Department of Environmental Biology

Revegetation of coal mine dumps to ameliorate effects of acidic seepage

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This thesis is presented as of the requirements for
the award of Degree of Doctor of Philosophy
of the Curtin University of Technology

September 2001

To everyone who believed in me

Declaration

I declare that all work presented in this thesis is that of myself alone unless otherwise acknowledged. The contents of this thesis has not been submitted previously, in whole or in part, in respect of any other academic award.

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28th September 2001

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Acknowledgements

This dissertation was not the sole work of a single student, but one with much assistance, guidance and support from a multitude of people. I hope I can remember all those who have assisted me in the past few years and can properly praise them for their efforts.

The opportunity to make use of the Collie sites was through the co-operation of the managements of Griffin Coal and Wesfarmers Coal. In particular I acknowledge the assistance of Ian Pigott, Peter Ashton and Jim Tyler. I must also thank CALM, in particular Tom Kenneally, for offering advice on selection of species and growth requirements on the coal overburden materials. I also wish to thank ACARP and members of the Collie ACARP project, in particular Prof. Bruce Philips and Prof. Louis Evans, for providing funds for my research. I also thank the Mulga Research Centre and Curitn Alumni for providing me with scholarships throughout my studies.

I must also thank my supervisors. To my main supervisor Professor John Fox, who has always encouraged me to work hard and has guided much of my research with critical yet experienced judgement. To my associate supervisor Dr John Koch, who gave me an independent and friendly advice which lead my research into finding valuable conclusions. And to my "unofficial" supervisor Augustine Doronila, a patient and diligent man who provided assistance and wise counsel (as well as much labour) to me while on the field.

I would also like to thank Dr John Clemens, Sarah Flynn, Dr Lorelle Lightfoot, Dr Robyn Simcock and Dr Mark Whitten for their personal communications and Dr Louise Egerton-Warburton, Dr Robyn Simcock, Dr Christina Cox and Suzanne Reichman for helping to correct my drafts.

There were many technical staff members who also assisted me in my thesis preparation. Peter Mioduszewski allowed me to develop a "green thumb" and was always on hand to look after my plants and assist my glasshouse trials (and his wife Satwant for saving my plants from drying out one hot day). Others were Ian Abercrombie, Ted Cockett, Lydia Kupsky, Jean Burgess, Enid Holt and Kathy Whiteman.

I must thank a plethora of other "slaves" that I have received assistance from, both on field trips and on campus. On field trips I had the pleasure of Zoè Benham, Kazuo Iiyama, Mangadas Lumban Gaol, Melinda Moir, Isaiah Mukema, Cyrus Naseri, Nicolyn Short, and Rodger Walker (plus several miscellaneous undergraduates) for doing mundane work in various grades of miserable weather. On campus I was able to slave drive Chantal Burrows, Mel Hopkins, Lara Jefferson, Thomas Lion, Tue Man Luong and Rachel Meissner. I also was able to rope in my friends Steven Fleming and Anthony Wright for recording data for me when I was too lonely to do it by myself.

Not having enough people to annoy in my own work, I also had to go bother fellow students to relieve me of thesis stress (that's my excuse anyway). Thanks to Lubomir Bisevac, Linda Broadhurst, Fiona Butson, Karl Brennan, Veronica Campagna, Dylan Korczynskyj, Aaron Gove, Sandra Griffin, Dr Philip Groom, Danäe Harman, Erin Lowe, Maze` Majer, Dave Mickle, Dr Reinier Mann, Isaiah Mukema, Jane Rosser, Chris Serginson, Tim Simmons, Jill Symington, Chris Siva, Tugiyono, Erich Volschenk, Jonathan Warden, Louise Williams and Roy Wittkuhn for putting up with me.

I also bothered many other people outside the university campus in the name of stress relief. Thanks to Lena Anderson, Jodee Lysaght, Vicki Preston and Dr Sharyl Williams for their support over the superhighway. And thanks to my friends Jim Allan, Yugon Chobanoff, Nicholas Eagle, Eun Kyoung Fleming, Cathy Harris, Craig and Jeffrey Iles, Richard and Kelly Kelly, Simon Kelly, Shireen Sampson and Chelsea Toy for putting up with a whining postgrad for so many years.

Neither last nor least, I must thank my family for all their love and support throughout all my years at university. To Ema, for following my every step; to Isa, for inspiring me to appreciate plants; to Arno, for always believing I could do this; to Lauri, for providing me shelter and to Jane for adding another doctor to the family.

And finally, I'd like to thank the ravens in the Curtin field trial area for testing my patience.

Thank you all.

Abstract

Species prescriptions are developed for revegetating abandoned acidic coal overburden seepage sites in the Collie region of Western Australia. The research involved selecting appropriate plant species and determining successful methods of enhancing revegetation. Candidate species were screened for tolerance to acidic overburden materials, local climate conditions and metal toxicity. Methods tested included improving spoil conditions and trialing an alternative method for seeding.

Twelve species of native plants were tested for tolerance in two acid overburden materials in pot and field trials. *Eucalyptus robusta* is the most tolerant, *Eucalyptus camaldulensis and Eucalyptus cladocalyx* are highly tolerant, *Eucalyptus rudis* and *Melaleuca hamulosa* demonstrate potential, provided adequate soil moisture is available.

An important growth restriction factor in acid soils is the presence of free aluminium ions. A glasshouse trial performed on seven species for tolerance to aluminium toxicity revealed *E. robusta* as most tolerant and *E. camaldulensis* and *Kunzea ericifolia* as highly tolerant. *E. rudis* and *M. hamulosa* are moderately tolerant, but *E. cladocalyx* and *Eucalyptus diversicolor* are very sensitive to aluminium.

Various methods were trialed to increase growth of seedlings transplanted on to acidic overburden sites. Both commercial cow manure and slow-release fertiliser tablets increase growth, whereas commercial potting mix and lime do not. Inoculation of plants with the ectomycorrhiza fungus *Pisolithus tinctorius* increases the amount of infection in roots but does not enhance plant growth.

Supplementary fertilisation is necessary to maintain growth (nitrogen) and restore chlorophyll production (phosphorus) in fast growing eucalypt seedlings planted into typical acidic spoils. Poor levels of nutrient availability in such acidic sites appear to be the primary factor in retarding growth. In the absence of supplementation, foliage reddening is observed in several species.

An alternative method of seeding dumps is fascining. Prepared dump surfaces may be covered with capsule-laden branchwood of myrtaceous species. Material of the locally available *Kunzea ericifolia* is effective in producing many seedlings. Subsequent seedling growth is enhanced with fertiliser and lime addition.

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Figure 1: Successful revegetation on acidic coal overburden (pH 4) adjacent to Ewington 2 lake (part of field trial in Chapter 2)

Chapter 1 - General Introduction

Introduction

Authorities within the Collie region of Western Australia seek the reduction of acidity in water bodies in the region. Abandoned overburden dumps and voids from previous coal mining activities over the past 100 years are scattered through residual jarrah forest and farmland. The sulphidic materials within the overburden, on being exposed to air, appear to have caused the soil to become acidic. Rainfall runoff and seepage are believed responsible for leaching acidic material into nearby old mine pits (voids). This is thought to be a major cause for the water bodies within the voids to become highly acidic.

The Collie project was funded during 1997 and 1999 to address this issue. Six separate sub-programs were involved. One sought to determine appropriate techniques for revegetating the acidic overburden surrounding the voids. Plants can reduce the volume of seepage from the dumps extracting water via the roots and transpiring this to the atmosphere via the leaves (Bell & Menzies 2000; Curry, Ritchie & Wilson 2000). In the longer term plants can reduce or even halt the generation of acid runoff by forming a topsoil barrier containing roots and litter. This reconstructed ecosystem should then be sustainable, requiring little management once established and being capable of self-renewal.

Acid mine drainage and acid soil

Acid sulphate soils result from the oxidation of ferric pyrite (FeS_2), a common constituent that is normally stable and inert. Such soil usually occurs sporadically and spatially within a profile (Marcar 1995). However, when exposed to oxygen and water, the pyrite may oxidise to form sulphate and hydrogen ions, leading to acid mine drainage (AMD) leaching from the material (Jackson 1991). The four sequential chemical reactions (numbered 1-4) that produce AMD are outlined below:

$$2FeS_2 + 2H_2O + 7O_2 \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (1)$$

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

$$8Fe^{3+} + S^{2-} + 4H_2O \rightarrow 8Fe^{2+} + SO_4^{2-} + 8H^+ \quad (3)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad (4)$$

As the soil pH becomes lower, aluminium ions are released and become dominant in cation exchange. As a result, basic cations such as calcium, magnesium and potassium are leached out (Simpson 1986). Aluminium can also generate further acidity through the following two step-wise reactions (numbered 5 and 6):

$$Al^{3+} + H_2O \text{ -> } AlOH^{2+} + H^+ \quad (5)$$

$$AlOH^{2+} + H_2O \text{ -> } Al(OH)_2 + H^+ \quad (6)$$

Mining of sulphidic minerals greatly increases the risk of acid production by breaking up the rock, causing a larger surface of pyritic material to be exposed to the weather. Water and oxygen can then react with the sulphides at a faster rate. Typical AMD from coal mining has low pH (as low as 2), and high sulphate (>2000 mg/l) (Environment Australia 1997).

Around the world, acid drainage is one of the most serious environmental problems within the mining industry (Jackson 1991; Environment Australia 1997; Biggs 1998). It can occur in mining for coal, precious metals, base metals and uranium. Acid mine drainage often continues long after mining activities have ceased. For example, abandoned mines over 2000 years old in Rio-Tinto (Spain) still produce AMD. The long-term damage due to acid drainage in Australia is unknown, as the impact has not yet been fully quantified (Environment Australia 1997). It has the potential to be a significant environmental issue.

Contamination of waterways by acid mine drainage is a serious problem at many active and abandoned coal mines. AMD will pollute the surrounding water and retard, or even

prevent, plant growth (Jackson 1991). In low pH conditions metals may revert into solute form and accumulate into toxic levels. Acid drainage usually runs off waste rock stockpiles, tailings and coal rubble. It can also occur from groundwater leaching from underground mine shafts or from open pit walls (Environment Australia 1997). The floor and sides of a void are quite impermeable, thus allowing the gradual accumulation of water within a void. Water, containing leached minerals from tailings and mine waste, can flow into the void, making the void water of low quality. As these voids are not frequently flushed, certain compounds, like sulphates may build up to unacceptable levels (Milnes 1995).

Western Australia's climate and limited physiognomic relief cause AMD to behave differently from other AMD sites. The lack of water and relatively low amount of runoff makes the AMD localised, thus not affecting large areas. However this localisation causes the problem to be more severe and persistent. Native vegetation, groundwater and site reclamation can be strongly affected. The limited rainfall can retard the AMD process, creating problem sites decades after mining has ceased in that area (Biggs 1998).

Effect of acid soil on plant growth

Poor plant growth in highly acidic soil can be due to a combination of acidity, toxicities and nutrient deficiencies and lack of beneficial micro-organisms, such as rhizobia and mycorrhiza (Howeler 1991). Acid soils may physically and chemically damage root system, thus reducing the ability of roots to penetrate the soil and access water and nutrients (Simpson 1986). In addition, plants weakened by growing in acidic soils are more vulnerable to microbial attack (Handreck & Black 1991) and disease (Simpson 1986). Furthermore, the acidic soils may become compacted to the extent of restricting root growth, which, in turn, reduces root exploration of the soil for nutrients and water (Fox & Doronila 1996).

Plants are negatively affected by acidity not only directly from hydrogen ion (H^+) and base concentrations but also due to secondary effects. The rate of organic decay, nutrient release and retention is affected as cation exchange capacity is reduced. Such secondary or indirect effects may result in a deficiency of essential mineral nutrients and/or the release of heavy metal elements to potentially toxic levels (Bradshaw & Chadwick 1980). Specifically, acidic soil is usually low in nitrogen, phosphorus, calcium and magnesium, and may be high in aluminium and manganese (Leeper & Uren 1993). In addition, many acidic soils are also deficient in the trace element molybdenum (Simpson 1986).

Controlling AMD

Reduction of acid mine drainage has not been fully accomplished on an operational scale other than by fully submerging the waste under water (Farmer & Richardson 1981). When stored underwater, sulphidic materials tend to be chemically unreactive. If deep enough, the water acts as an efficient oxygen barrier. It may also be chemically reduced from accumulating organic materials and biological activity (Environment Australia 1997). Water treatment is expensive and is only a temporary solution for AMD. However, acid drainage is continuous, so any water treatment must be undertaken frequently to ensure sufficient buffering capacity of the water is maintained (Jackson 1991).

Soil covers with vegetation may also provide a barrier between water, oxygen and highly sulphidic materials. Oxygen is best controlled if the pore space is saturated (Environment Australia 1997). However, thin soil coverings may dry and erode away, and thus expose the overburden. Such circumstances may reinitiate acid production. Even soils as thick as 1.2 m may become acidic from upward acidic seepage and diffusion (Jackson 1991).

In the event that topsoil is unavailable, then the overburden material itself may be all that is available and must be used as a barrier. Chemical and physical properties also need to be properly examined and if possible, ameliorated. A range of possible techniques are

available to increase the organic matter and nutrient status of the material, such as adding organic matter, lime, inorganic fertilisers, soil conditioners, green manure, mulch and planting nitrogen fixing plant species (Environmental Protection Agency 1995).

Study site - Collie

The town of Collie and the Collie basin are located on the Darling Plateau, approximately 160 km SSE of Perth at latitude 33^0 26' S and longitude 116^0 12' E (Figure 1.1). The town is the centre of the only coal mining activity in Western Australia and currently has a population of approximately 8,500 (Collie Shire Council *pers. comm.).*

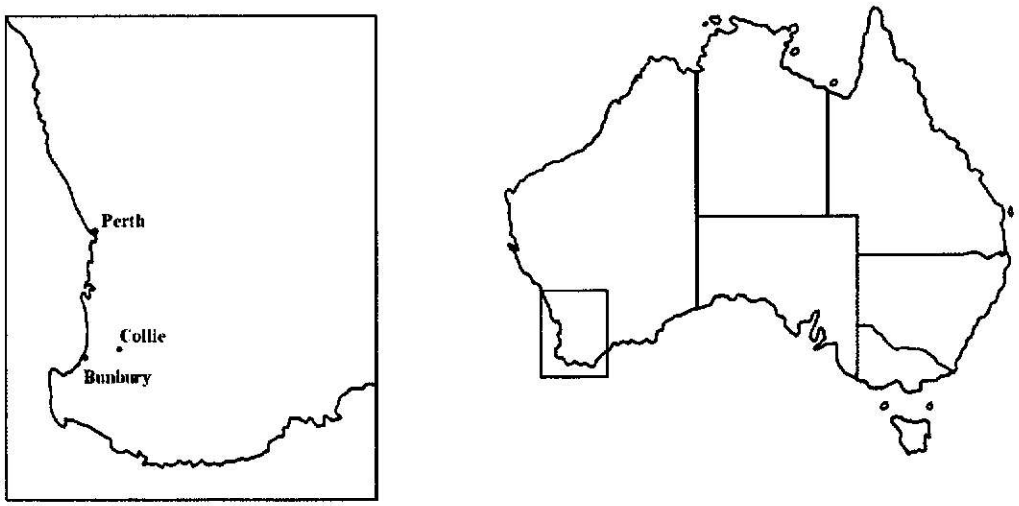

Figure 1.1: Location of Collie within Australia

The Collie basin is an area of approximately 220 km^2 and is divided into 3 sub-basins – Cardiff, Shotts and Muja (Figure 1.2). The natural vegetation within the basin was described by Heddle, Loneragan & Havel (1980) as comprising 3 associations – Collie, Cardiff and Muja complexes. The vegetation on all three sub-basins is sensitive to dieback (*Phytophthora cinnamomi* Rands) and about 50% of the Collie basin is infected (Bradshaw *et al.* 1989). Soils mostly comprise highly weathered material with exhausted levels of nutrients (Gilkes, Scholz & Dimmock 1973).

The Collie complex is an open forest of *Eucalyptus marginata* Donn ex Smith (jarrah), *E. calophylla* Lindley (marri) and *Allocasuarina fraseriana* (Miq.) Johnson (western forest sheoak) with a range of understorey species able to cope with sand and lateritic gravel.

The Cardiff complex is an open *Banksia* woodland with *Nuytsia floribunda* (Labill.) R. Br. Ex Fenzl and understorey species such as *Petrophile linearis* R. Br., *Adenanthos obovatus* Labill. and *Eremaea* species.

The Muja complex is an open woodland of *Melaleuca preissiana* Schauer and *Banksia littoralis* R. Br. *Eucalyptus patens* Benth. trees are found in drier areas while *Melaleuca* species occur in wetter sites.

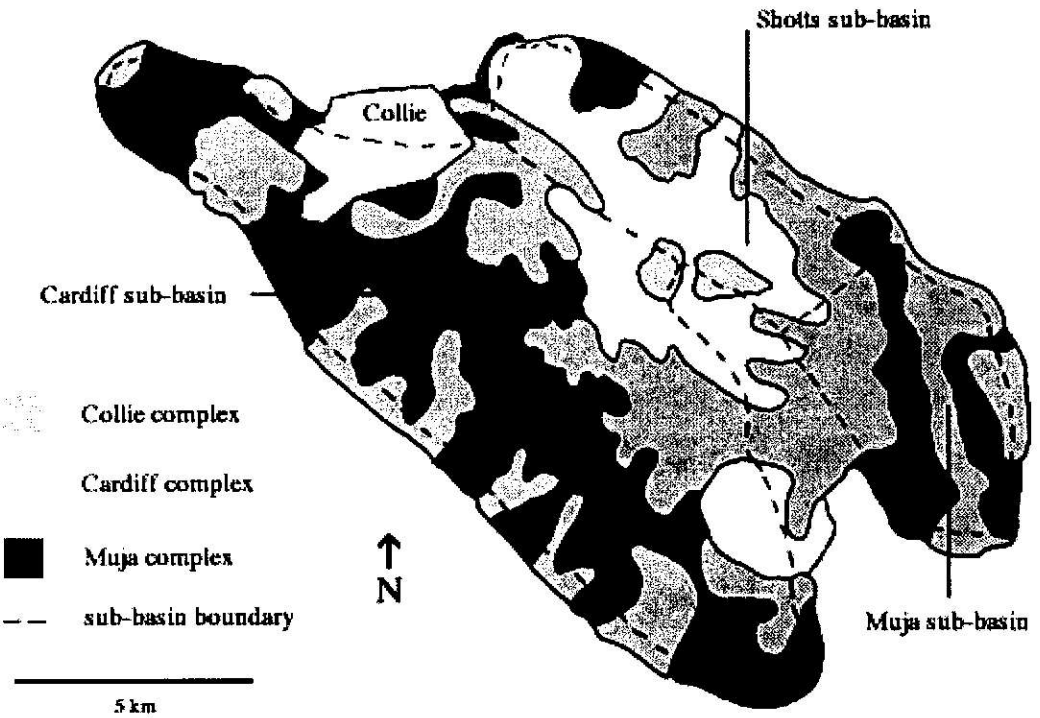

Figure 1.2: The vegetation complexes and sub-basins within the Collie basin (compiled from Heddle *et al.* 1980 and Stedman 1988)

The south-west region of Australia has a typically warm Mediterranean climate. Rainfall at Collie exceeds 120 mm month^{-1} during May to August, with 17-22 rain days a month (Figure 1.3). Over the last 102 years, the mean annual rainfall has been 950.2 mm and mean number of rainy days per year 142.2. The summer mean monthly maximum reaches ~ 30^0 C in January and February, while the winter mean monthly minimum temperature is ~ 5^0 C in July (Figure 1.4). Such rainfall and temperature patterns result in an effective growing season from mid-April to October (Bureau of Meteorology 2001).

