seed (P<0.001).

6.1 The Building blocks for this project and new questions

The work of Marcar (CSIRO, Canberra, 1989-2003) and Bell (1994- 2000) and Atwell *et al* (2010) were particularly inspirational for this project. The field trials of Sun and Dickinson (1995), Dunn *et al* (1994) and Hoy *et al* (1994) also provided useful building blocks for looking at the success of seed sourced from riparian habitats on saline soils.

Eucalyptus seed sourced from acid habitats showed a higher germination than other habitat types, under conditions that represented soils with moderate salinity. The results also suggest that when a species is salt hardy, as opposed to merely salt tolerant, the effect of seed source habitat is less important. For salt hardy species, seed source habitat may only be a factor to be considered for rehabilitation of highly degraded sites. Examples of such sites could be frost-prone areas with combined conditions of waterlogging with moderate salinity, such as found in the Upper Shoalhaven or combination of other factors highly acidic, saline topsoils calcareous subsoils, or for high salinity sites. However, when species are less salt tolerant, such as in the case of *E. pauciflora*, then seed tree habitat becomes more important.

Most of the previous research examining the effect of provenance variation has focused on saline and waterlogged provenances and generally found that seed sourced from saline habitats performed best in saline conditions (Sands, 1981; Niknam and McComb, 2000;

Stirzaker *et al*, 2002). Marcar (1993) suggests that when waterlogging is a combined factor with salinity, this may not always be the case and certainly, for the medium salinity treatment in this experiment (MS), the seed sourced from waterlogged habitats performed well for some of the species. The inclusion of seed sources from acid habitats, particularly waterlogged and acid, would be useful in future research seeking highly salt tolerant provenances.

6.2 Rehabilitating Areas of Land Degradation

The importance of habitat has been shown to be a highly significant factor when sourcing seed for rehabilitation degraded soils ($P<0.001$). Selecting which habitat type is best depends on whether the rehabilitation site has a single detrimental factor or multiple stressors. The degree of salt tolerance of the chosen species is another variable to consider because less salt tolerant species have shown a higher variability in salinity and waterlogging tolerance.

The performance of acid habitat seed sources in these trials is a strong argument for using local species as a starting point for rehabilitation in the Upper Shoalhaven. The soils in the Upper Shoalhaven Catchment are naturally acidic and with the combination of extreme frost, the local environment has produced highly tolerant seeds and seedlings. Each local environment where soil degradation is occurring will have its own set of problems and challenges. Where local species are targeted for use and research into rehabilitation, then unexpected solutions may be found, such as in this project.

6.3 Further Research

The acid habitat seed sources are definitely worthy of further investigation. Of the species tested, several are worthy of further research for salt tolerance and also provenance variation, particularly: *A melanoxylon, C. littoralis, E. blakelyi* and *E. ovata*. *E. pauciflora* is useful species for frost-prone areas and further research into potential salt tolerant provenances is also warranted.

The investigation of acid habitats in frost-prone areas for sourcing of possible highly salt tolerant seedlings would make a useful research project, as would further investigation into provenance variation in salinity and waterlogging tolerance for species sourced from frost-prone environments.

The effect of using a biologically active fertiliser as an aid to germination in saline soils is worthy of further investigation, particularly for *Acacia* and *Casuarina* species.

6.4 Summary

For successful rehabilitation in areas of environmental extremes, there are many factors to be considered. The trials undertaken in this project demonstrate the strong possibility that seed tree habitat influences a seedling's ability to cope with environmentally stressful situations. For highly salt tolerant species, there may be a need to look at success on waterlogged sites, particularly when this is an interactive effect in the field. For moderately to mildly salt tolerant species, seed source habitat could be a much more important factor for provenance selection. Rehabilitation sites need to be assessed and provenances selected which have been shown to cope with all of the site's parameters and detrimental factors. Results from this project could assist to some degree, with seed sources for *E. pauciflora* and *E. ovata*.