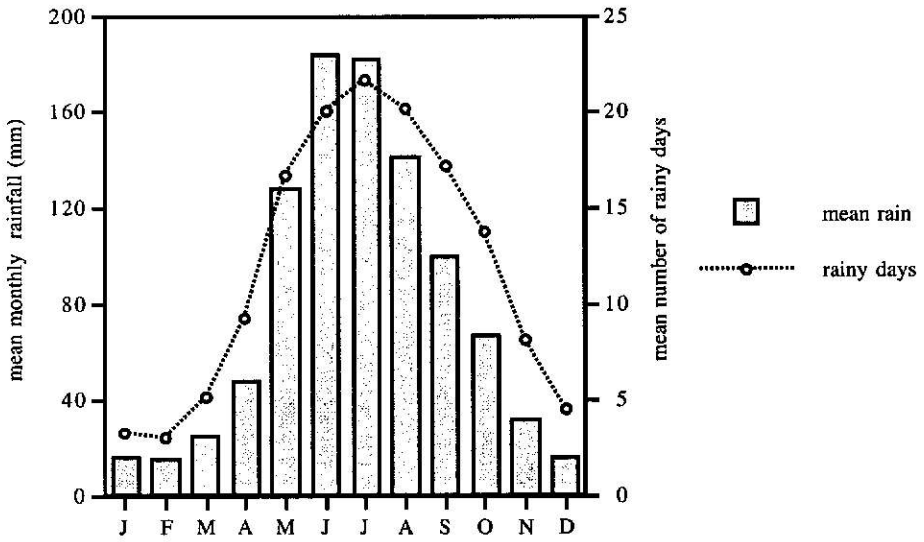

Figure 1.3: Mean monthly rainfall and number of rainy days at Collie (1900-2001) (Bureau of Meteorology 2001).

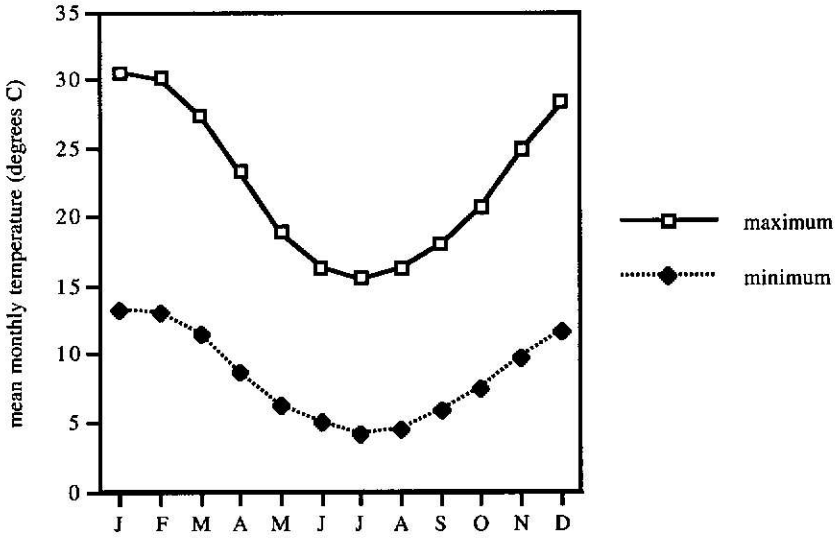

Figure 1.4: Mean monthly maximum and minimum temperatures at Collie (1900-2001) (Bureau of Meteorology 2001).

Coal was discovered near the Collie River in 1883. Permian coal occurs to depths of 1000 m. There are 55 significant seams of coal, the thickest being 15 m, but most are less than 4 m in thickness. Collie coal is a sub-bituminous non-coking coal with specific energy of 20.8 MJ kg^{-1}. It is high in moisture (25.9%) but low in sulphur (0.45%) and ash (4.6%). The coal rock is coarse to granular, with successions of cross-bedded sandstone, shale, claystone, siltstone and conglomerate with intercalated seams of sub-bituminous coal (LeBlanc Smith 1990).

The coal was first mined in 1893, but production did not exceed 1 million t yr^{-1} until 1965 (Bradshaw *et al.* 1989). There are currently 2 mining companies – Griffin and Wesfarmers Coal (formerly Western Colleries Limited). The bulk of the coal is supplied to Western Power for the Muja power plant, which supplies electricity to the majority of the state. Coal is also transported to nearby industries such as Worsley Alumina. In recent years, coal has been primarily mined using open-cut techniques, which can retrieve more than 80% of the coal, compared to less efficient underground techniques that retrieve only 45 to 65%. Open cut mining was first introduced in Collie in 1943 at Stockton. At completion of mining in 1960, the Stockton void filled with groundwater and was developed as a recreational area (Stedman 1988).

All areas currently disturbed by mining are rehabilitated, as required by legislation. Final dump areas are landscaped, topsoiled and revegetated (Bradshaw *et al.* 1989). However, prior to the last 25 years, the history of environmental management had generally been poor. Specifically, many former coal mining sites were simply abandoned without any attempt made at revegetation. In addition, mining voids filled with ground water and became lakes (Figure 1.5). The water quality in these lakes declined in the 1970's with problematic water quality and by the 1980's was generally poor (Stedman 1988). Many of these lakes occupying the voids at Collie currently possess pH levels of 3.5 - 5.0.

An important source of this acidity in the lakes is the surrounding overburden material (Figure 1.6). Collie overburden is typically of low pH (3.0 to 4.5), the phosphorus level is less than 1 ppm, and the material is depleted in organic matter. This soil tends to be

waterlogged in winter and bakes hard in summer. Such low pH levels indicate the potential for toxic levels of aluminium (Al). The spoils have little physical structure, can reach high temperatures, dry out rapidly and crust after rainfall (Hughes & Fox 1993). Spoils also tend to be compacted due to wheeled machinery, often making it almost impenetrable for root growth, water and oxygen (Bradshaw & Chadwick 1980).

Despite more than forty years of exposure, less than 10 percent of the older Collie dumps have been naturally colonised. No colonisation has occurred where soil pH is less than 3.5. Only when the pH is more than 5.0 has any significant cover developed (Bartle & Riches 1978; Collie Coal Mines Rehabilitation Committee 1981). Even then, species diversity is much less on dump soils than in the surrounding forest. Low abundance and diversity results from poor early plant growth being more sensitive to acidic or high Al soils. Excessive soil hardness also contributes to lack of growth. Poor root growth may be mainly responsible for the failure of initial establishment in many species (Koch 1984).

Rehabilitation

Natural colonisation by vegetation on barren soils with acidic or metal toxic properties may take as long as 50 to 100 years (Bradshaw 1988). This colonisation may not be sufficient to reduce acid production and seepage. Human intervention may enhance the revegetation process where an overburden surface is a barrier to air and water. Vegetation cover may break the cycle of pyritic material being continuously exposed by erosion. Topsoil and vegetation may provide an adequate oxygen barrier to the pyritic material, preventing acidification (Farmer & Richardson 1981). Plants act as biological water pumps, and have the potential to remove large amounts of water from the soil by transpiration (Leeper 1967). As a plant community establishes on the overburden, the demand by the roots and micro-organisms may reduce the level of oxygen. This may act as a barrier to reduce or even prevent sulphide oxidation. Substantial liming and successful vegetation can also control the pH of the spoil (Sorenson *et al.*1980).

Figure 1.5: Ewington 2 lake, an abandoned void where the water pH was 4.5 in July 1998

Figure 1.6: Sparsely colonised overburden surrounding Ewington 2 lake

Revegetating a site depends on the physical and chemical conditions of the soil and the selection of plant species suited to those conditions (Jackson 1991). Restoration is the attempt to return the site to its original condition before the disturbance. This may be straightforward where the former land use is farm land, but more difficult when it was a "natural" system. Restoration is virtually impossible in many cases, as the original ecosystem has taken several millennia to develop (Bradshaw 1988). The disturbance of the land surfaces can alter the conditions for vegetation growth so much that it may not be possible to return the pre-existing plant community to the original conditions (Fox 1984).

Rehabilitation may serve as a cheaper and easier goal than restoration. Rehabilitation can be defined as slightly short of restoration, where the goal is to achieve a similar, often simpler version of the original environment (Bradshaw 1988). The success of rehabilitation upon mining wastes depends upon the control of physical, chemical and climatic processes (Fox & Tacey 1994). Mine wastes are nutrient deficient, sterile, compacted and high in toxic elements. Mining rehabilitation is site specific; each waste material and climate condition determines the extent and type of revegetation (Fox 1984).

There are four broad approaches to reduce the acidity of soils. The first is to minimise acid production by reducing acidifying inputs and any nutrient losses. The second is to select species and varieties that are adapted to the specific acidic soil conditions to maximise soil recycling of nutrients and water. The third is to apply ameliorants to the soil to reduce acidification. The fourth is to supply inputs to assist plant survival and growth, such as trace elements and beneficial microbes (Helyar 1991). Revegetation encompasses the second and fourth approaches. This can be simplified into two objectives:

1) to select suitable species
2) to select suitable methods

Revegetation research

The success of a revegetation effort depends on the properties of the soil and the selection of plants suited to those soil conditions (Jackson 1991). Pilot trials are desirable not only to test the species, but also to assess the best techniques to be employed (Fox & Tacey 1994). The initial success of rehabilitation relies on abiotic factors. Long-term success, however, depends upon the vegetation (Tongway 1998).

Growing plants tolerant to acid soils is an alternative to establishing vegetation on acidic soils when inputs, such as lime and fertiliser, are limited. Though the pH of a soil may be adjusted by chemical means to a level optimal for most plant species, it is wiser to choose species that are tolerant of the existing pH (Koch 1984; Sheat & Schofield 1999). A comprehensive ecological analysis is necessary to select the most appropriate species for the particular environment. Species should be selected to perform for a long term in a difficult environment with minimal management (Bartle & Shea 1978). Indigenous species may not survive in the altered soils. Species that have similar growth forms to the original vegetation and can tolerate the overburden conditions and the climate are the most appropriate to use (van Leeuwen 1994; Environmental Protection Agency 1995).

Choice of species must be practical, and suitable for the soil and climatic conditions. Some species types may only be grown if certain site conditions can be overcome, such as overcoming nutrient deficiency, providing drainage and breaking of hardpans. Overburden dumps tend to hold less moisture than natural soils, so species need to be selected that can tolerate drought conditions rather than whether it is simply native to that area (Handreck & Black 1991). Species should also be chosen to maximise organic matter production in the soil, to improve early establishment and longevity of the vegetative cover (Richardson 1980).

Plants may develop different strategies to cope with the range of stress factors existing in acidic soils. Screening a plant for tolerance of merely one of these factors is inappropriate for predicting adaptation of that species to acidic soils (Marschner 1991).

Mechanisms for acid soil tolerance may be within the rhizosphere (for example, secretion of organic acids and other compounds), within the root (for example, membrane transport or the protection of vital metabolic processes) or in the leaves (for example, immobilisation of Al) (Marcar 1995).

The tolerance, growth and propagation requirements of each candidate species must be considered in context with the physical and chemical properties of the site before field trials begin (Lamont 1978). There are two categories in which to assess performance of plant species: the direct measures of performance in meeting major objectives (such as tree growth and water consumption) and; using parameters to indicate successful adaptation to the environment (such as root habit and tolerance of soil water deficiency). Both categories should be used in evaluating trees and stands (Bartle & Shea 1978).

There are two ways of determining appropriate species. The first is to establish comprehensive arboreta of all promising species on the most important site types. This method, though providing a systematic format in evaluating all candidate species, can take decades to achieve results. The second method is to determine suitable species by conducting immediate evaluations on species in existing vegetation. This method may be difficult to apply as the stands may have been established for a variety of reasons and so be deficient in treatment, sites or species. As a result, some appropriate species may be excluded (Bartle & Shea 1978). Species used should be rigorously tested for site tolerance and ability in achieving the long-term goals of the site (Tacey, Olsen & Watson 1977). Growth limiting factors should also be tested with glasshouse trials (Jackson 1991).

Species selection

Most of the studied species are eucalypts. The ecological range of eucalypts in Australia is enormous. Over 75% of all Australian forests are dominated by eucalypt species. There are over 600 species of eucalypts (Johnson 1973). Some are distributed widely, others restricted to localised niches. Many species have a large level of genetic variation, and ecotypes may display clinal and disjunct variation within and between local populations (Florence 1996).

Eucalypts have developed special features under alternating extreme environmental conditions (first wet and fertile, then dry and infertile). These features allow eucalypts to tolerate unfavourable conditions, then grow rapidly when favourable conditions occur. These adaptations, not all present in a single species, include epicormic shoots, coppice, an indeterminate shoot pattern, lignotubers, fire-resistant bark and mechanisms to tolerate drought and insect attack. Some eucalypts can handle environmental stress. Some species are drought adapted, others cold adapted or fire resistant (Read 1994). Some eucalypts can survive drought by controlling water loss through the stomata or by growing deep roots to reach water unavailable to other plants (Eldridge *et al.* 1994). Fast growing eucalypts consume much water, either through deep penetrating roots that can reach the watertable, or by spreading lateral roots around the tree (Westoby 1989).

Eucalypts can be successful in exotic conditions because many of the restricting factors that occur in natural forests may not exist in the new location (for example, herbivorous insects and diseases). There may be favourable conditions on an exotic site, such as more nutrients and rainfall. Successful management, such as protection from fire, weed control and proper spacing, can further increase eucalypt survival and growth. Many of the sites where eucalypts are planted are degraded, being foreign to the native flora. Besides eucalypts, few native tree species can adapt to the degraded conditions. Eucalypts generally can perform better than the native flora (Eldridge *et al.* 1994).

This thesis did not examine the local dominant canopy species *E. marginata*, as this species has been observed to be a poor coloniser on the Collie coal mine dumps. *E. marginata* lacks stomatal control (Doley 1967) and requires deep rooting to access water (Abbot & Loneragon 1986), thus is highly vulnerable to water stress. These factors make this species not appropriate for revegetating Collie overburdens, which are often dry and compacted in summer.

Method Selection

Soil conditions may be improved to increase the success of revegetation. The major properties in these spoils that may affect seedling survival and establishment are soil pH, low organic matter content, toxic metals and poor nutrient levels. Methods of overcoming these factors are outlined below and are further discussed in the experimental chapters.

The low nutrient levels of mine spoils may be improved with inorganic fertilisers. These may be applied as granules for short-term nutrition or as slow release fertiliser tablets for long-term nutrition (Sheat & Schofield 1999). Increasing the level of nutrients in the soil may improve seedling survival by increasing plant growth to a size where the roots may access the subsurface moisture before the hot summer months dry out the surface soil. Faster plant growth will also hasten the rate of revegetation.

The most common method of treating acidity is with crushed limestone (Handreck & Black 1991). Increasing the soil pH improves soil structure (Leeper & Uren 1993) and allows the plants to benefit from organic matter longer by lowering the decay of organic matter (Boynton 1980). Liming can also increase soil fertility by supplying calcium and magnesium, improving the bioavailability of nutrients and also retaining the nutrients within the soil (Handreck & Black 1991). It can also lower the presence of heavy metals ions to non-toxic levels (Aitken, Dickson & Moody 1998) and reduce damage to fine roots (Helyar 1991).

Soil organic matter content may be improved by adding green and animal manures, sewerage sludge and other wastes. Organic matter improves the physical soil structure of the soil such as increasing drainage, aeration and retaining moisture (Traynor 1980). It also improves the chemical properties of the soil by increasing and buffering the soil pH, increasing nutrient levels (Davey & Krause 1980) and lowering metal toxicity (Magdoff 1992). It may also increase the amount of beneficial microbial life such as mycorrhiza (Davey & Krause 1980).

Mycorrhizal fungi attach to plant roots and act as root substitutes, exchanging nutrients and water for food. This symbiosis may be more efficient in increasing nutrient (Archer, Hodges & LeHunt 1993) and moisture uptake (Davies *et al.* 1996) than bare roots alone. Mycorrhiza may be more tolerant of metal toxicity, pathogens, acidity and high temperatures (Smith & Read 1997).

Format and objectives of thesis

The overall aim of this thesis is to determine methods of reclaiming acidic coal spoils with fast growing tolerant species. The priority of this research is to establish vegetation, to abate further soil and water acidification. The final land use was to be a closed forest that was at least similar, but not necessarily identical, to the surrounding vegetation.

The research constituting this thesis has two principal objectives: 1) to select the most suitable species for revegetating the acidic areas of coal overburden material surrounding void lakes at Collie; and 2) to determine appropriate methods of improving revegetation techniques for these difficult acidic coal overburdens. This research will contribute to the development of methods for the revegetation of perimeters of lake edges of coal mine voids, particularly in the Collie region. Such techniques should contribute to amelioration of acidic runoff into the void lakes and in developing techniques to revegetate acidic sites around the world.

The first part of the thesis focuses on selection of suitable plant species. Thirteen candidate species were tested in both a pot trial and a field trial to determine those species that were capable of fast growth and of tolerating the hostile conditions of various acidic overburdens (Chapter 2). Seven species were also tested for tolerance to aluminium, a metal that can be present as a free cation in acidic coal overburden in levels that can be toxic to plants (Chapter 3).

The second part of the dissertation examines suitable methods of revegetation. Five types of planting amendments were tested to determine whether straightforward amelioration was appropriate for the conditions occurring in acidic coal spoil. These methods included adding growth substrates, such as crushed limestone, potting mix and cow manure, around seedlings (Chapter 4); resupply of straight nitrogen and phosphorus fertiliser to established seedlings (Chapter 5), applying combinations of crushed limestone and slow release fertiliser to seedlings (Chapter 6); inoculating seedlings with the mycorrhizal fungus *Pisolithus tinctorius* Pers. (Coker & Couch) (Chapter 7) and using seed bearing branch material of *Kunzea ericifolia* (Sm.) Heynh as a mulch and seed source (Chapter 8). The overall findings, conclusions and recommendations are discussed in Chapter 9. A protocol for rehabilitating acidic overburdens is outlined in Appendix 1.

Chapter 2 – Selection of species for acidic sites around voids

Introduction

There are two empirical approaches to selecting appropriate species for revegetating sites: growing seedlings in pot trials and field trials. A pot trial can be a useful technique to gather information on (often little known) species at the germination and early establishment stages. In general, differences in pot and field trials can be expected depending on the amount of rainfall that the field trial receives. Pot trials are designed to test plant growth response in soils under optimal soil moisture conditions. In the field, the soil is generally drier, so growth may be slower (Bell 1981). Pot trials have the advantage of being short term and the faster growth should allow differences between treatments to be detected earlier. Entire seedlings are readily harvested, allowing for biomass measurements. Finally, pot trials remove the factor of climate, thus the only factor affecting plant growth is the soil medium. By testing species in glasshouse trials for tolerance to particular soil conditions, appropriate species may be selected relatively rapidly for revegetation of particular soil types tested.

Pot trials alone may not be sufficient to predict species growth. While pot trials aim to detect the responses of plant species to particular soil conditions in the short term, field trials are necessary to determine species responses to the site conditions, including the soil type, over a longer period (Eldridge *et al.* 1994). Field trials are also desirable to further test results obtained by pot trials and to develop broad scale, practical techniques. It is important to recognise early in the research the sources of possible variations in pot and field trials (Bell 1981). By combining the results of pot and field trials, suitable species can be selected more definitively than by either trial alone. Space constricts field trials and plot size is usually a compromise in the amount of uniform land available and the desirability of a relatively compact site representative of the conditions that require testing. The block must be small enough to encompass very little environmental variation. This may be achieved using smaller than normal tree spacings when the trial is of short duration (2-3 years) and when it is to be used for selecting suitable species and not for assessing yield (Eldridge *et al.* 1994).

Restoration or rehabilitation of mined lands at Collie represents some special challenges. In particular, the soils tend to be highly acidic and demonstrate variable levels of aluminium toxicity. For example, young *E. calophylla* trees growing on acidic hotspots develop severe trunk taper. It is believed that the taper is a sign of stress from growing in the acidic spoils. One possible cause of the taper is aluminium sequestration. A plant species may tolerate exposure to high levels of a metal by accumulating it within the main stem or trunk. This can be achieved by having differential metal compartmentalisation patterns that store accumulated metal ions in organs or sub-cellular compartments where no sensitive metabolic activity takes place (Verkleij & Schat 1990; Alloway 1995).

Two experiments, a pot trial and a field trial, are referred to in this chapter which examine growth of several plant species in Collie soils. The specific objective was to determine which species are tolerant and relatively fast growing in acidic soils from two contrasting overburden sites where plant growth had been poor. The null hypotheses are:

$H_0$1: Overburden soils do not differ significantly in chemical properties, compared with local control paddock soil.

$H_0$2: Species grown in pots containing overburden do not significantly differ in growth from plants in paddock soil (control).

$H_0$3: Acidic coal overburden sites do not differ significantly in soil pH and moisture.

$H_0$4: Plant species do not significant differ in survival, physical or physiological parameters when grown at various coal overburdens sites.

$H_0$5: Plant species do not indicate typical symptoms of aluminium sequestration when grown at various coal overburden sites.

Method

Most of the species used in the experiments were grown from seed collected from Collie dumps. Other local species were also included to attain a balance of local to eastern states species. A list of seed sources and brief descriptions of the studied species is located in Appendix 2. In January 1997, seeds from thirteen species were germinated in sterilised coarse sand. Nine species were from the genus *Eucalyptus – E. camaldulensis* Dehnh., *E. cladocalyx* F. Muell., *E. gomphocephala* D.C., *E. grandis* Hill ex Maiden, *E. maculata* Hook (syn. *Corymbia maculata* (Hook) Hillis and Johnson), *E. resinifera* Smith, *E. robusta* Smith, *E. rudis* Endl. and *E. wandoo* Blakely. Three species were shrubs - *Callistemon speciosus* (Sims) D. C. (syn. *Callistemon glaucus), Calothamnus rupestris* Shauer and *Melaleuca hamulosa* Turcz. The grass species *Neurachne alopecuroidea* R. Br. was also included as a potential groundcover species, as this species was observed to grow well near the voids. Seeds of all species, except *E. gomphocephala*, were collected from plants on revegetated Collie dumps in November 1996.

At 2 wks, sufficient seedlings for Experiments 1 and 2 were transplanted into peat pots of dimensions 5 x 5 x 6 cm filled with Naturegrow™ general potting mix (Appendix 3). Each plant was fed every two weeks with 20 mL Thrive™ liquid fertiliser (Appendix 3). Soils used for the pot trial were from a hotspot at the Griffin Coal mining entrance (hereafter 'Muja'), Ewington 2 lake (hereafter 'Ewington'), and a paddock adjacent to Ewington (hereafter 'control') whose soil was sandy loam in texture. Muja and Ewington were also the sites for the field trial.

Ewington was mined within the year of 1960 and then left. Its location next to a swampy area made mining the coal difficult and only 27,174 tonnes of coal were removed (Stedman 1988). The void filled with water shortly after mining ceased and became a lake with a pH of ~ 4.5. During mining, the overburden material was deposited around the lake. The dumps have become acidic and have only scarce colonising vegetation.

The south lakeside is approximately 15 to 20 m in width between the lake and dumps. It is comprised of acidic overburden of sandy loam texture.

In 1980, an area of 4 ha at Muja was covered with coal overburden and then initially revegetated with pasture species. In 1990, the revegetation was converted into trees and shrubs with sown native understorey species and using hand-planted *Eucalyptus* species. This dump was then divided into three separate areas: no understorey species sown, understorey species sown, and remnant pasture species. In the first mentioned area, a localised patch of acidic overburden (termed a hotspot) developed. The hotspot was approximately 220 square metres in size, sandy clay loam in texture and contained only a few *Juncus kreusii* plants. This hotspot is surrounded by *Eucalyptus* trees on the west, south and east sides, and cleared ground with a fence on the north side.

Data were tested for homogeneity with boxplots and scatterplots. Differences in each parameter and for each species were analysed using analysis of variance (ONEWAY) and Fisher's tests where appropriate.

Experiment 1 - Pot trial

This pot trial was conducted at the Curtin University field trial area (FTA) in Bentley, Western Australia. In May 1997, batches of soil from each soil type were taken to the FTA and mixed separately in an electrically-driven cement mixer to ensure homogeneity. Three hundred and sixty 2.5 L pots were double lined with small garbage bags. Sub-samples of each soil type were retained for analysis of pH, conductivity and nutrient levels by the CSBP Plant and Soil Analytical Laboratory (Appendix 4). The pots were divided into three sets of 120 – one for each soil type - and then filled with 2 L of soil. One seedling was then individually planted per pot with ten replicate pots used for each species and soil type, except that only five replicates for each soil type were used for *E. camaldulensis* and *E. rudis* because of a shortage of seedlings.

The trial ran for 28 weeks. Heights, crown and stem diameters of each plant were measured on May 22nd, July 3rd, August 15th, September 30th and December 8th 1997. During the trial, any weeds that emerged from the control soil were removed (no weeds were observed from the two overburden soils). The *Eucalyptus* and shrub seedlings were then harvested and separated into leaves, stems and roots, while the grass, *Neurachne alopecuroidea,* was divided into leaves and roots. Plant parts were dried to a constant weight at 60^{0}C for 48 hours, after which the dry weights were recorded. Plant tissue of the four poorest performing species were then tested at CSBP Plant & Soil Analytical Laboratory for chemical analysis to determine if nutrient deficiency was a contributing cause to the lack of growth (Appendix 4). Aluminium concentrations were not determined. Within each species, 4 seedlings were randomly selected and paired to obtain 2 samples of leaves and stems (n=2). Temperature ranged from 18 to 36° C during the experiment.

Experiment 2 - Field trial

In June 1997, sets of 10 plants from each species were planted in a Latin Square Design at the two acidic coal overburden sites Ewington and Muja (Appendix 5) and at an edge of an adjacent paddock (control). Seedlings were planted at 1 x 1 m spacing and were protected from animals with plant guards. A month after planting, each plant was given 10 g of Osmocote Plus™ 9 month slow release fertiliser prills and 10 g of crushed limestone (Appendix 3). Weeds invaded the control site, resulting in the death of all seedlings in the first 3 months of the trial through competition for space, nutrients and water. There was no significant presence of weeds at the two acidic sites for the entire period, and most seedlings survived.

Species assessment was by growth. Plants were measured at the beginning of the experiment for height, crown widths and stem diameter. Measurements were taken every 3 mo for a 24 mo period. Stems of all eucalypts from both sites were measured in September 1998 (15 months) and October 1999 (28 months) to obtain trunk volumes. Stem height measurements of eucalypts in June 1998 and 1999 were divided by basal

diameters to give an index of taper. Twelve soil samples (5 to 15 cm) were taken in a grid pattern of four N-S columns at 4 m spacing and 3 W-E rows of 6 m spacing in Dec 1998 and July 1999 from both sites. Soils were weighed, dried in a 40^0 C oven for 10 days and weighed again to enable percentage moisture to be calculated. 20 g of each sample were then placed into flasks with 80 mL deionised water and mixed in an orbital shaker at 200 rpms for 12 hours and then measured for pH and electrical conductivity.

Leaf water potentials of six species were tested at pre-dawn and midday in March 1999 (summer) and July 1999 (winter) with a PMS Instrument Co™ Model 1000 pressure chamber to determine if differences in soil moisture at sites affected moisture status of plants. Control measurements were taken from well hydrated plants of the same species grown in pots at the FTA on the Curtin campus.

Results

Experiment 1 - Pot trial

Chemical analysis

All three soils were very low in nutrient levels and did not differ significantly in levels of ammonium, potassium or conductivity (Table 2.1). The control soil was generally more fertile, with an order of magnitude higher level of nitrate, and significantly more phosphorus and iron but had much less sulfur. The control soil also contained significantly less aluminium and was significantly less acidic. Organic carbon was low in all soils (≤ 1.0%) but was significantly lowest in the Ewington soil. Therefore $H_0 1$ is rejected: overburden soils differ significantly in chemical properties from the control paddock soil.

Table 2.1: Chemical analysis of soils (5 to 15 cm depth) by sites (SD in brackets)

Characteristics (unit)	Control (n=4)	Muja (n=5)	Ewington (n=4)	*P*
Nitrate (mg/kg)	14.0 (1.4) a	1.0 b	2.1 (1.2) b	<0.001 (***)
Ammonium (mg/kg)	1.8 (0.5)	3.2 (1.3)	2.5 (2.4)	0.418 (NS)
Phosphorus (mg/kg)	9.5 (1.3) a	5.0 (0.7) b	4.0 (1.6) b	<0.001 (***)
Potassium (mg/kg)	31.0 (5.0)	32.8 (8.1)	33.5 (9.0)	0.892 (NS)
Sulfur (mg/kg)	8.0 (0.7) b	48.1 (26.9) a	59.1 (13.2) a	0.007 (*)
Iron (mg/kg)	347.8 (60.2) a	203.2 (35.2) b	152.0 (41.0) b	<0.001 (***)
Aluminium (mg/kg)	2.6 (0.3) b	29.1 (8.0) a	32.6 (13.4) a	0.001 (**)
Organic C (%)	1.00 (0.10) a	0.95 (0.30) a	0.20 (0.05) b	<0.001 (***)
Conductivity (mSm^{-1})	0.159 (0.011)	0.102 (0.078)	0.134 (0.049)	0.361 (NS)
pH (H_2O)	5.4 (0.1) a	4.2 (0.6) b	4.7 (0.4) b	0.006 (**)
pH ($CaCl_2$)	4.7 (0.1) a	3.9 (0.3) b	4.2 (0.2) b	0.001 (**)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
different letters in a row indicate significant differences between sites

Growth over time

Heights, crown diameters and stem diameters of the tested species varied in response to the different soils by 28 wks (Figures 2.1 to 2.3; Tables 2.1 to 2.2). *E. camaldulensis, E. cladocalyx, E. maculata, E. robusta* and *E. rudis* seedlings were not significantly different when grown in the different soils. *E. gomphocephala* plants in control soil were significantly greater in height and stem diameter than those in Muja soil but not seedlings in Ewington soil (P = <0.001 and 0.027 respectively). *E. grandis* plants growing in the control soil were also significantly taller than those grown in the Muja soil (P = 0.023) and had significantly smaller crown diameters in the Ewington soil (P = 0.016).

Only one *M. hamulosa* control seedling survived, but this plant was just taller than the mean height of those grown in Ewington soil; the latter being significantly greater in height, stem diameter and crown diameters than those grown in Muja soil (P = 0.011, <0.001, 0.018 respectively). *C. speciosus* and *C. rupestris* plants growing in control soil were significantly taller than seedlings grown in Ewington soil (P = 0.002, 0.011); the latter species also had significantly larger crown diameters and stem diameters (P = 0.041, 0.008) when grown in Ewington soil. *E. resinifera* seedlings did not differ significantly in height but had both smaller crown and stem diameters (P = 0.001; <0.001) when grown in either overburden material. *E. wandoo* plants growing in control soil were significantly taller than seedlings grown in Ewington soil (P = 0.008) and had both smaller crown and stem diameters (P = 0.003, 0.002) when grown in either overburden material.

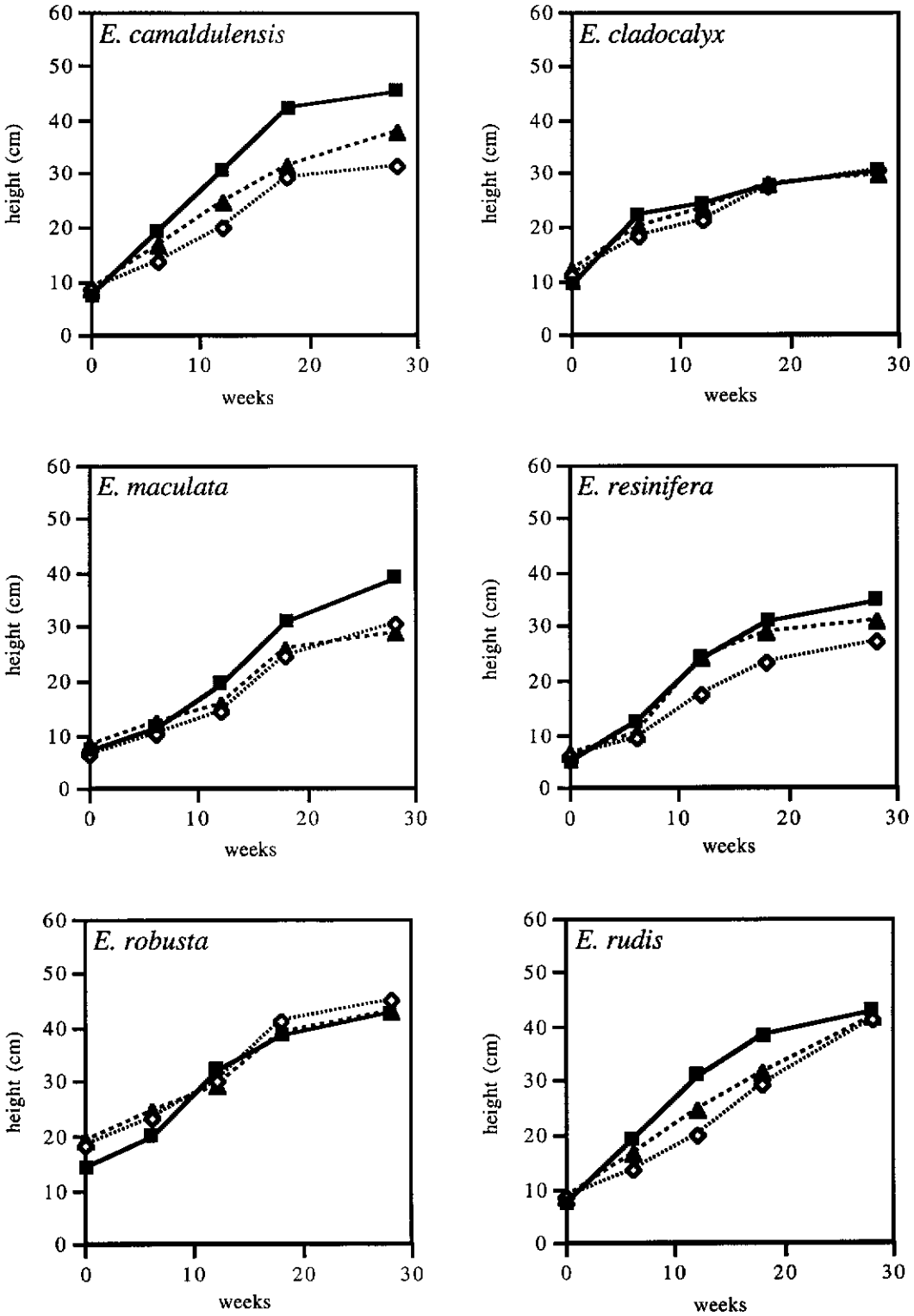

Figure 2.1: No significant differences in mean heights (cm) of *E. camaldulensis*, *E. cladocalyx*, *E. maculata*, *E. resinifera*, *E. robusta* and *E. rudis* seedlings grown over 28 wks in various soils (■ = control, ◇ = Muja, ▲ = Ewington).

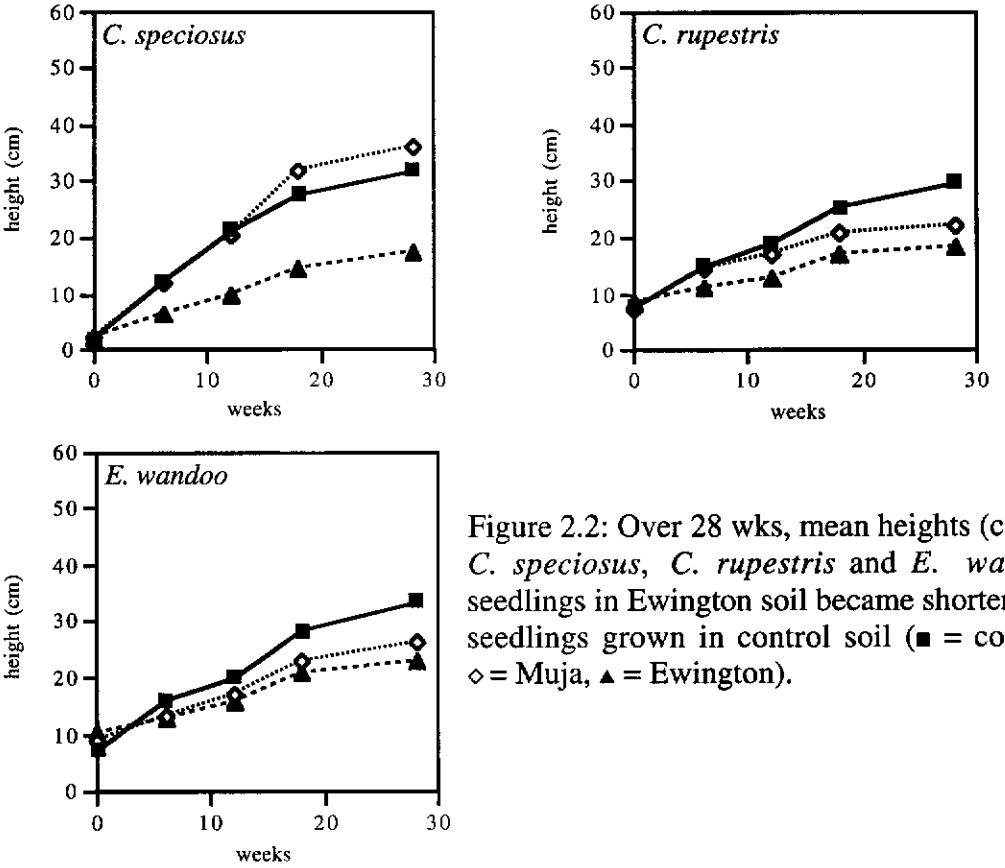

Figure 2.2: Over 28 wks, mean heights (cm) of *C. speciosus*, *C. rupestris* and *E. wandoo* seedlings in Ewington soil became shorter than seedlings grown in control soil (■ = control, ◇ = Muja, ▲ = Ewington).

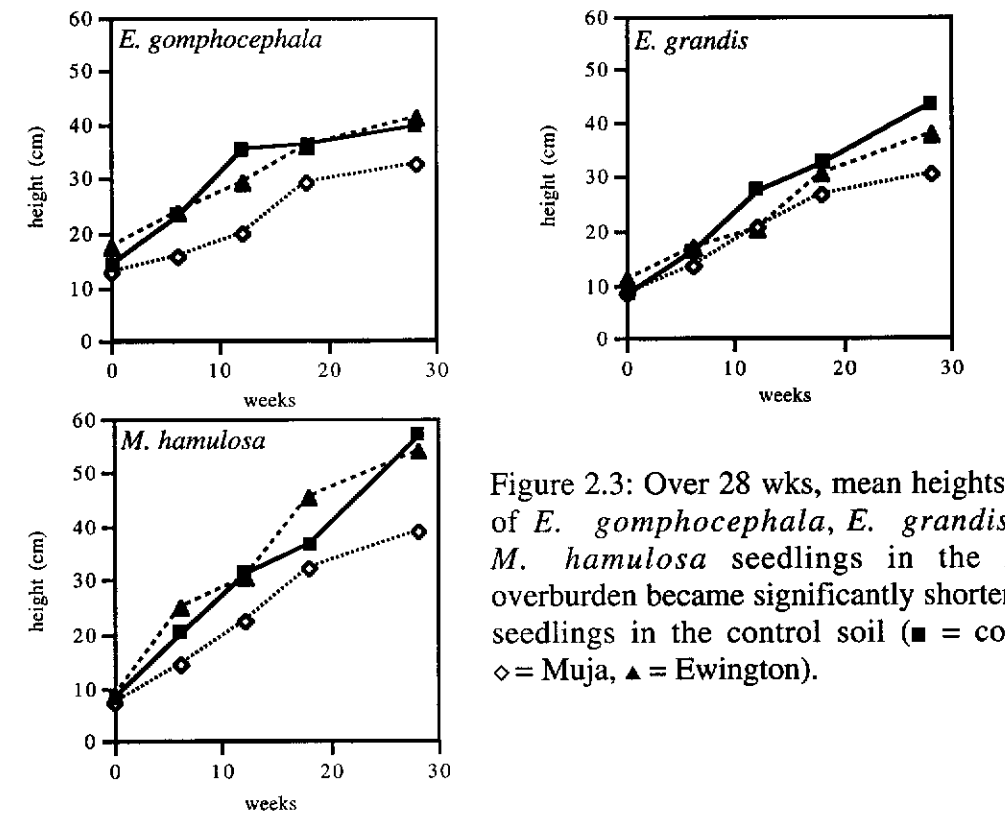

Figure 2.3: Over 28 wks, mean heights (cm) of *E. gomphocephala*, *E. grandis* and *M. hamulosa* seedlings in the Muja overburden became significantly shorter than seedlings in the control soil (■ = control, ◇ = Muja, ▲ = Ewington).