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APPENDICES

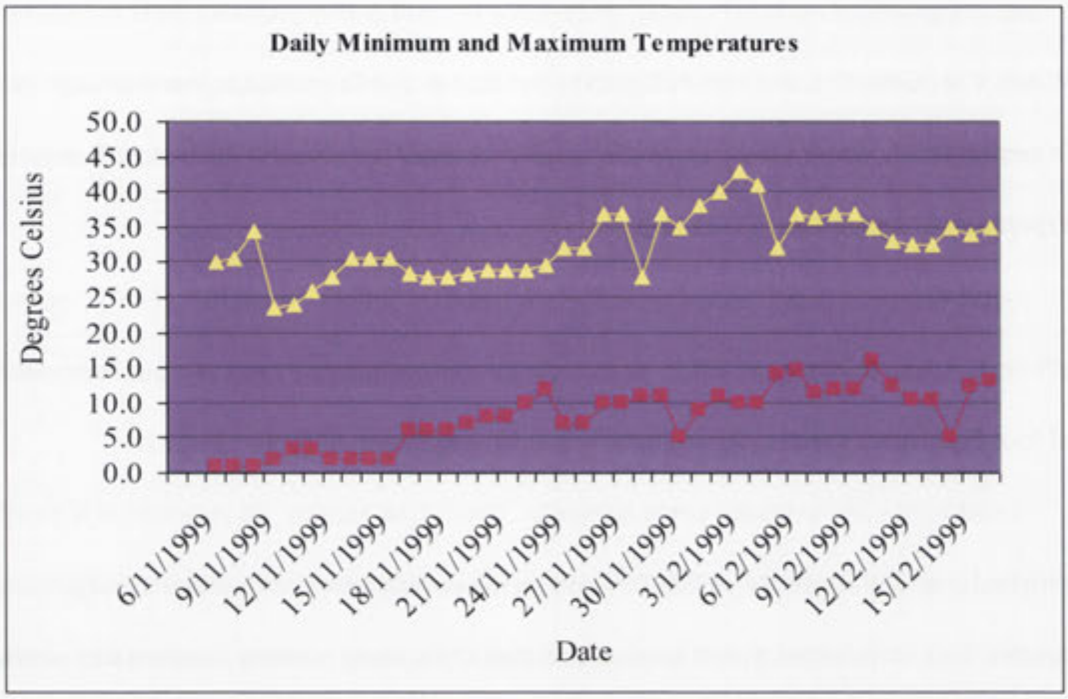

Figure 22. Graph of Daily Temperatures

Table 21. Final Radicle Protrusion Results - individual species

Species	**Habitat Types**							**Treatment Average**
Eucalyptus pauciflora								
Habitat	D	DA	HS	LS	MS	W	WA	
Treatment								
C	0.21	0.44	0.29		0.36	0.26	0.22	**0.30**
LS	0.20	0.43	0.28		0.36	0.26	0.21	**0.29**
MS	0.06	0.15	0.08		0.11	0.07	0.06	**0.09**
PS	0.08	0.21	0.12		0.17	0.11	0.09	**0.13**
W	0.35	0.61	0.45		0.54	0.42	0.36	**0.45**
WS	0.29	0.55	0.38		0.47	0.35	0.30	**0.39**
Habitat Average	**0.20**	**0.40**	**0.27**		**0.33**	**0.25**	**0.21**	**0.28**
E.ovata								
Habitat	D	DA		LS	MS	W		
C	0.90	1.00		0.80	0.80	1.00		**0.90**
LS	0.95	1.00		0.90	0.90	0.95		**0.94**
MS	0.85	0.90		1.00	0.85	1.00		**0.92**
PS	0.90	1.00		1.00	0.90	0.90		**0.94**
W	0.95	1.00		0.85	0.80	1.00		**0.92**
WS	0.90	1.00		0.90	0.90	0.92		**0.92**
Habitat Average	**0.91**	**0.98**		**0.91**	**0.86**	**0.96**		**0.93**
Acacia melanoxylon								
Habitat	D					W		
Treatment								
C	0.54					0.86		**0.70**
LS	0.64					0.91		**0.78**
MS	0.52					0.86		**0.69**
PS	0.72					0.93		**0.83**
W	0.24					0.63		**0.44**
WS	0.28					0.69		**0.48**
Habitat Average	**0.49**					**0.81**		**0.65**