Table 2.2: Mean crown diameters and standard deviations of seedlings at 28 wks (cm)

Species	n	Control	n	Muja	n	Ewington	*P*
E. camaldulensis	3	21.0 (1.4)	6	20.1 (5.1)	6	19.8 (6.5)	0.968 (NS)
E. cladocalyx	6	23.5 (2.1)	9	19.3 (4.0)	10	21.2 (5.0)	0.263 (NS)
E. gomphocephala	7	39.7 (10.2)	10	32.8 (7.7)	10	41.0 (7.8)	0.180 (NS)
E. grandis	7	28.3 (5.4) a	9	28.5 (5.2) a	9	21.7 (4.4) b	0.016 (*)
E. maculata	8	31.0 (5.2)	10	27.4 (5.3)	9	25.3 (5.1)	0.100 (NS)
E. resinifera	7	31.0 (5.2) a	10	22.6 (4.3) b	9	22.1 (4.6) b	0.001 (**)
E. robusta	9	26.4 (8.2) a	9	18.4 (4.8) b	9	22.6 (3.6) ab	0.037 (*)
E. rudis	4	14.2 (8.1)	4	15.6 (1.7)	4	15.9 (4.0)	0.901 (NS)
E. wandoo	7	21.1 (3.3) a	9	15.0 (3.5) b	6	15.9 (3.0) b	0.003 (**)
C. speciosus	7	16.0 (3.0)	6	17.8 (3.5)	10	11.2 (2.5)	0.100 (NS)
C. rupestris	10	25.2 (3.5) a	10	22.2 (3.7) ab	7	18.9 (6.8) b	0.041 (*)
M. hamulosa	1	30.0 (0) ab	9	12.0 (4.0) b	9	21.6 (10.2) a	0.023 (*)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Similar letters in rows indicate no significant differences, a indicates largest species

Table 2.3 Mean stem diameters and standard deviations of seedlings at 28 wks (cm)

Species	Control	Muja	Ewington	*P*
E. camaldulensis	4.72 (0.48)	3.18 (0.65)	4.00 (0.97)	0.077 (NS)
E. cladocalyx	4.33 (0.32)	3.88 (0.68)	3.76 (0.55)	0.263 (NS)
E. gomphocephala	4.17 (0.47) ab	3.64 (0.82) b	4.60 (0.66) a	0.027 (*)
E. grandis	5.33 (1.56)	4.21 (0.73)	4.56 (0.91)	0.157 (NS)
E. maculata	5.44 (1.23)	4.42 (0.89)	4.22 (1.03)	0.055 (NS)
E. resinifera	5.43 (1.22) a	3.71 (1.14) b	3.17 (0.62) b	<0.001 (***)
E. robusta	4.61 (0.71)	3.93 (0.37)	4.26 (0.53)	0.074 (NS)
E. rudis	5.72 (0.50) a	3.69 (0.77) b	4.13 (0.77) ab	0.041 (*)
E. wandoo	4.46 (0.70) a	3.48 (0.56) b	3.20 (0.62) b	0.002 (**)
C. speciosus	4.37 (2.5)	4.13 (0.58)	3.26 (0.28)	0.165 (NS)
C. rupestris	5.58 (1.48) a	3.99 (0.35) b	3.99 (1.30) b	0.008 (**)
M. hamulosa	3.80 (0) ab	2.17 (0.63) b	3.63 (0.66) a	0.001 (**)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Similar letters in rows indicate no significant differences, a indicates largest species
(n values same as in Table 2.2)

Analysis of dry weights of leaves, stems and roots produced results that were also similar to growth measurements (Tables 2.4 to 2.6). The leaf, stem or root weights of *E. cladocalyx, E. robusta* and *E. rudis* did not show any significant difference from the control plants, whereas all three dry weights of *M. hamulosa* plants grown in the Muja overburden were significantly lighter than plants grown in the Ewington soil. In contrast, *E. maculata, C. rupestris* and *C. speciosus* seedlings were not lighter when grown in the Muja spoil but were lighter when grown in the Ewington spoil. *E. maculata* plants growing in the Ewington overburden had significantly lighter foliage and stems than the control plants. *C. rupestris* plants grown in the Ewington spoil had significantly lighter foliage than both the Muja and control plants and lighter stems than the control plants. *C. speciosus* plants grown in the Ewington overburden had significantly lighter leaves, stems and roots to both the Muja and control plants.

Several species produced less dry weight when grown in the two acidic overburdens than in the control soil. Both overburden treatments depressed *E. grandis* foliage and roots weights compared with controls. *E. camaldulensis* plants were significantly lighter in Muja spoil than the paddock control for all three weights while the Ewington treatment plants were only significantly lighter in the leaf and stem weights. *E. gomphocephala* plants grown in both spoils had significantly lighter stems and roots. The grass *N. alopecuroidea* were significantly lighter in both leaf and root when grown in both spoils.

Other species were less obvious in variation of dry mass. *E. resinifera* plants grown in the Ewington spoil had significantly lighter leaves, stems and roots while the Muja treatment only had significantly lighter stems and roots than the control plants. Likewise, *E. wandoo* plants grown in the Ewington spoil had significantly lighter stems and roots while the Muja treatment only had significantly lighter stems than the control plants.

Table 2.4 Mean dry weights and standard deviations at 28 wks

Species	Control	Muja	Ewington	*P*
E. camaldulensis	10.40 (4.24) a	4.40 (1.58) b	5.62 (1.73) b	0.013 (*)
E. cladocalyx	7.48 (1.64)	7.43 (1.43)	7.42 (1.59)	0.998 (NS)
E. gomphocephala	12.37 (6.02) a	6.96 (2.02) b	7.73 (1.12) b	0.011 (*)
E. grandis	8.70 (1.83) a	5.80 (2.25) b	5.02 (1.00) b	0.003 (**)
E. maculata	12.16 (2.10) a	9.10 (2.74) b	7.38 (1.55) b	0.001 (**)
E. resinifera	8.55 (1.50) a	5.99 (2.52) b	5.30 (1.59) b	0.006 (**)
E. robusta	6.03 (1.54)	6.00 (1.20)	5.14 (1.05)	0.339 (NS)
E. rudis	6.47 (1.03)	7.00 (1.11)	7.58 (1.09)	0.441 (NS)
E. wandoo	6.86 (1.97) a	4.64 (1.98) b	3.11 (1.71) b	0.004 (**)
C. speciosus	2.20 (0.36) a	2.43 (0.34) a	0.95 (0.35) b	0.001 (**)
C. rupestris	3.24 (0.42) a	3.38 (1.03) a	1.67 (0.86) b	0.001 (**)
M. hamulosa	0.80 (0) ab	0.27 (0.17) b	0.81 (0.38) a	0.004 (**)
N. alopecuroidea	0.68 (0.38) a	0.16 (0.08) b	0.24 (0.14) b	0.024 (*)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Similar letters in rows indicate no significant differences, a indicates heaviest species
(n values same as in Table 2.2)

Most of the candidate species did not differ in trunk taper at the end of the experiment (Table 2.5). However, *E. resinifera* plants had significantly larger relative basal stem diameters while *C. speciosus* plants had significantly smaller tapers when grown in the Ewington soil. Therefore, $H_0$2 is accepted for *E. cladocalyx* and *E robusta*, and to a lesser extent, *E. camaldulensis*, *E. maculata* and *E. rudis* but is rejected for the other species. *E. gomphocephala* and *M. hamulosa* were significantly smaller in growth parameters when grown in Muja overburden, *C. speciosus* and *C. rupestris* were significantly smaller in Ewington overburden while *E. grandis*, *E. resinifera*, *E. wandoo* and *N. alopecuroidea* were significantly smaller in both overburdens.

Table 2.5: Mean height/stem diameter ratios and standard deviations at 28 wks

Species	Control	Muja	Ewington	*P*
E. camaldulensis	96.8 (8.4)	99.9 (16.9)	93.9 (6.8)	0.721 (NS)
E. cladocalyx	71.4 (9.2)	80.1 (11.4)	80.1 (11.9)	0.396 (NS)
E. gomphocephala	56.1 (7.2)	43.4 (6.7)	51.9 (6.4)	0.087 (NS)
E. grandis	90.4 (38.4)	71.2 (14.8)	87.5 (25.6)	0.295 (NS)
E. maculata	76.1 (26.7)	71.2 (15.4)	71.6 (18.6)	0.857 (NS)
E. resinifera	66.6 (20.5) b	75.9 (16.7) b	97.1 (11.2) a	0.003 (NS)
E. robusta	91.9 (21.3)	114.1 (10.1)	102.2 (20.5)	0.071 (NS)
E. rudis	82.1 (28.3)	104.9 (29.8)	103.0 (18.1)	0.214 (NS)
E. wandoo	77.0 (23.1)	76.9 (15.4)	70.9 (17.6)	0.797 (NS)
C. speciosus	75.2 (6.3) a	87.4 (10.0) a	53.9 (6.7) b	0.005 (**)
C. rupestris	54.7 (12.3)	55.1 (17.3)	43.0 (5.7)	0.190 (NS)
M. hamulosa	150.0 (0)	180.2 (28.1)	147.8 (39.6)	0.156 (NS)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Similar letters in rows indicate no significant differences, b indicates species with taper
(n values same as in Table 2.2)

Final mean heights of each *Eucalyptus* species varied within overburdens, but not in the control soil (Table 2.6). *E. robusta, E. rudis, E. gomphocephala* and *E. camaldulensis* were ranked first to fourth in descending height for both acidic spoils. *E. maculata* and *E. cladocalyx* were ranked near the middle in the Muja soil but among the shortest in the Ewington soil. In contrast, *E. grandis* and *E. resinifera* were ranked in the middle in the Ewington soil, but among the shortest in the Muja soil. *E. wandoo* was the shortest species for both spoils.

Table 2.6: Species ranked from tallest (top) to shortest (bottom) by mean hts at 28 wks

Control (cm)	Muja (cm)	Ewington (cm)
E. camaldulensis	*E. robusta* a	*E. robusta* a
E. grandis	*E. rudis* a	*E. rudis* a
E. robusta	*E. gomphocephala* b	*E. gomphocephala* a
E. rudis	*E. camaldulensis* b	*E. camaldulensis* ab
E. gomphocephala	*E. maculata* b	*E. grandis* a
E. maculata	*E. cladocalyx* b	*E. resinifera* bc
E. resinifera	*E. grandis* b	*E. cladocalyx* c
E. wandoo	*E. resinifera* b	*E. maculata* cd
E. cladocalyx	*E. wandoo* b	*E. wandoo* d
0.404 (NS)	<0.001 (***)	<0.001 (***)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Similar letters in columns indicate no significant differences, a indicates tallest species

There was a generally higher mortality rate in seedlings grown in the control soil than in the overburden soils (Table 2.7). Just over a third of the seedlings died in the control soil, whereas less than one fifth of the seedlings died in either overburden. *M. hamulosa*, and to a lesser extent, *E. cladocalyx, E. gomphocephala, E. grandis, E. resinifera* and *C. speciosus,* lost more seedlings in the control soil than in the overburden material. Only *N. alopecuroidea* had more mortalities in both acidic overburdens, and *E. wandoo* and *C. rupestris* in the Ewington overburden than the control.

Table 2.7: Mortality of seedlings in soils grown over 28 weeks

Species	control	Muja	Ewington
E. camaldulensis	2/5	0/6	0/6
E. cladocalyx	4/10	1/10	0/10
E. gomphocephala	3/10	0/10	0/10
E. grandis	3/10	1/10	1/10
E. maculata	2/10	0/10	1/10
E. resinifera	3/10	0/10	1/10
E. robusta	1/10	1/10	1/10
E. rudis	1/5	0/4	0/4
E. wandoo	3/10	1/10	4/10
C. speciosus	3/10	4/10	0/10
C. rupestris	0/10	0/10	3/10
M. hamulosa	9/10	1/10	1/10
N. alopecuroidea	7/10	9/10	8/10
Mortality	41/120 (34.2%)	18/120 (15.0%)	20/120 (16.7%)

Chemical analyses of plant leaves, stems and roots

E. gomphocephala performed poorly in both overburden materials. Plants grown in the Ewington soil had significantly less leaf iron and stem magnesium and sulfur (Table 2.8). Plants from both overburdens had significantly less stem calcium and chlorine. There were no significant differences in levels of all other elements tested (Appendix 6).

Table 2.8: Levels of nutrients in *E. gomphocephala* at 28 weeks (n=2)

Nutrient	control	Muja	Ewington	F	*P*
Leaf					
Fe (ppm)	37.7 (1.2) a	34.8 (1.2) a	23.5 (2.1) b	11.04	0.041 (*)
Stem					
Ca (%)	0.74 (0.05) a	0.28 (0.05) b	0.23 (0.01) b	91.90	0.002 (**)
Mg (%)	0.30 (0.01) a	0.25 (0.04) ab	0.16 (0.03) b	11.08	0.041 (*)
Cl (%)	0.92 (0.08) a	0.39 (0.08) b	0.56 (0.01) b	36.12	0.008 (**)
S (%)	0.09 (0.01) b	0.30 (0.08) a	0.08 (0.01) b	13.99	0.030 (*)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
different letters in a row indicate significant differences between sites

E. grandis performed poorly in the Ewington material. Plants were found to have significantly lower levels of magnesium in the stems and calcium in the stems but more iron in the leaves and stems and copper in the stems (Table 2.9). There were no significant differences in levels of all other elements tested (Appendix 6).

Table 2.9: Levels of nutrients in *E. grandis* at 28 weeks (n=2)

Nutrient	control	Ewington	F	*P*
Leaf				
Fe (ppm)	30.25 (0.07)	52.65 (3.46)	83.56	0.012 (*)
Stem				
Ca (%)	0.45 (0.04)	1.11 (0.01)	493.22	0.002 (**)
Mg (%)	0.29 (0.01)	0.19 (0.01)	150.19	0.007 (**)
Cu (ppm)	1.90 (0.28)	3.05 (0.21)	21.16	0.044 (*)
Fe (ppm)	44.4 (7.1)	80.8 (2.6)	45.56	0.021 (*)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

E. wandoo performed poorly in both overburden materials. Plants indicated significantly lower levels of leaf iron and root zinc and sulphur when grown in either overburden material (Table 2.10). Plants grown in the Muja material also were lower in leaf potassium, stem zinc and sulphur. There were no significant differences in levels of all other elements tested (Appendix 6).

Table 2.10: Levels of nutrients in *E. wandoo* at 28 weeks (n=2)

Nutrient	control	Muja	Ewington	F	*P*
Leaf					
K (%)	0.86 (0.09) a	0.53 (0.09) b	0.70 (0.03) ab	9.74	0.049 (*)
Fe (ppm)	65.2 (8.4) a	36.4 (4.5) b	27.9 (4.7) b	20.26	0.018 (*)
Stem					
Zn (ppm)	55.0 (5.6) a	17.7 (1.4) b	41.2 (1.6) ab	58.21	0.004 (**)
S (%)	0.28 (0.11) a	0.18 (0.07) b	0.63 (0.08) a	14.56	0.029 (*)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
different letters in a row indicate significant differences between sites

Calothamnus rupestris performed poorly in Ewington overburden. Plants were very low in many nutrients in the leaves and had significantly more leaf iron and copper in all plant parts (Table 2.11). There were no significant differences in levels of all other elements tested (Appendix 6).

Table 2.11: Levels of K and Na in *C. rupestris* at 28 weeks (n=2)

Nutrient	control	Ewington	F	*P*
Leaf				
K (%)	1.05 (0.03)	0.61 (0.01)	522.35	0.002 (**)
Ca (%)	0.60 (0.01)	0.10 (0.01)	1826.31	0.001 (**)
Mg (%)	0.32 (0.01)	0.21 (0.01)	37.73	0.025 (*)
Na (%)	0.80 (0.01)	0.21 (0.01)	5965.20	<0.001 (***)
Cl (%)	2.69 (0.08)	0.28 (0.01)	2021.42	<0.001 (***)
Cu (ppm)	2.40 (0.14)	3.85 (0.07)	168.20	0.006 (**)
Zn (ppm)	19.2 (0.28)	8.8 (0.4)	832.00	0.001 (**)
Fe (ppm)	26.55 (9.55)	59.65 (0.64)	23.94	0.039 (*)
Stem				
Cu (ppm)	1.75 (0.50)	7.85 (1.91)	19.13	0.048 (*)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Judd, Attiwell & Sands (1996) calculated a range of 6 essential nutrients within foliage of members of the *Symphomyrtus* subgenus growing in native forests from over 500 references. Comparisons with the range of foliage levels of the three tested species (all members of *Symphomyrtus*) indicated lower levels of several nutrients when grown in all 3 soil types but were excessive in other nutrients (Table 2.12). Foliage from *E.*

gomphocephala seedlings were lower in N and Ca in all 3 soils, were lower in K in control and Ewington soils, lower in Mg in control and Muja soils and lower in Mn in the Muja soil. However, plants in the Ewington soil were higher in P. Foliage from *E. grandis* seedlings was lower in N and K in both soils and lower in Mn in Ewington soils, but also higher in P in both soils. Foliage from *E. wandoo* plants from all 3 soils was lower in Mg and Mn, lower in Ca in both overburden soils and lower in N in the Ewington soil. However, control plants had higher levels of P and K and Ewington plants had higher levels of P.

Table 2.12: Mean foliar concentrations and SD of essential nutrients of *Symphyomyrtus* species (Taken from Judd, Attiwell & Adams 1996) compared to three tested eucalypt species

species	n	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)
Symphomyrtus		1.21 (0.04)	0.069 (0.003)	0.71 (0.03)	1.00 (0.07)	0.26 (0.01)	610 (70)
E. gomphocephala							
Control	2	0.89 (0.05) ↓	0.08 (0.01) ✓	0.64 (0.03) ↓	0.67 (0.02) ↓	0.21 (0.02) ↓	482 (84) ✓
Muja	2	0.98 (0.10) ↓	0.08 (0.01) ✓	0.57 (0.16) ✓	0.57 (0.12) ↓	0.20 (0.03) ↓	445 (73) ↓
Ewington	2	1.08 (0.01) ↓	0.09 (0.01) ↑	0.52 (0.01) ↓	0.55 (0.08) ↓	0.23 (0.05) ✓	514 (48) ✓
E. grandis							
Control	2	0.51 (0.05) ↓	0.31 (0.11) ↑	0.45 (0.21) ↓	0.89 (0.12) ✓	0.27 (0.10) ✓	451 (130) ✓
Ewington	2	0.51 (0.01) ↓	0.23 (0.02) ↑	0.43 (0.04) ↓	1.02 (0.08) ✓	0.23 (0.03) ✓	198 (5) ↓
E. wandoo							
Control	2	1.30 (0.14) ✓	0.10 (0.01) ↑	0.86 (0.09) ↑	0.63 (0.06) ✓	0.18 (0.01) ↓	325 (44) ↓
Muja	2	1.11 (0.09) ✓	0.08 (0.02) ✓	0.53 (0.09) ✓	0.55 (0.03) ↓	0.18 (0.04) ↓	253 (56) ↓
Ewington	2	1.05 ↓	0.14 (0.05) ↑	0.70 (0.03) ✓	0.46 (0.06) ↓	0.24↓	359 (26) ↓

↓ = below 1st SD; ✓ = within 1st SD; ↑ = above 1st SD

Experiment 2 - Field trial

Soil properties

Soil acidity varied between the two sites (Table 2.13). The Muja site was constantly more acidic than Ewington in both summer and winter. The Ewington site was wetter than Muja in summer but winter rains increased soil moisture at Muja. Conductivity levels were relatively constant at both sites and are considered too low to affect plant growth. Thus H_o3 is rejected: acidic coal overburden sites do differ in soil pH and moisture.

Table 2.13: Mean and SD of summer and winter soils (5 to 15 cm depth) of spoils

Time of Year	n	Mean	SD	*F*	P
pH					
December 1998 (summer)					
Muja	12	3.51	0.27	11.08	0.003 (**)
Ewington	12	3.80	0.12		
July 1999 (winter)					
Muja	12	3.45	0.3	40.8	<0.001 (***)
Ewington	12	4.28	0.3		
Moisture (%)					
December 1998 (summer)					
Muja		4.2	1.4	22.0	<0.001 (***)
Ewington		11.0	4.8		
July 1999 (winter)					
Muja		11.1	2.3	2.98	0.098 (NS)
Ewington		13.7	4.8		
Conductivity ($\mu\Omega s^{-1}$)					
December 1998 (summer)					
Muja		20.5	8.2	1.25	0.276 (NS)
Ewington		17.3	5.7		
July 1999 (winter)					
Muja		22.0	7.8	2.76	0.111 (NS)
Ewington		14.8	12.6		

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Water potential

In March 1999, all tested species at Muja had significantly less water potential than at Ewington or the controls before dawn ($P < 0.001$ for all) (Figure 2.4). At midday, some species also indicated stress at Ewington. *E. cladocalyx* and *E. maculata* plants at Muja had significantly higher water potentials than those at Ewington and the controls (P = 0.015 and 0.001 respectively). *E. camaldulensis* plants at both overburden sites had significantly lower water potentials than the controls (P = 0.033). *E. rudis* plants at Ewington had significantly higher water potentials than the controls and Muja (P = 0.015 and 0.028 respectively). *E. robusta* and *E. wandoo* did not differ significantly between sites.

Measurements at August 1999 of plants at both overburden sites did not indicate any significant differences in water potential from the control at both pre-dawn and midday (Figures 2.5). The soil moisture content at both overburden sites was thought to be adequate for the plants despite evapotranspiration during the winter months (Table 2.15). Seedlings grown on acidic spoils do not significantly differ in water potentials over time.

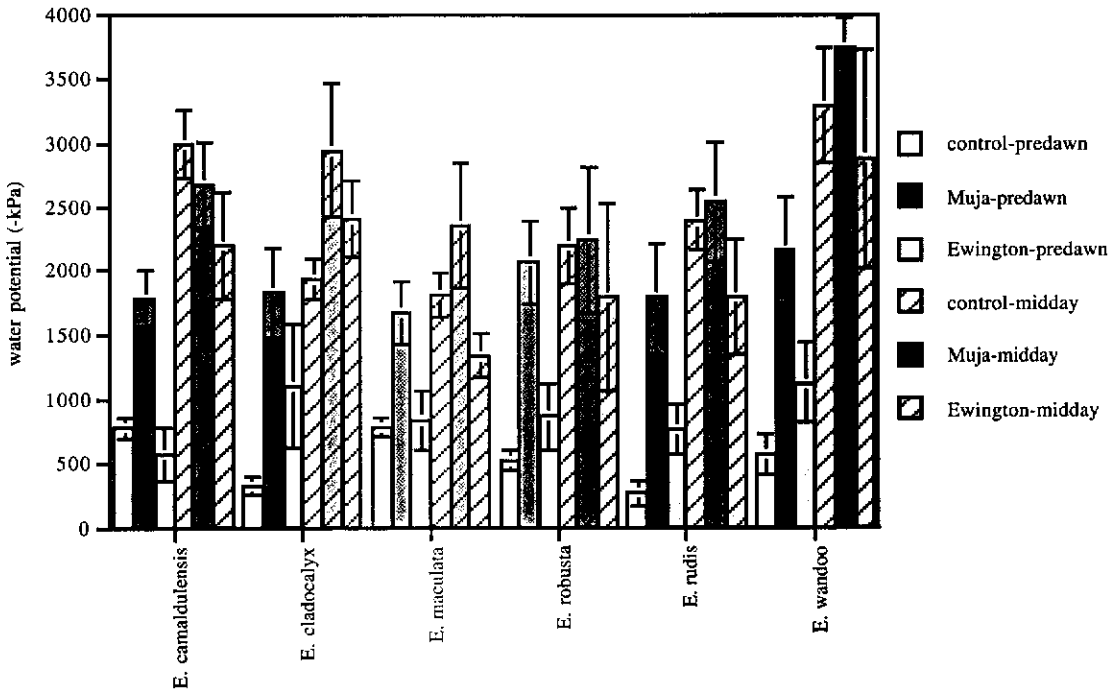

Figure 2.4: Mean water potential and SE (kPa) of plants in Collie spoils March 1999 (n=3)

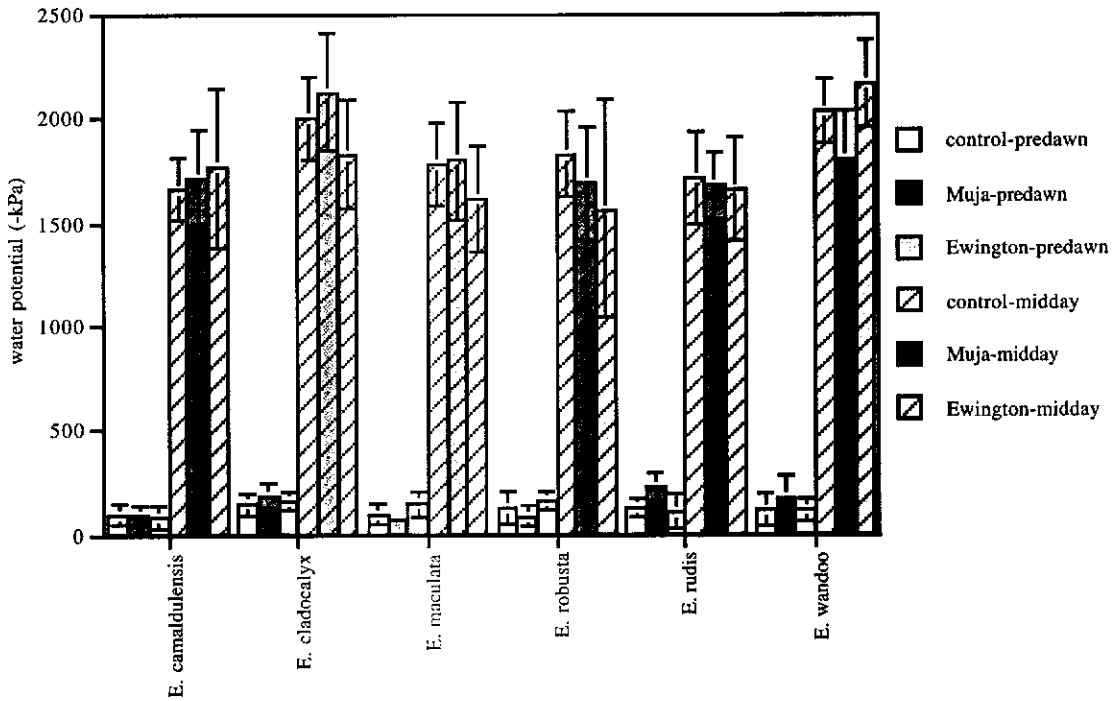

Figure 2.5: Mean water potential and SE (kPa) of plants in Collie spoils August 1999 (n=3)

Seedling survival and growth

By the end of the experimental period, (24 months), survival was variable at both sites. Muja had a higher mortality (20.0%) while that at Ewington was relatively low (10.8%) (Table 2.14). The grass *N. alopecuroidea* had completely died out at both sites during the first 12 months. *C. speciosus* lost half of its seedlings at Muja and one seedling at Ewington. *M. hamulosa* lost three seedlings at Muja and one at Ewington. *E. grandis E. maculata, E. resinifera* and *C. rupestris* lost one or two seedlings each at Muja but none at Ewington.

Table 2.14: Mortalities of seedlings grown in acidic coal overburdens for 24 months

Species	Muja	Ewington
E. camaldulensis	0/5	0/5
E. cladocalyx	0/10	0/10
E. gomphocephala	0/10	0/10
E. grandis	1/10	0/10
E. maculata	2/10	0/10
E. resinifera	1/10	0/10
E. robusta	0/10	0/10
E. rudis	0/10	0/5
E. wandoo	0/10	1/10
C. speciosus	5/10	1/10
C. rupestris	2/10	0/10
M. hamulosa	3/10	1/10
N. alopecuroidea	10/10	10/10
Mortality	24/120 (20.0%)	13/120 (10.8%)

Mean heights, crown diameters and basal stem diameters of planted individuals over the first year (June 1997 to June 1998) indicated that over half the species grew faster at the Ewington site than at the Muja site (Figure 2.7 to 2.12). *E. camaldulensis, E. cladocalyx* and *E. robusta* did not vary significantly in height, crown or stem diameter between the two overburden sites. *E. gomphocephala,* and *Calothamnus rupestris* did not vary in height or crown diameters between the two overburden sites but had significantly greater stem diameters at Ewington ($P = 0.042$ and 0.032 respectively). *E. grandis, E. maculata, E. resinifera, E. rudis, E. wandoo, Callistemon speciosus* and *Melaleuca hamulosa* plants

had grown significantly taller in heights (P = <0.001, 0.007, 0.017, 0.044, 0.013, 0.005 and 0.006 respectively), crown diameters (P = <0.001, 0.024, 0.010, 0.011, <0.001, 0.015, and 0.010 respectively) and stem diameters (P = <0.001, 0.006, 0.013, 0.018, <0.001, <0.001, and <0.001 respectively) at Ewington than at Muja. No species were larger at the Muja site.

During the next 12 months (June 1998 to June 1999), many species at Ewington exhibited reduced growth while those at Muja continued to grow steadily. *E. camaldulensis, E. gomphocephala, E. grandis, E. robusta, E. rudis* and *C. rupestris* did not vary significantly in height and crown and stem diameters between the two sites. *E. cladocalyx* had grown significantly taller at the Muja site (P = 0.003) but did not vary in crown or stem diameters between sites. *E. maculata, E. resinifera* and *C. speciosus* seedlings at the Ewington site were significantly taller (P = 0.002, 0.004 and 0.027 respectively) and had significantly larger stem diameters (P = <0.001, 0.004, 0.037 respectively). *E. wandoo* no longer significantly varied in height but still had significantly larger crown and stem diameters at Ewington (P = 0.026 and 0.007 respectively). *M. hamulosa* also no longer significantly varied in height and crown stem diameters but still had significantly larger stem diameters at the Ewington site (P = 0.002).

Several species varied in stem volumes during the experimental period. *E. maculata* and *E. wandoo* had significantly larger stem volumes at Ewington than at Muja in both September 1998 (P = 0.013 and <0.001 respectively) (Figure 2.13) and October 1999 (P = 0.001 and 0.003 respectively) (Figure 2.14). *E. grandis* seedlings had significantly larger stem volumes at Ewington in September 1998 (P <0.001) and *E. resinifera* was also significantly larger at Ewington in October 1999 (P = 0.004). *E. cladocalyx* seedlings had significantly larger volumes at Muja (P = 0.044). Therefore for six species H_o4 is rejected: *E. maculata, E. resinifera, E. wandoo, E. speciosus* and *M. hamulosa* survive less and are significantly smaller when grown at Muja than at Ewington while *E. cladocalyx* grows less at Ewington than at Muja. For the remaining six species, there is no significant difference in growth between Muja and Ewington.

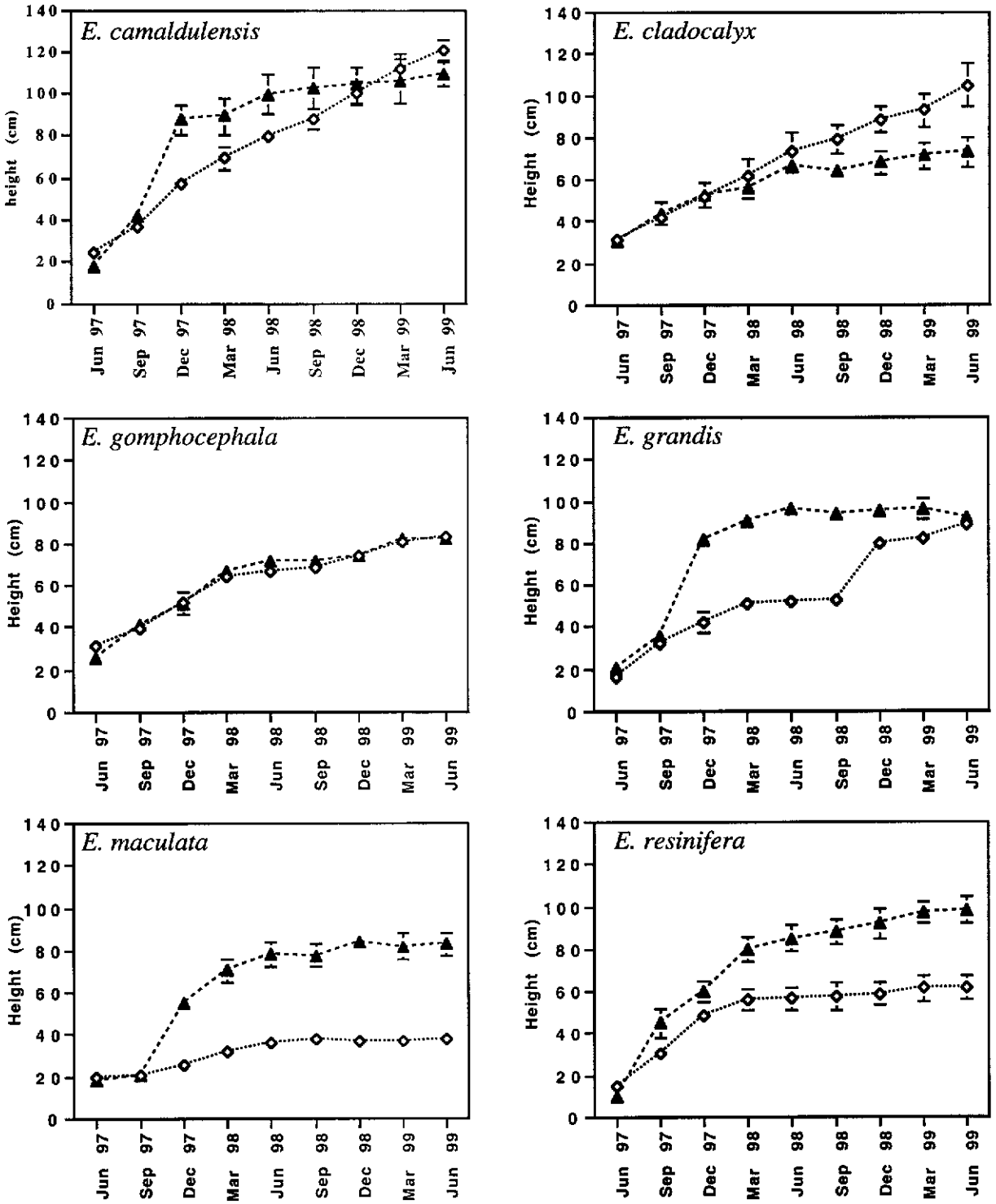

Figure 2.7: Mean heights (cm) and standard errors of *E. camaldulensis, E. cladocalyx, E. gomphocephala, E. grandis, E. maculata* and *E. resinifera* plants growing on acidic sites (◇Muja, ▲ Ewington)

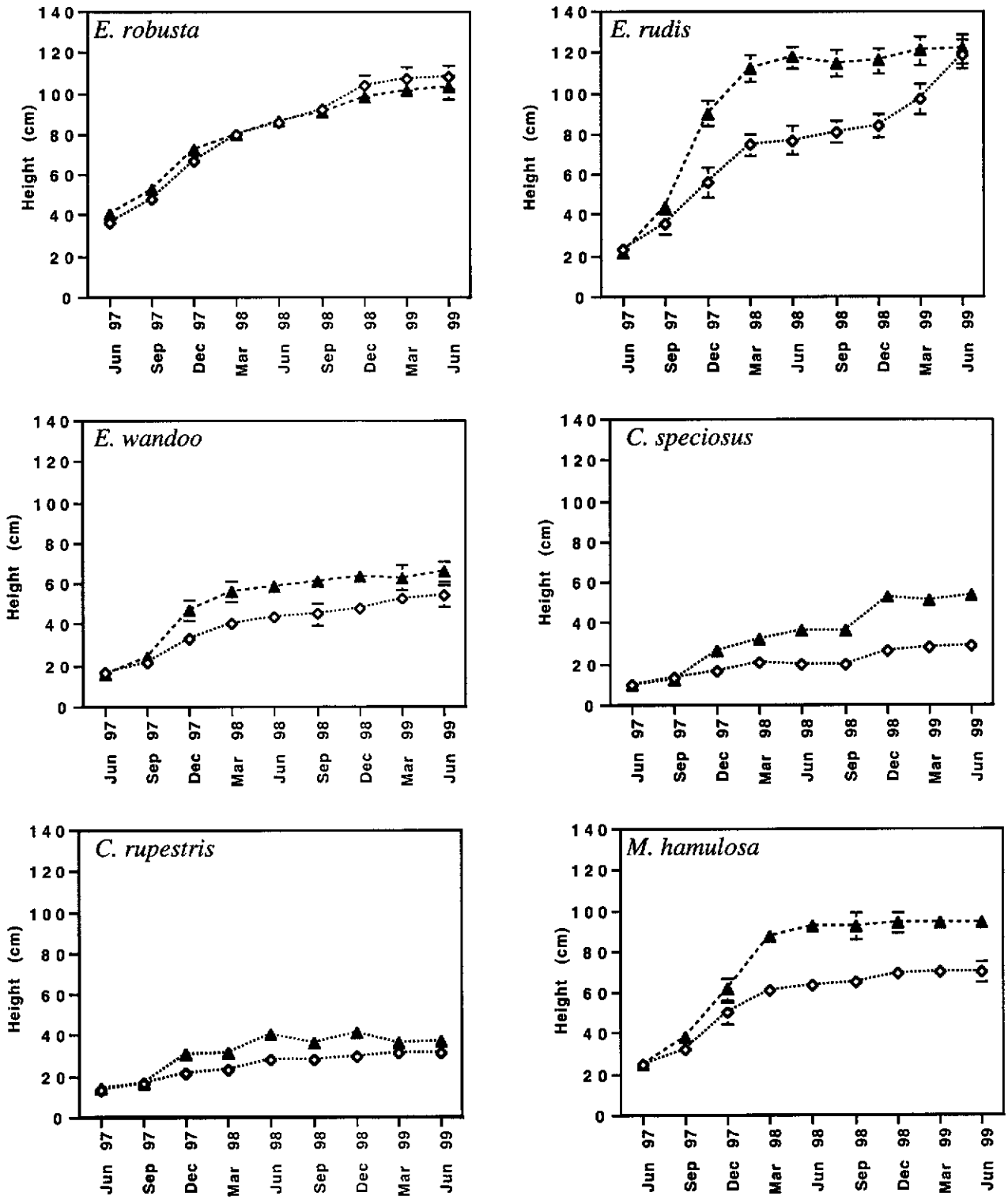

Figure 2.8: Mean heights (cm) and standard errors of *E. robusta, E. rudis, E. wandoo, C. speciosus, C. rupestris* and *M. hamulosa* plants growing on acidic sites (◇Muja, ▲ Ewington)

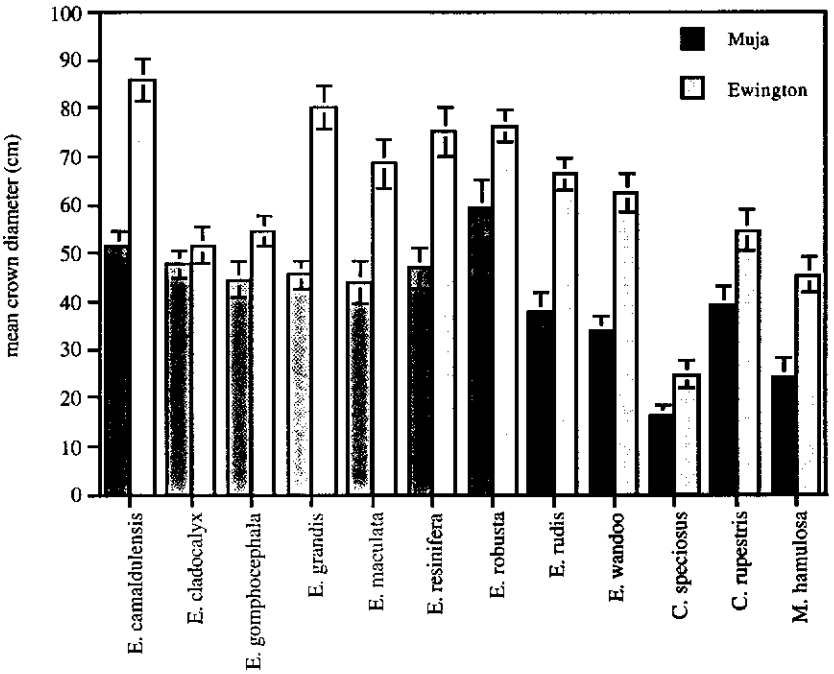

Figure 2.9: Mean crown diameters (cm) and standard errors of planted species in Collie overburdens September 1998