Acacia decurrens								
Habitat			HS	LS				
C			0.90	1.00				0.95
LS			0.95	0.80				0.88
MS			0.75	0.50				0.63
PS			0.80	0.90				0.85
W			0.95	0.70				0.83
WS			1.00	0.60				0.80
Habitat Average			0.89	0.75				0.82
Casuarina littoralis								
Habitat	D	DA	HS	LS	MS	W	WA	
C	0.66	0.50	0.80	0.90	0.80	0.71	0.80	0.74
LS	0.79	0.61	0.75	0.75	0.60	0.81	0.75	0.72
MS	0.15	0.50	0.40	0.15	0.40	0.07	0.40	0.30
PS	0.66	0.75	0.65	0.95	0.20	0.60	0.60	0.63
W	0.40	0.47	0.75	0.55	0.40	0.66	0.65	0.55
WS	0.70	0.71	0.90	0.85	0.80	0.71	0.85	0.79
Habitat Average	0.56	0.59	0.71	0.69	0.53	0.59	0.68	0.82
E.dives								
Habitat		DA		LS	MS	W		
C		0.63		0.41	0.74	0.37		0.54
LS		0.67		0.45	0.78	0.41		0.58
MS		0.10		0.04	0.16	0.04		0.09
PS		0.51		0.29	0.63	0.26		0.42
W		0.76		0.56	0.84	0.52		0.67
WS		0.78		0.58	0.85	0.54		0.69
Habitat Average		0.58		0.39	0.67	0.36		0.62
E.aggregata								
Habitat					MS			
C					0.65			0.65
LS					0.68			0.68
MS					0.40			0.40
PS					0.80			0.80
W					0.75			0.75
WS					0.60			0.60
Habitat Average					0.65			0.65

E.blakelyi								
Habitat				LS				
C				0.81				0.81
LS				1.00				1.00
MS				0.81				0.81
PS				1.00				1.00
W				1.00				1.00
WS				0.86				0.86
Habitat Average				0.91				0.91
Casuarina stricta								
Habitat	D				MS			
C	0.80				1.00			0.90
LS	0.70				1.00			0.85
MS	0.50				1.00			0.75
PS	0.75				1.00			0.88
W	0.60				1.00			0.80
WS	0.70				0.80			0.75
Habitat Average	0.68				0.97			0.83
Leptospermum flavescens								
Habitat		DA			MS		WA	
C		1.00			0.10		0.95	0.68
LS		0.90			0.20		0.85	0.65
MS		0.79			0.20		0.85	0.61
PS		0.95			0.10		0.95	0.67
W		1.00			0.20		0.90	0.70
WS		0.89			0.20		0.75	0.61
Habitat Average		0.92			0.17		0.88	0.66

Average SED=0.04

Table 22. Whole Project Species and Provenance list

Eucalyptus pauciflora
Sandholes Rd Jillamatong
Jillamatong creekside
Jillamatong creekside – old
"Nithdale" Shoalhaven River
"Woodlands" # 2
Northangera Rd
Halls Lane
Long Flat
Tantulean Creek
Kings Hwy, Goulburn ' T ' section

Acacia melanoxylon
Currawan Creek
Braidwood - Goulburn Rd
Kings Hwy
Brick Kiln Crossing
Tantulean Creek
"Mornington" creek
Ballalaba

Casuarina littoralis
Wallace's Gap
Woodlands # 2
"Roscommon"
MR's property – Windellema
Pretty Beach – Kioloa
Michael's property - Mongarlowe
Mornington Creek - Ballalaba
"Woodlands" # 1
20.7km from Mongarlowe - Charley's Forest
Rainforest margin, Cabbage Tree Creek