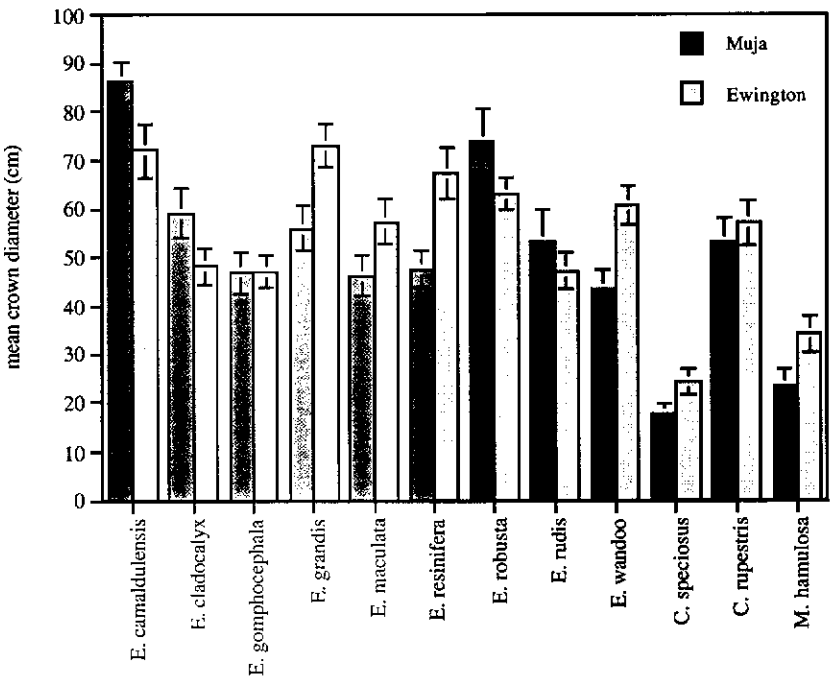

Figure 2.10: Mean crown diameters (cm) and standard errors of planted species in Collie overburdens October 1999

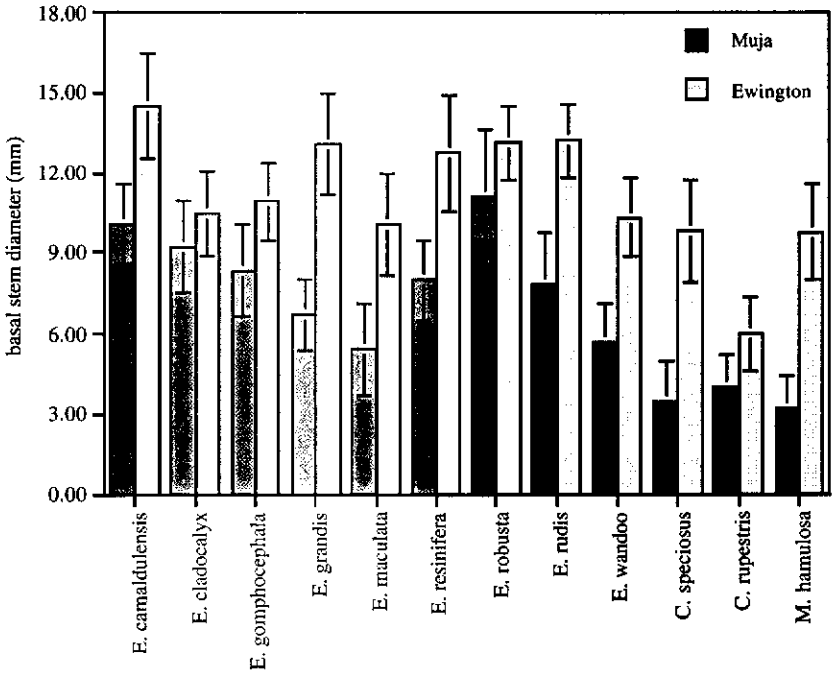

Figure 2.11: Mean basal stem diameters (mm) and standard errors of planted species in Collie overburdens September 1998

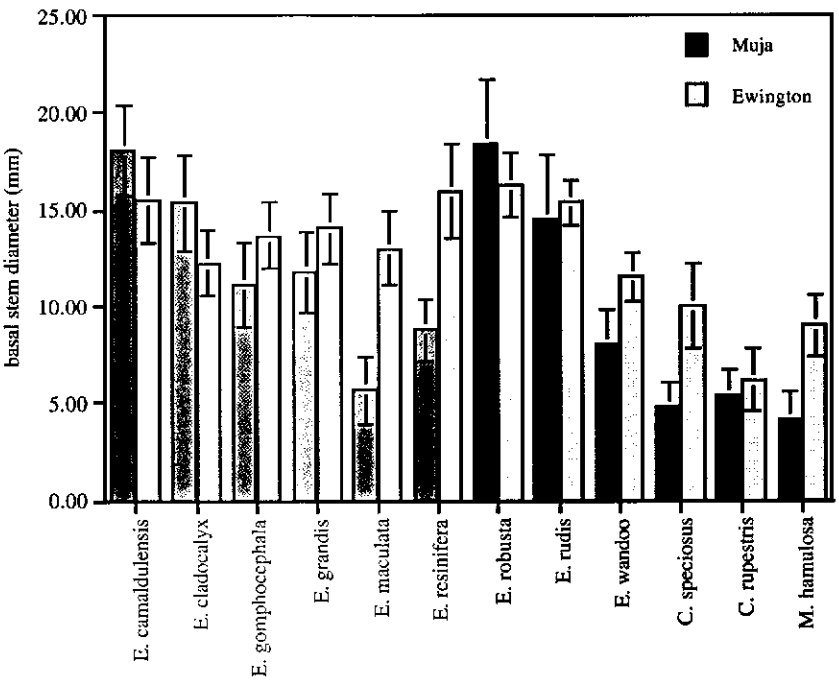

Figure 2.12: Mean basal stem diameters (mm) and standard errors of planted species in Collie overburdens October 1999

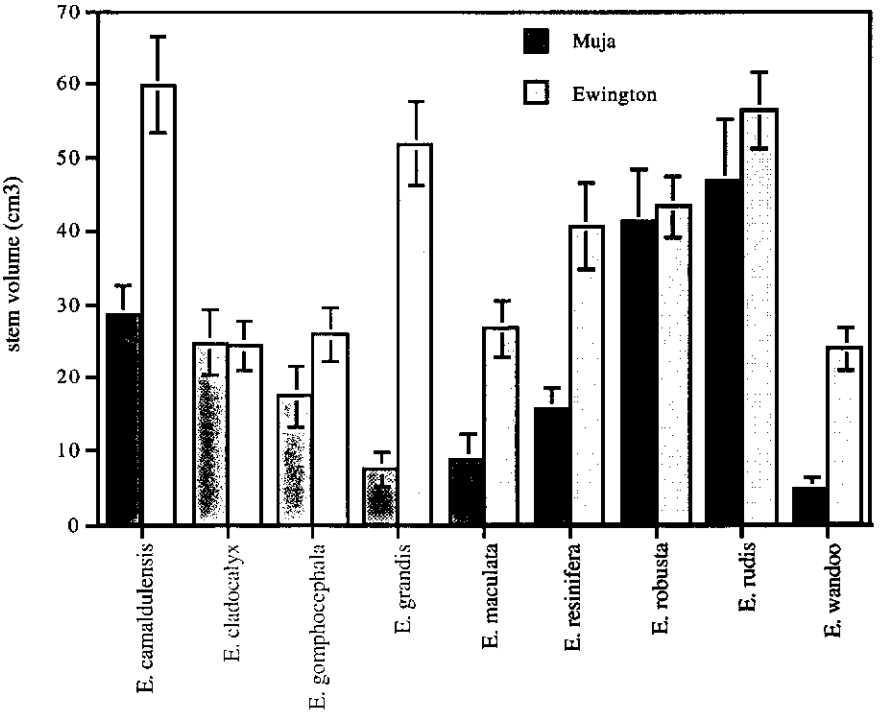

Figure 2.13: Extracted mean stem volumes (cm^3) and standard errors of planted eucalypt species in Collie overburdens September 1998

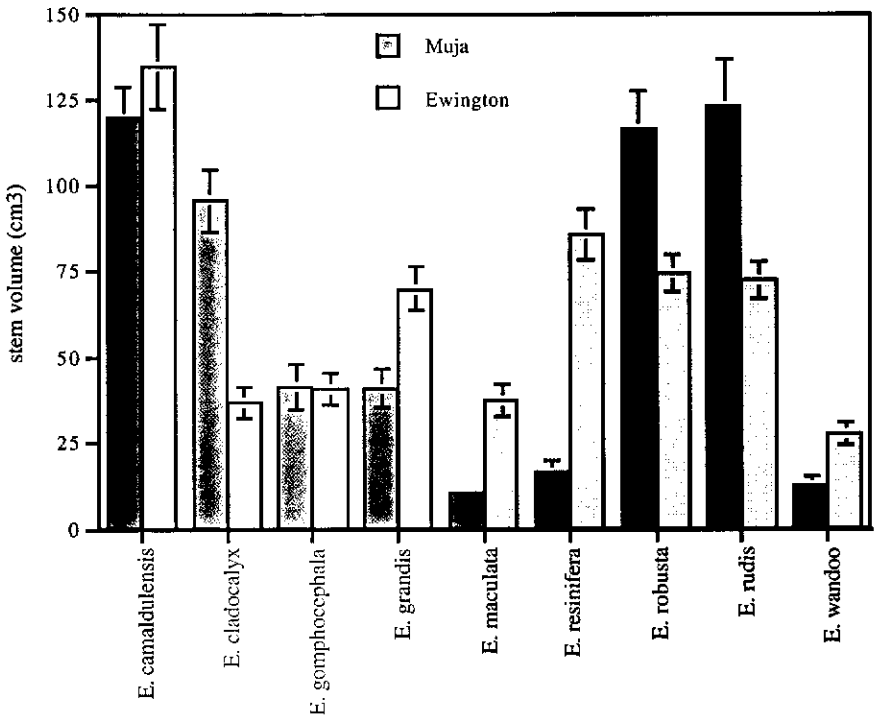

Figure 2.14: Extracted mean stem volumes (cm^3) and standard errors of planted eucalypt species in Collie overburdens October 1999

Stem taper

Most eucalypt species did not differ significantly in height/basal stem diameter ratios between sites in June 1998 (Figure 2.15). *E. cladocalyx, E. gomphocephala* and *E. wandoo* plants at Muja had significantly lower ratios at Ewington than at Muja (P = 0.003, 0.042 and 0.040 respectively). June 1999 data gave similar results (Figure 2.16). *E. gomphocephala* and *E. wandoo* seedlings still had significantly smaller ratios at Ewington (P = 0.0011 and 0.038 respectively) but *E. cladocalyx*, along with the other eucalypt species, did not vary between sites. Thus $H_0$5 is rejected: *E. gomphocephala* and *E. wandoo* seedlings indicated possible aluminium sequestration by developing tapered growth on Ewington overburden.

Final heights

A comparison of final *Eucalyptus* seedling heights indicated highly significant differences between species for each site (P < 0.001 for both; Table 2.15). *E. camaldulensis, E. robusta,* and *E. rudis* were the tallest species at both sites. *E. cladocalyx* and *E. resinifera* were the tallest species at Ewington but the shortest at Muja. *E. grandis* was one of the taller species at Muja but one of the shortest at Ewington. *E. gomphocephala* was ranked near the middle for both sites. Both *E. maculata* and *E. wandoo* were the shortest at both sites.

Table 2.15: Species ranked from tallest (top) to shortest (bottom) by final mean heights

Muja	Ewington
E. camaldulensis a	*E. rudis* a
E. rudis a	*E. camaldulensis* ab
E. cladocalyx a	*E. robusta* abc
E. robusta ab	*E. resinifera* abcd
E. grandis abc	*E. grandis* bcde
E. gomphocephala bc	*E. gomphocephala* cdef
E. resinifera cd	*E. maculata* def
E. wandoo d	*E. cladocalyx* ef
E. maculata d	*E. wandoo* f
<0.001 (***)	<0.001 (***)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Similar letters in columns indicate no significant differences, a indicates tallest species

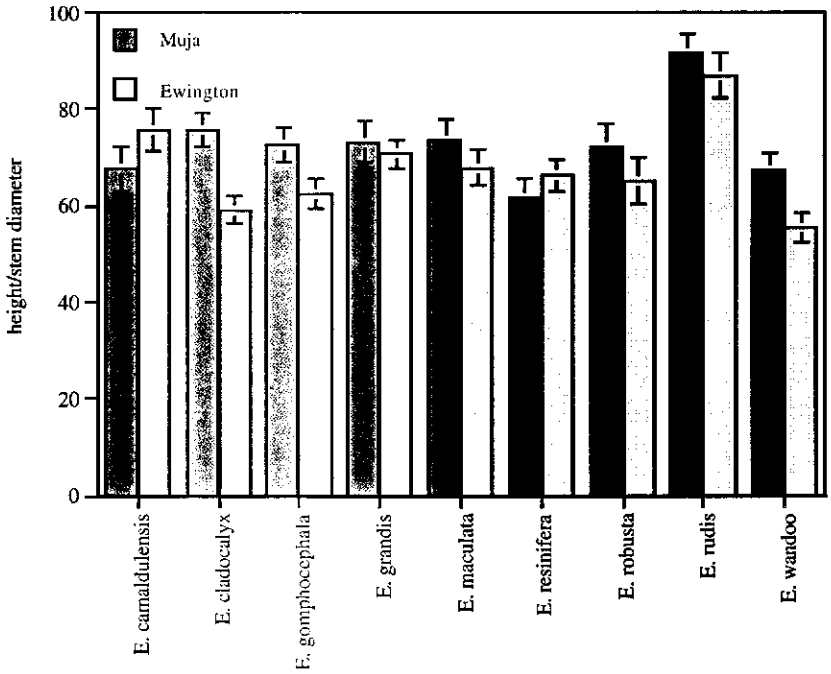

Figure 2.15: Mean height/stem diameter ratios and standard errors of plant eucalypt species in Collie overburdens June 1998

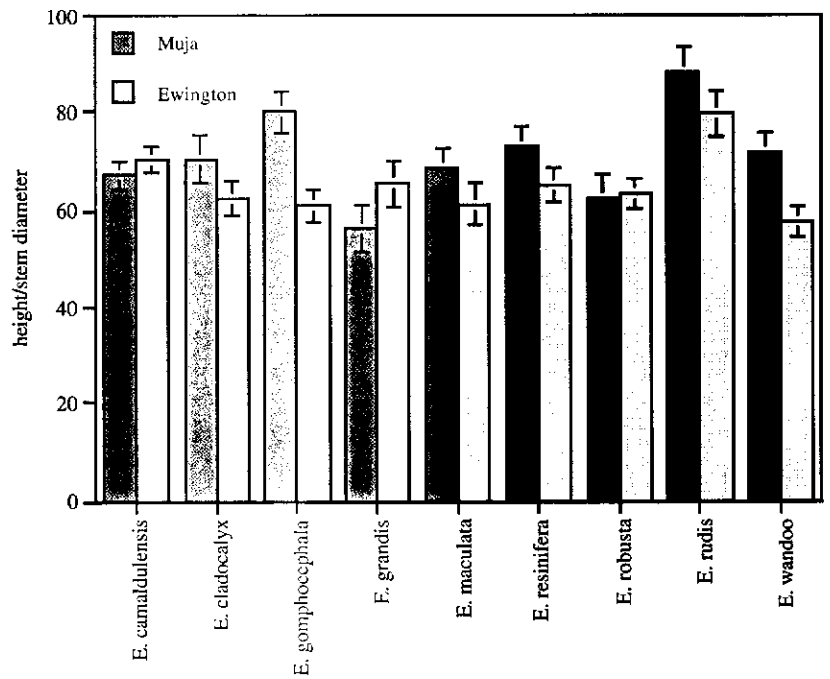

Figure 2.16: Mean height/stem diameter ratios and standard errors of plant eucalypt species in Collie overburdens June 1999

Relative growth rate

For both sites, there was much variation between the eucalypt species in relative growth rate (RGR) during the first 12 months (Figures 2.17 and 2.18). At Muja, *E. camaldulensis*, *E. grandis*, *E. resinifera*, *E. rudis* and *E. wandoo* all had significantly higher RGRs than the other species ($P = 0.003$). The species ranking at Ewington was similar; *E. camaldulensis*, *E. grandis*, *E. maculata*, *E. resinifera* and *E. rudis* were all significantly greater in RGR than the other species ($P < 0.000$). RGR was generally higher at the Ewington site than at the Muja site.

However, the RGRs of all species decreased greatly and did not significantly vary within either site during the following 12 months ($P = 0.140$ and 0.141 for Muja and Ewington respectively). The decrease in RGR was more pronounced at Ewington; none of the species had RGR values above 0.08.

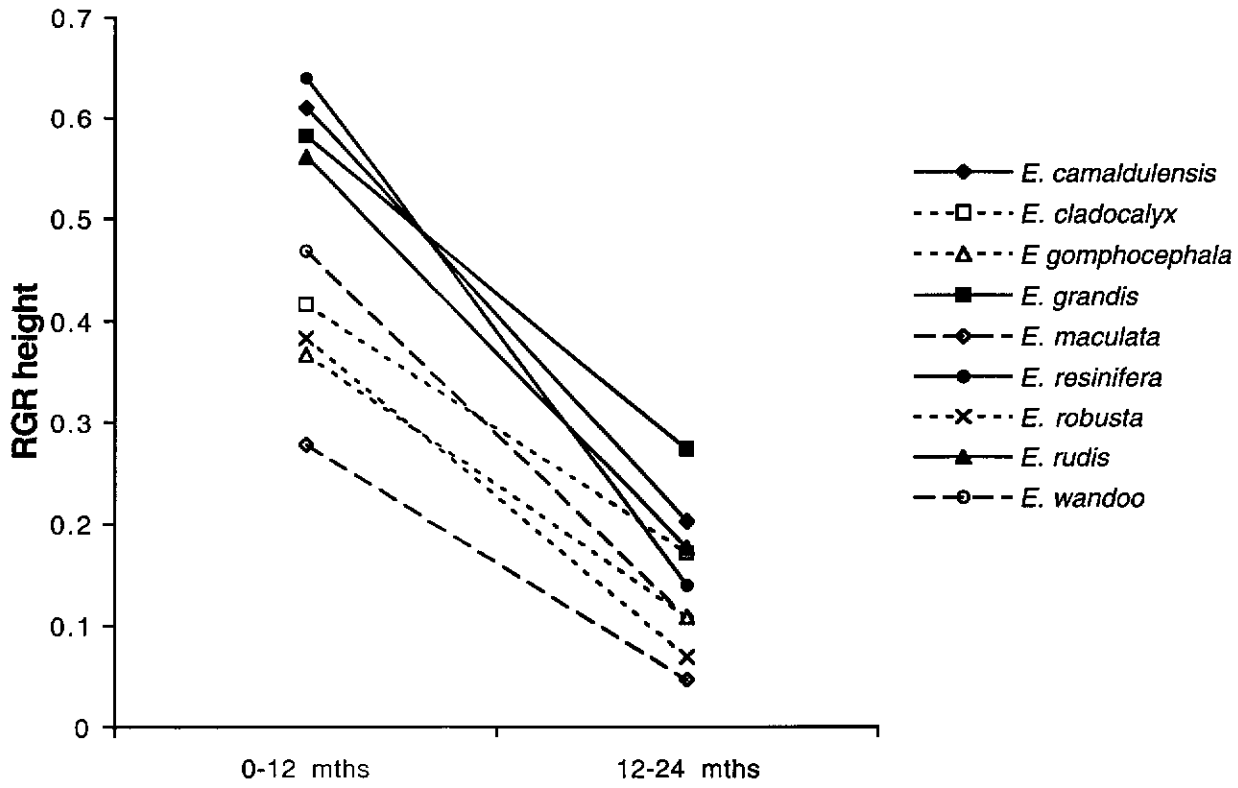

Figure 2.17: Annual RGR of eucalypt species grown at Muja over 24 months

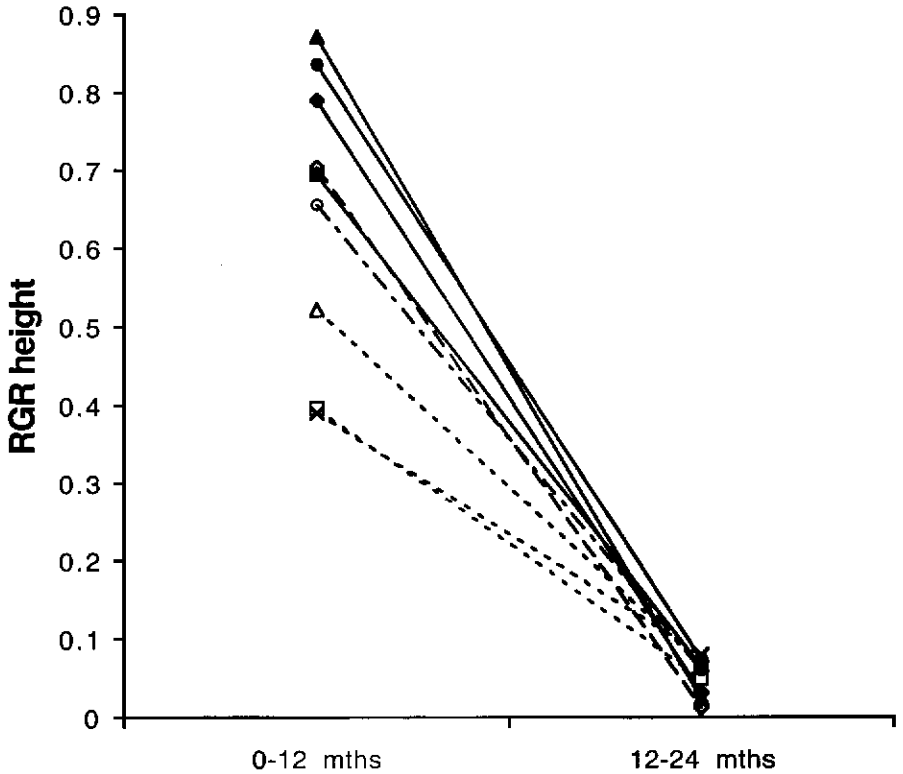

Figure 2.18: Annual RGR of eucalypt species grown at Ewington over 24 months

Discussion

The most important chemical properties that separated overburden spoil materials from the control paddock soil were acidity, low levels of nutrients and high levels of heavy metals. Growth responses of the candidate species would have been influenced by each species' tolerance or sensitivity to these hostile factors. All three materials generally had very low levels of nutrients with each of nitrogen, phosphorus and potassium levels all below marginal levels required for wheat in south-western Australia (Peverill, Sparrow & Reuter 1999). The foliage from the three poorest performing *Eucalyptus* species appeared deficient in many nutrients, particularly nitrogen, regardless of soil type. It is likely that all the candidate species grew poorly due to the low nutrient levels in all three soils tested. This suggests that differences in growth could have been detected earlier if a more fertile control soil had been used. Further studies should involve testing the soil for cation exchange capacity to indicate uptake potential of nutrients such as potassium, magnesium, calcium and sodium to further examine the true nutrient status of these soils.