Eucalyptus rubida
Mt. Gillamatong
"Warragunnia" Mayfield Rd
Wallaces Gap

Acacia decurrens
Warri bridge - Shoalhaven River
"Warragunia" Mayfield Rd.
"Woodlands" #2

Eucalyptus ovata
Kings Hwy - Glbn ' T ' section
Mulloon Creek
Long Flat
Tantulean Creek
Glbn Rd Creek
Glbn Rd
Halls Lane
Mongarlowe swamp
Sandholes Rd Jillamatong

Leptospermum flavescens
Pretty Beach – Kioloa
20.3km from Mongarlowe
Stuarts Crossing Rd
20.7km from Mongarlowe

Eucalyptus dives

Woodlands # 1
Tantulean Creek
"Bobar" - Kings Hwy
Little Bombay parking spot
Nerriga Rd 7.2km along dirt

Casuarina cunninghamiana
Casuarina Sands - Cotter

Mark's property - Araluen

Casuarina stricta

Mt Majura

Pretty Beach – Kioloa

Eucalyptus mannifera
Wallace's – Nunlef
Bobar
Nerriga Rd 7.2km along dirt
Mt Majura

Eucalyptus radiata
O'Connell's - Jerrabutgulla
Northangera Rd

Eucalyptus aggregata
Little Bombay Saltpan
Jembaicumbene

Eucalyptus blakelyi
Woodlands #1

Eucalyptus maidenii
Sawyers Ridge Rd

Callistemon seiberi
"Mornington" creek
Ballalaba

Field Trial Results

Variation to Salinity and Waterlogging

The seedlings grown from seed collected from a range of different parent tree habitats were trialled in field plantings across the Shoalhaven River Upper Catchment. The results showed very clearly that parent tree habitat was a major factor in seedling survival on degraded sites. The results presented in this thesis are from "Nunlef", a property situated near Braidwood with saline affected land that covered several hectares. The only seedlings that survived were from parent trees with habitats of environmental stress *i.e.* waterlogging (W); waterlogging plus acidity (WA); and waterlogging plus salinity (WS). The seedlings from these parent habitats survived in the saline soil, but did not grow past the two or four leaf stage. Dry (D) and Dry and acid (DA) habitat seed had zero survival rates in the highly saline soil.

Table 23 below shows survival results of *Eucalyptus ovata* and *E.pauciflora* seedlings planted into two blocks. The lower block had lower soil salinity levels than in the upper block, but waterlogging was an additional factor. Seedlings showed a higher rate of survival in the lower block compared to the highly saline upper block, but were unable to grow past the two or four leaf stage when checked five years after planting. *E.ovata*, the waterlogged parent habitat (W) seedlings and waterlogged with salinity parent habitat (WS) seedlings had the highest survival on this site. For *E.pauciflora*, the W seedlings had the highest survival rate. The survival rates of *Eucalyptus* seedlings from W, WA and WS parent habitats suggest these provenances have potential for revegetation of waterlogged and low salinity sites.

Table 23. Survival Rates In Field Trials

Species and Provenance	Seed Tree Habitat	Upper Block %survival	Lower Block %survival	Overall % Survival
Eucalyptus ovata				
Mongarlowe swamp	Waterlogged	28.57	58.33	43.45
Mulloon Creek	Waterlogged	28.57	25.00	26.79
Kings Hwy, Glbn ' T '	Medium salinity	0.00	33.33	16.67
Tantulean Creek	Waterlogged	0.00	33.33	16.67
Goulburn Road Creek	Waterlogged and low salinity	35.71	50.00	42.86
Halls Lane	Dry	21.43	Not tested	21.43
Eucalyptus pauciflora				
Northangera Road	Dry and Acid	0.00	20.00	10.00
Woodlands	High salinity	10.00	20.00	15.00
Halls Lane	Dry	0.00	30.00	15.00
Ballalaba Nithdale	Waterlogged and acid	10.00	30.00	20.00
Jillamatong Creek	Waterlogged	10.00	40.00	25.00
Sandholes Rd	Dry and Acid	0.00	30.00	15.00