Soil moisture is considered to be an additional factor in mortality and growth rates in both trials. Plants in the pot trial did not differ in the amount of supplied water between treatments. However, plants in the control soil quickly grew larger than those in the acidic spoils and had become rootbound in the small pots. During the final few months of the trial (November to December), the control plants may have transpired much of the soil moisture and suffered water stress. This may have caused the high fatalities amongst the control plants. Larger pots (> 2 L) should be used in future experiments.

Plants in the field trial appeared to be vulnerable to soil moisture deficit, particularly in summer and especially at the Muja site. The rainfall provided enough water to prevent water stress in winter. However, these findings are preliminary and further work is required on turgor loss point, water table levels, rainfall and relative humidity to properly determine the nature of the summer conditions affecting the studied species.

Plants at the Ewington site grew faster in the first year presumably due to the significantly higher available soil moisture in summer. However, growth slowed as the limited fertiliser supplied was depleted. Plants at Muja grew at a slower rate over the first year where soil moisture was much lower in summer. The slower rate of growth allowed the plants to continue growing into the second year, as the fertiliser supply may have lasted longer. The use of fertilisers on acidic overburdens is further examined in Chapters 4 to 6.

E. robusta did not differ in any physical and physiological measurements and indicated fast growth in both the pot trial and field trials. Koch (1984) reports that *E. robusta* grows well when planted on overburden sites (pH 4.6). *Eucalyptus robusta* was a successful species in acid sulphate soil trials in New South Wales (de Jong 2000). These experiments suggest that *E. robusta* is the most tolerant, and appropriate species, for growing on acidic coal overburdens in Collie.

E. camaldulensis showed fast growth and some tolerance to both overburdens and also did not significantly vary in any physical or physiological measurements between the acidic sites. Koch (1984) reports that seedlings of this species suffers no mortalities and is relatively tolerant and fast growing in acidic overburdens. However, Fox, O'Dea & Patroni (1985) reports that *E. camaldulensis* seedlings suffered high mortality and grew less in acidic overburdens of pH 3.9 than in ameliorated overburdens with pH higher than 5.3. It is possible that the ameliorated overburden material used by Fox *et al.* (1985) provided better growth conditions than the paddock soil used in this experiment. The overburden used could also have been more hostile to plant growth than the materials used in this experiment. *E. camaldulensis* is also considered to be a tolerant and appropriate species for growing on acidic coal overburdens in Collie.

E. cladocalyx indicated fast growth and some tolerance to both overburdens in both trials. In the field trial, this species exhibited taper differences at 12 months, but this was lost by 24 months. Leggate (1980) and Koch (1984) also report that *E. cladocalyx* grows

moderately well when planted in overburden soils. This species is also considered appropriate for revegetating acidic soils.

Although *E. rudis* did not differ in growth in the pot trial between soil types, it differed in response in the field trial. This species appears to have suffered moisture stress to have grown less at the Muja site than at the Ewington site. *E. rudis* seedlings have been previously reported as acid tolerant when grown in pots containing acidic coal materials (Fox & Doronila 1992) and when planted on acidic sites (Koch 1984). The species is believed to be tolerant of acidic overburden materials, provided there is adequate soil moisture.

The high mortality of control *Melaleuca hamulosa* seedlings in the pot trial allowed only crude comparisons be made with those in the overburden materials. However, the species still exhibited the same suppression in growth in the Muja overburden and good growth in Ewington material as in the field trial. This species may only be appropriate for revegetating the less acidic and moister sites similar to Ewington.

E. grandis, E. gomphocephala and *E. resinifera* indicated some reduced growth in both overburden materials. In the pot trial, *E. grandis* is believed to have suffered calcium and magnesium deficiency and possible copper and iron toxicity in the Ewington soil. In the pot trial, *E. resinifera* revealed moderate to fast growth and some tolerance to both overburdens, but in the field trial, it grew better at the Ewington site than at Muja. *E. gomphocephala* had lower nutrient levelss when grown in both spoils and a more extreme taper at the Ewington site, suggesting sensitivity to aluminium toxicity. Koch (1984) found that *E. gomphocephala* was tolerant but *E. resinifera* grew poorly in acidic soils. However, Fox *et al.* (1985) also concluded that *E. gomphocephala* seedlings grew better when coal overburdens were limed to a pH of more than 5.3. It is believed that these species may require extra management such as in liming or fertilising, if they were to be used in revegetating the spoil materials, so on this criterion they are not appropriate species.

The shrub species *Calothamnus rupestris* and *Callistemon speciosus* did not indicate high tolerance to acidic overburdens. *C. rupestris* demonstrated moderate tolerance and growth at both sites in both trials but is thought to have suffered much nutrient deficiency and possibly copper and iron toxicity. *Callistemon speciosus* in Ewington material grew less in the pot trial; in the field trial it grew less well in the Muja material, indicating possible moisture stress. These species are thought to be inappropriate for revegetating acidic overburdens.

In the pot trial, *Calothamnus rupestris* in Ewington overburdens had significantly larger tapers and high levels of copper in the stem. Aluminium and copper toxicity symptoms in wheat are similar, which suggests that the mechanism for toxicity for both metals may also be similar (Wheeler, Power & Edmeades 1993). It is possible that the taper is a result of copper accumulation within the stem. Collie overburdens should be analysed for copper concentrations to determine if this metal may be a significant factor in preventing some plant species revegetating certain acidic sites.

E. wandoo, E. maculata and the grass *N. alopecuroidea* grew poorly in both acidic soils in both trials. *Neurachne alopecuroidea* was highly sensitive to both overburdens; all seedlings perished at both sites in the field trial and most died in the pot trial. *E. wandoo* seedlings may have sequestered aluminium into the stems, which may have resulted in slower growth in the field trial. Koch (1984) and Doronila & Fox (1990) report poor to moderate growth for *E. wandoo* and *E. maculata* on overburden soils. These three species are thought to be poorly adapted to growing in the hostile overburden, and therefore not appropriate revegetation species.

In summary, acidity, low nutrients and high metal levels negatively affected seedling growth in acidic soils. Soil moisture was an additional factor in depressing growth on acidic sites. The exotic species *E. robusta,* and to a lesser extent *E. camaldulensis* and *E. cladocalyx,* have shown the greatest potential adaptation to growth on acidic sites. Local species *E. rudis* and *M. hamulosa* are appropriate for acidic overburdens, provided that soil moisture is adequate. Species found not to be suitable were *E. gomphocephala, E. grandis, E. maculata, E. resinifera, E. wandoo, C. rupestris, C. speciosus* and *N. alopecuroidea.* The physiology and ecology of the candidate species are further discussed in Chapter 9.

Chapter 3 – Determining the aluminium tolerances of selected plant species

Introduction

All metals are toxic to plants when present in high concentrations, but some metals are toxic at concentrations much lower than others. When selecting appropriate plant species for rehabilitating acidic soils, the possible factor of metal toxicity needs to be considered. Heavy metals, whether essential micronutrients or not, can exert toxic effects on plants at relatively low concentrations (Verkleij & Schat 1990). Species selected for rehabilitation of particular acidic sites need to be tolerant of the concentrations of any potentially toxic metallic ions present at the site.

Aluminium is an important, potentially toxic, metal which is not essential for plant nutrition, yet it is the third most abundant element in the earth's crust (Traynor 1980). In low pH soils, aluminium may be released from insoluble aluminium complexes, such as kaolinite and gibbsite, to forms that are toxic to plants: Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$ and $AlO_4Al_{12}(OH)_{24}(H_2O)_{24}^{7+}$ (known as Al_{13}) (Atwell, Kreidemann & Turnbull 1999). Aluminium is the most important growth-reducing agent affecting plants in acidic soils (McCormick & Steiner 1978; Göransson & Eldhuset 1991) and can be absorbed by active uptake, like any cation (Traynor 1980).

Aluminium toxicity can cause retardation of root growth and plant productivity (Aniol 1991). Aluminium toxicity symptoms expressed in shoots are more likely to be due to root growth inhibition, rather than a direct effect of aluminium on the shoots (Rengel 1992). Many studies have indicated that this element can reach toxic levels to plants growing in soils of pH < 5.0 and exert a negative effect on the mineral nutrient status of the plant (Kulhavy & Cervaná 1991; Marschner 1991; Matsumoto 1991; Kasran, Shamsuddin & Edwards 1992; Sandison 1994). Excess aluminium in soils can also damage the roots sufficiently to render plants vulnerable to drought (Foy, Chaney & White 1978).

Studies have found much variation on the KCl extractable Al levels in coal overburdens at Collie. Most soils tested indicate levels less than 50 ppm Al. Forest soils in the Collie region range from 0 to 8.5 ppm Al (Koch 1984). Muja overburdens have been reported to vary from less than 0.01 ppm to 36.0 ppm (Dames & Moore 1983; Coulson 1996). In contrast, patches of overburden elsewhere around Collie have been recorded with over 100 ppm and even up to 453 ppm Al (Koch & Bell 1983).

Plants may have evolved different strategies to tolerate the range of stress factors existing in acidic soils. Screening a plant for tolerance of only one of these factors is insufficient for predicting adaptation of that species to acidic soils (Marschner 1991). Plants can only be declared tolerant of the particular acidic soil used in the screening. Screening plants for tolerance to acidic soils is also limited by the wide diversity of soil types and by the complex character of resistance to soil acidity (Aniol 1991; Blamey *et al.* 1991). Also, the uptake of metals by plants in pot trials may be greater than that from plants in the same soil in the field. Factors that may affect results are differences in microclimate and soil moisture. The roots of container-grown plants may be only in contaminated soils while roots of field plants may explore less contaminated material at different depths (Alloway 1995).

Nutrient solutions overcome most of the above mentioned problems by providing a precise control of the composition of the root media. Nutrient solutions can remove soil characteristics that may hamper the experiment, such as buffer ions and lack of nutrients. It also allows the response of the plant to a single toxicity factor to be examined by adding solutions containing only one toxic metal (Aniol 1991). However, high concentrations of nutrients may interfere with the examination of plant tolerance in solution cultures, so low nutrient levels should be applied to ensure proper toxicity is achieved (Blamey *et al.* 1991). Soil solutions can only be used to test for species tolerance but not avoidance. This may explain the poor correlation of nutrient solution screening and growth performance on acidic soils. Plants may grow a shallow root

system, where nutrients and moisture may be available during the growing season, so shoot growth is unaffected (Marschner 1991).

Many annual plant species have been tested for Al tolerance. Two varieties of the snap bean *Phaseolus vulgaris* display toxicity symptoms at 20 ppm Al by 12 days (Naidoo 1976). *Piptatherum miliaceum* was retarded at 25 ppm Al (Zavas, Syneonidis & Karaglis 1996). *Danthonia linkii* and *Microlaena stipoidies* root growth decline by up to 70% with more than 1000 ppm Al (Crawford & Wilkens 1998). *Agrostis tenuis* shows stunted growth at 54 ppm Al (Clarkson 1969). Some work has been carried out on trees and shrubs. *Gastrolobium bilobum* is tolerant to Al levels up to 500 ppm Al in solution (Fox & Doronila 1992). *Paraserianthes lophantha* tolerates up to 200 ppm Al and *Acacia decurrens* tolerates up to 500 ppm Al (Hughes 1991). Silver birch (*Betula pendula*) and Scots pine (*Pinus sylvestris*) show decreased growth at 270 and 800 ppm Al respectively (Göransson and Eldhuset 1991). Cotton root growth is stunted from concentrations of 225 ppm Al, depending on the type of soil used (Adams & Lund 1966). *Lotus corniculatus* is reduced in growth more than *Lotus pedunculatus* at 430 ppm Al (Blamey *et al.* 1991).

The objective of the experiment was to test a range of species for tolerance to the phytotoxic metal aluminium ion Al^{3+}. The experiment sought to determine if the presence of aluminium in overburden soil is an important factor to be considered in rehabilitation of hotspots and to determine tolerance of these species to high levels of aluminium.

The null hypotheses are:

H_o1: Aluminium does not significantly affect growth of seedlings.

H_o2: There is no significant difference in growth between *Eucalyptus* species in aluminium treatments applied.

Method

Preliminary trial to determine a suitable sand-based potting mix

A preliminary trial was conducted for 7 wks (July 1999 - September 1999) to determine an appropriate soil medium for the toxicity experiment. Thirty five 490 mL (10 x 7 x 7 cm) pots were lined with plastic bags and separated into 5 treatments with 7 replicates. Treatments trialed were: pure coarse sand; 3:1 coarse sand/ fine sand; 1:1 coarse sand/ fine sand; 1:3 coarse sand/ fine sand; and, pure fine sand. Four wk-old *E. rudis* seedlings were planted into pots and lightly watered daily. 20 mL of half strength Thrive™ solutions were applied once a fortnight (Appendix 3). Temperature ranged from 16 to 24° C. Initial seedling heights did not significanty differ between treatments.

Plant survival, health and height were compared at seven weeks. Plants growing in 3:1 coarse/ fine sand and 1:1 coarse/ fine sand mixes were significantly taller than those growing in coarse sand alone (Table 3.1). Survival was best with the 1:1 coarse/ fine sand mix (7/7), followed by the other mixed sands (5/7); then coarse sand (4/7); and, lastly, the fine sand (3/7). From this analysis, it was determined that the 1:1 coarse/ fine sand mix would be the most appropriate soil medium in which to conduct toxicity experiments.

Table 3.1: Mean heights (cm) and survival of *E. rudis* seedlings in sand mixes at 7 wks

Mean Height (cm)	n	Mean	SD	F	*P*
pure coarse sand	4/7	13.1 b	1.7	6.04	0.003 (**)
3:1 coarse/ fine sand	5/7	16.9 a	0.9		
1:1 coarse/ fine sand	7/7	17.1 a	1.7		
1:3 coarse/ fine sand	5/7	16.0 ab	1.6		
pure fine sand	3/7	14.3 ab	1.5		

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Fishers LSD test was used to distinguish between treatment measurements

Field capacity of the 1:1 sand mix was calculated using twenty pots (750 mL; 15 cm height x 8cm dia). Pots were lined with paper towel and filled with 800 g dry 1:1 mixture. The pots were then thoroughly soaked with water and left to drain for 24 hours. The sand from each pot was then weighed and the dry weight subtracted from the wet weight. The mean field capacity for 50.50 sand mix was found to be 23.03% (SD = 1.23). This field capacity was used in the following experiment .

Al toxicity experiment

Seven species were tested for tolerance to aluminium. Five species (*E. camaldulensis, E. cladocalyx, E. robusta, E. rudis* and *Melaleuca hamulosa*) are identified in Chapter 2 as highly tolerant to acid soils. *Kunzea ericifolia* (Smith) Heynh is fast growing and occurs as thickets on acidic soils around Ewington 2 lake. *Eucalyptus diversicolor* F. Muell. is also fast growing and is a valuable timber species. Seeds of all species, except *E. diversicolor*, were collected from plants on revegetated Collie dumps (Appendix 3).

In October 1999, seeds of *Eucalyptus camaldulensis, E. cladocalyx, E. diversicolor, E. robusta, E. rudis, Kunzea ericifolia* and *Melaleuca hamulosa* were germinated in a glass house. Two hundred and eighty 750 mL pots (15 cm height x 8 cm diameter) were lined with 2 small freezer bags and filled with 800g of dry 1:1 coarse/fine sand. Forty equal sized seedlings from each species were individually planted into the pots and set into four treatment groups of ten replicates. Initial seedling heights did not significanty differ between replicate sets.

Four categories were established to determine the tolerance range of plants tested. Plants sensitive to aluminium may be defined as those that display toxicity symptoms in solutions with less than 100 parts aluminium per million parts soil (100 ppm). Low tolerant species display symptoms in solutions less than 200 ppm. Highly tolerant

species only display symptoms when grown in 400 ppm Al solutions. Very highly tolerant plants display no symptoms when grown in 400 ppm Al solutions.

In November 1999, Al solutions were prepared to give each pot one of four ppm soil levels of aluminium sulphate $Al_2(SO_4)_3$ dissolved in 184 mL distilled water to attain field capacity (Table 3.2). Al solutions were applied only once during the experiment to maintain contant Al levels in the closed pots. $AlCl_3$ was not used, as the triple level of chloride ions may not have fully evaporated before the application, and be highly toxic to plants. $Al_2(SO_4)_3$ was chosen instead to further recommend the findings with particular spoils in Collie, which have considerable levels of sulphate present.

Table 3.2: Aluminium toxicity treatment sets per tested species

Treatment	n	$Al_2(SO_4)_3$ given (g) per 184 mL	To make Al treatment in 800 g soil
Control	10	0	0 ppm
Low Al	10	0.08	100 ppm
Medium Al	10	0.16	200 ppm
High Al	10	0.31	400 ppm

One week after transplanting, the plants received 20 mL of full strength Optimum Grow™, general purpose hydroponic solutions A and B (Appendix 3) so that the effects of the Al treatments would not be confounded by deficiencies of other mineral nutrients.

Further applications of the hydroponic solutions were applied at 3 wk intervals to maintain low but adequate nutrition during the experiment. Only insignificant portions of the aluminium ions would have complexed, as soluble phosphorus levels supplied were low (0.48 mg during the entire experiment), so nutrients should not have affected the toxicity of the treatments.

Pots were weighed every 2 days and field capacity retained. Measurements of ht (cm) were made fortnightly for all seedlings. Leaf numbers were counted on the eucalypt seedlings while the shrubs *K. ericifolia* and *M. hamulosa* were measured for mean crown diameter. Maximum and minimum temperatures during the twelve week trial were 41^0

C and 16^0 C respectively. After 12 wks, seedlings were harvested. All but two seedlings from each treatment for each species were dried in a 60^0 C oven for 72 hrs and dry weights obtained.

The remaining two seedlings were cut into shoots and roots. Using a modified method of Crawford & Wilkens (1998) to test for aluminium accumulation or adsorption, these roots were washed with deionised water and stained using 0.02% hematoxylin solution (w/v) in distilled water for 60 min at room temperature. The stain was made 1 hr beforehand: 1.0 g of hematoxlyn and 0.1 g $NaIO_3$ were added to 1000 mL of deionised water and stirred with a magnetic stirrer for 60 min to dissolve and partially oxidise the hematoxylin. Roots were then washed again and stored in deionised water. Stained roots were then placed in petri dishes and photographed using 100 ASA film.

Data were tested for homogeniety with boxplots and scatterplots. Statistical analysis was performed by transforming percentage values to obtain normal distributions, then testing with one-way ANOVAs and Fisher's LSD post-hoc test. Transformation involved dividing percentage values by 100 to obtain proportional values between <0.001 and 1.000, which was square rooted then arc sined (Sokal & Rolf 1981).

The shrub species were not compared for relative growth rates with the *Eucalyptus* species because variation in the nature of growth made the comparison difficult.

Results

Seedling growth over time

All species had 100% survival in all treatments. Increased concentrations of applied aluminium were associated with a decline in growth and dry weights of most species tested (Figures 3.1 - 3.3). *E. robusta* seedlings did not differ significantly between treatments for the entire period, though seedlings that received 400 ppm Al tended to be smaller than the other three treatments. Seedlings of *E. camaldulensis* became significantly shorter in the 400 ppm Al treatment at 6 wks ($P = 0.001$), and final stem diameters and dry weights were all significantly smaller ($P = 0.001$ and <0.001 respectively).

Seedlings of three species were smaller in the heavier treatments but not in the lighter treatments. *M. hamulosa* became significantly shorter in the 400 ppm Al treatment at 6 wks ($P = 0.007$) and significantly smaller dry weights ($P = 0.026$). *K. ericifolia* was significantly shorter at 400 ppm after 4 wks ($P = 0.029$) and by 12 wks had significantly smaller stem diameters ($P < 0.001$). *E. rudis* seedlings in the 200 and 400 ppm Al treatments were significantly shorter at 6 wks ($P = 0.002$), had fewer leaves in the 100 ppm treatment ($P = 0.005$) and less dry weight in the 400 ppm Al treatment ($P = 0.026$).

Seedlings of two species were smaller in all Al treatments than the control seedlings. All *E. cladocalyx* in all the treatments receiving aluminium were significantly smaller at 4 wks and had fewer leaves at 12 wks than controls ($P = <0.001$ for both). *E. diversicolor* seedlings in the treatments receiving aluminium were significantly smaller at 2 wks and had significantly lighter dry weights than controls ($P = 0.006$ and 0.004 respectively). Therefore H_o1 is rejected: Al significantly reduces growth of seedlings.

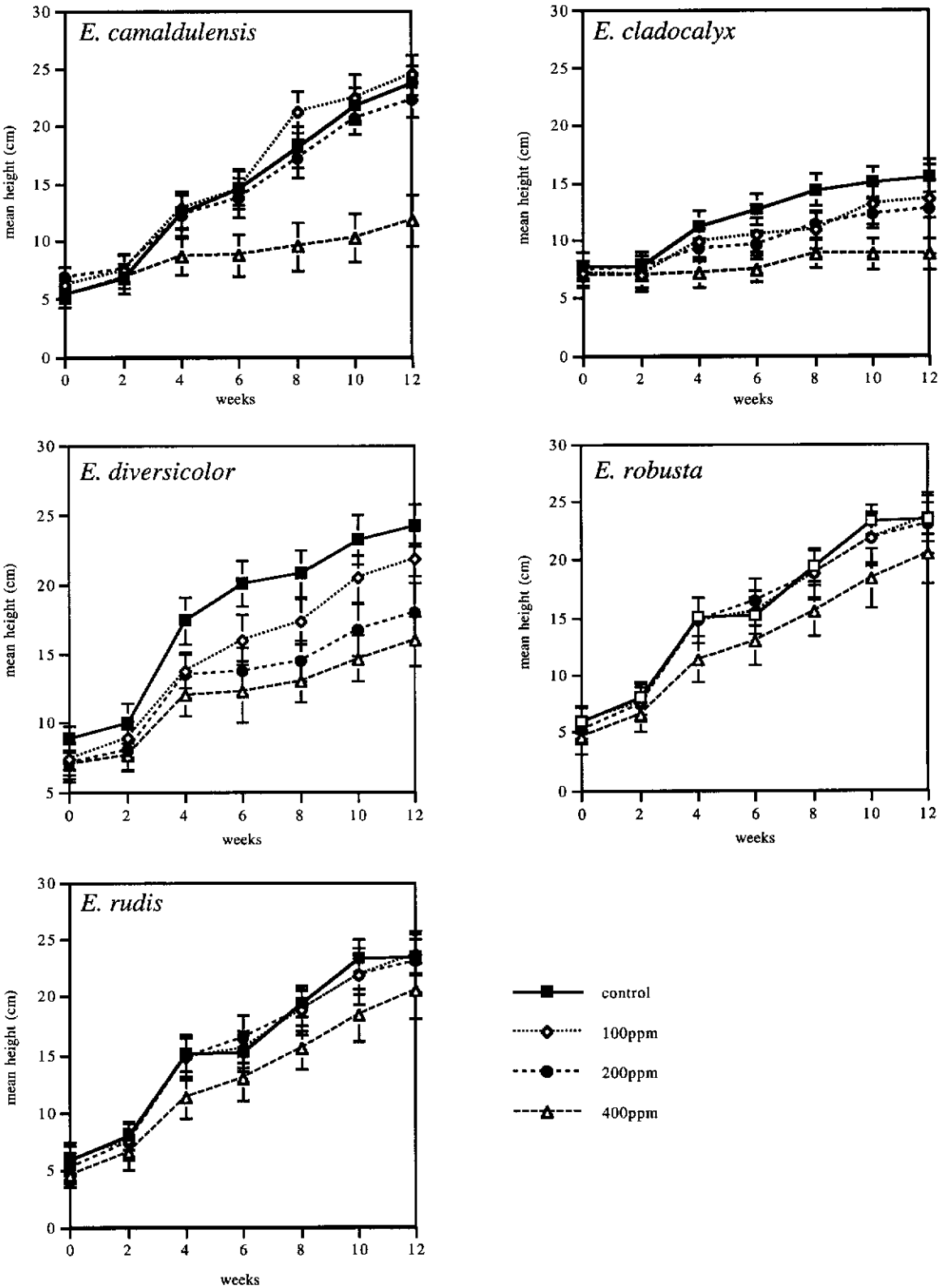

Figure 3.1: Mean heights (cm) and SE of eucalypt seedlings in Al solutions over 12 wks

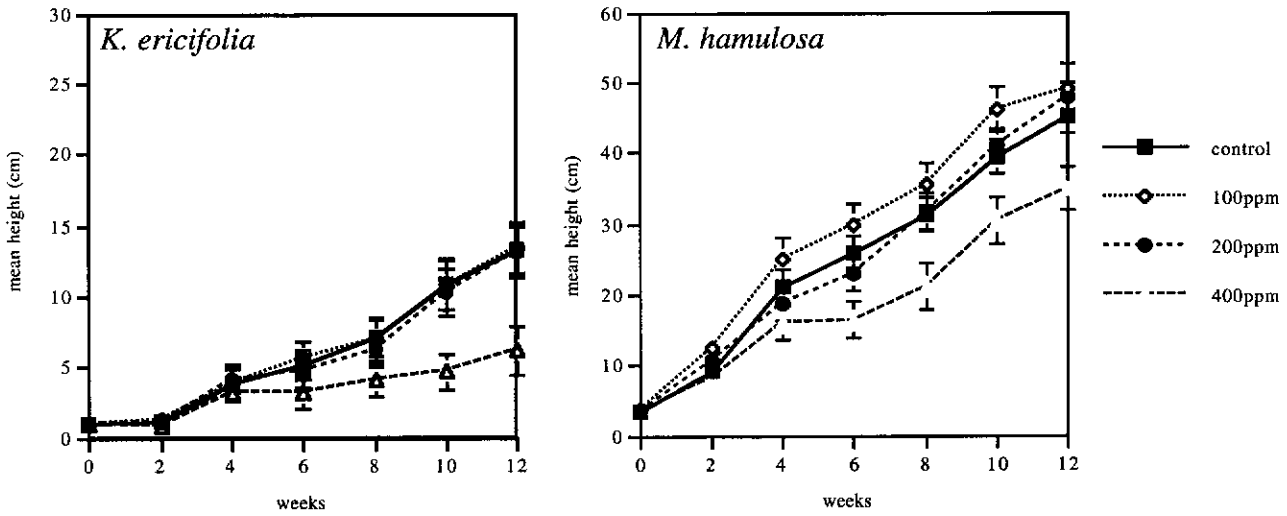

Figure 3.2: Mean heights (cm) and SE of shrub seedlings in Al solutions over 12 wks

Table 3.3: Mean dimensions and SE of seedlings in Al solutions at 12 wks

Species	0 ppm	100 ppm	200 ppm	400 ppm	*P*
Number of leaves					
E. camaldulensis	19.7 (2.4)	18.3 (2.3)	19.3 (3.5)	18.0 (3.7)	0.640 (NS)
E. cladocalyx	24.4 (6.2) a	19.8 (3.0) b	18.8 (5.1) b	10.5 (1.4) c	<0.001
E. diversicolor	57.6 (16.7)	56.6 (18.8)	50.4 (13.2)	37.0 (13.3)	0.055 (NS)
E. robusta	23.8 (1.7)	21.5 (3.5)	2.8 (4.4)	20.0 (3.0)	0.148 (NS)
E. rudis	23.6 (6.7) a	17.8 (2.9) b	15.2 (4.1) b	14.8 (5.2) b	0.005 (**)
Crown diameter (cm)					
K. ericifolia	6.1 (1.9) a	5.0 (1.7) b	5.5 (1.5) b	2.3 (1.0) b	0.009 (**)
M. hamulosa	4.3 (2.1)	3.9 (0.9)	4.4 (1.2)	3.1 (1.3)	0.246 (NS)
Stem diameter (mm)					
E. camaldulensis	2.16 (0.37) a	2.04 (0.35) a	2.07 (0.36) a	1.26 (0.30) b	0.001 (**)
E. cladocalyx	1.77 (0.34) a	1.63 (0.35) a	1.62 (0.22) a	0.82 (0.11) b	<0.001 (***)
E. diversicolor	2.41 (0.48) a	2.14 (0.27) ab	1.98 (0.42) b	1.85 (0.18) b	0.027 (*)
E. robusta	2.17 (0.37)	2.05 (0.54)	2.10 (0.28)	1.96 (0.46)	0.792 (NS)
E. rudis	1.90 (0.24) ab	2.21 (0.34) a	1.70 (0.45) bc	1.51 (0.44) c	0.007 (*)
K. ericifolia	0.74 (0.16) a	0.82 (0.09) a	0.80 (0.13) a	0.39 (0.14) b	<0.001 (***)
M. hamulosa	1.68 (0.25)	1.74 (0.32)	1.90 (0.19)	1.48 (0.39)	0.061 (NS)
Dry weight (g)					
E. camaldulensis	1.91 (0.48) a	2.13 (0.49) a	1.83 (0.56) a	0.54 (0.45) b	<0.001 (***)
E. cladocalyx	0.98 (0.14) a	0.92 (0.19) a	0.99 (0.26) a	0.34 (0.16) b	<0.001 (***)
E. diversicolor	2.68 (0.42) a	1.98 (0.60) b	1.83 (0.91) b	1.41 (0.58) b	0.004 (*)
E. robusta	1.89 (0.39)	1.76 (0.81)	1.92 (0.63)	1.32 (0.51)	0.192 (NS)
E. rudis	1.81 (0.33) a	1.79 (0.66) a	1.25 (0.56) ab	0.94 (0.48) b	0.005 (**)
K. ericifolia	0.32 (0.13) a	0.39 (0.11) a	0.40 (0.15) a	0.14 (0.19) b	0.001 (**)
M. hamulosa	0.93 (0.30) a	1.06 (0.33) a	0.99 (0.33) a	0.58 (0.32) b	0.026 (*)

Analysis of variance ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05
Fishers LSD test: Similar letters in rows indicate no significant difference

Hematoxylin staining

Prepared roots were observed for signs of purple staining that would indicate the binding of hematoxylin stain with aluminium ions in the plant tissue. In general, the accumulation or adsorption of aluminium was observed as stunted root tips with purple colouring (Figure 3.3). Root tips in control plants did not demonstrate any coloration. In contrast, stunted purple roots were seen in *E. cladocalyx* and *E. diversicolor* in all Al treatments; in *E. rudis* and *M. hamulosa* in the 200 and 400 ppm Al treatments; and, in *E. camaldulensis* and *K. ericifolia* and very faintly in *E. robusta* at the highest level used.

Figure 3.3: Roots of *E. cladocalyx* seedling exposed to 200 ppm Al with small root tips

Comparison of Species

Comparisons among *Eucalyptus* species at each aluminium level at 12 weeks demonstrated that significant differences occurred in height and dry mass accumulation (Figures 3.4 and 3.5). *E. camaldulensis* was amongst the tallest only up to the 200 ppm treatment and had the highest dry weights in all treatments. *E. cladocalyx* had significantly smaller and lighter seedlings in all treatments. *E. diversicolor* was one of the tallest and heaviest species in the control, 100 ppm and 400 ppm Al treatments, but was not one in the 200 ppm treatment. The *E. robusta* control seedlings were one of the tallest species for all treatments not one of the heaviest. *E. rudis* seedlings were also one of the tallest for all treatments, but was only moderate in biomass.

The ranked order of mean heights in the 400 ppm Al treatment, was *E. robusta* > *E. diversicolor* > *E. rudis* > *E. camaldulensis* > *E. cladocalyx.* The ranked order of dry weights was similar: *E. diversicolor* > *E. robusta* > *E. rudis* > *E. camaldulensis* > *E. cladocalyx.* In general terms of plant size, *E. robusta* and *E. diversicolor* were the largest species, *E. camaldulensis* and *E. rudis* of moderate size and *E. cladocalyx* the smallest species. Therefore $H_0 2$ is rejected: There are significant differences in growth between *Eucalyptus* species in the aluminium treatments.

M. hamulosa was the tallest species at the end of the experiment in all treatments so is considered to be a fast growing species despite demonstrating stress. In terms of height and weight *K. ericifolia* was intermediate to the control and 100 ppm treatments and was smaller in the 200 and 400 treatments, so is considered to be intermediate in growth rate.

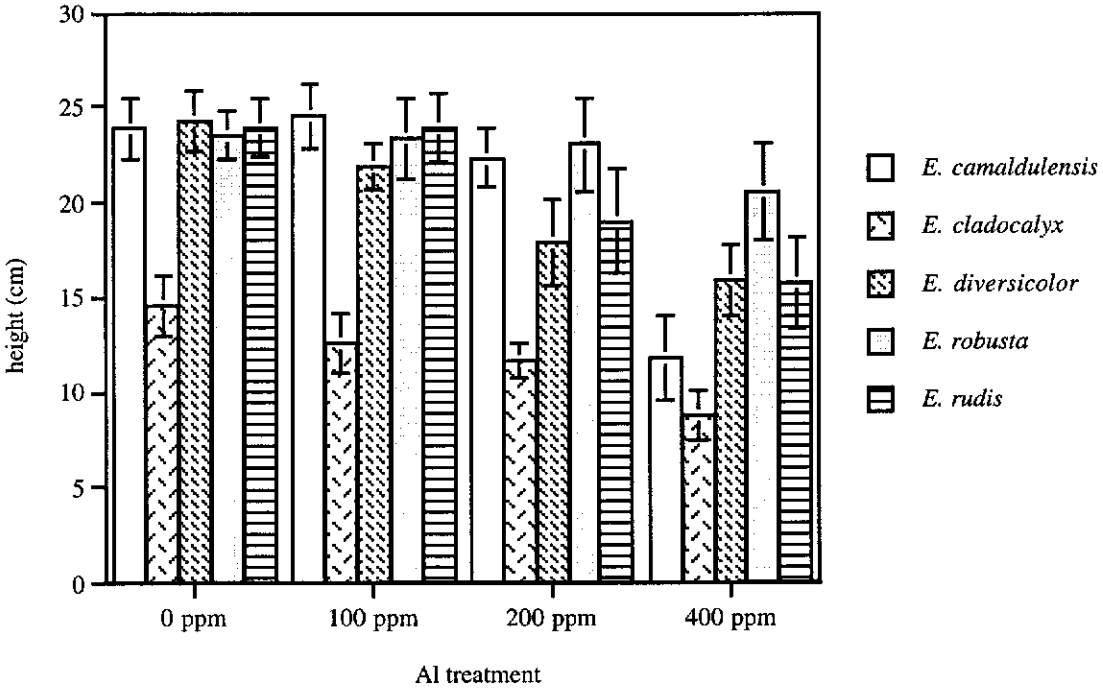

Figure 3.4: Mean heights (cm) and SE of eucalypt seedlings after 12 wks exposed to Al

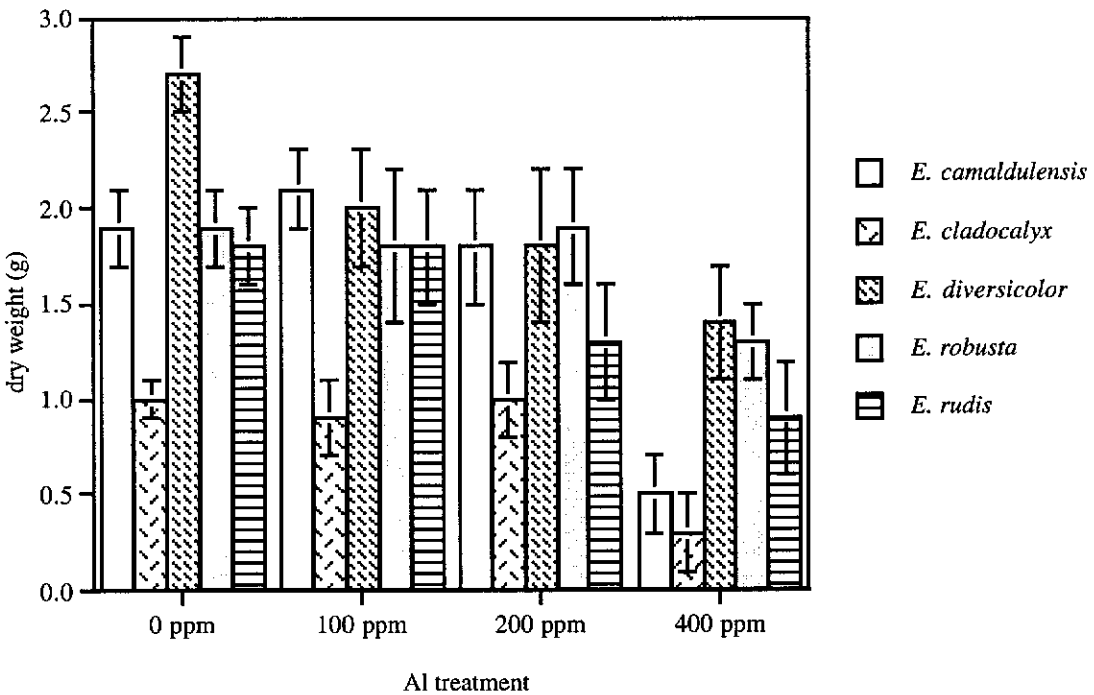

Figure 3.5: Dry weights (g) and SE of eucalypt seedlings after 12 wks exposed to Al

Discussion

Levels of tolerance to Al^{3+} varied greatly amongst the tested species. Interestingly, responses differed between *E. camaldulensis* and *E. rudis*, suggesting that even closely related species are likely to differ. Revegetating soils with free aluminium ions present requires selecting species that are tolerant to the metal level of each site. Three categories were used to designate responses – high, moderate and low tolerance. Species in these categories may, in turn, be considered to be appropriate for revegetating soils of high, moderate and low Al levels.

This trial has determined that *Eucalyptus robusta* and *E. camaldulensis* are relatively fast growing and the most tolerant of the tested species to Al. *E. robusta* never differed in any measurement regardless of Al^{3+} treatment. There was only a slight hematoxylin stain present in the roots from the 400 ppm treatment, suggesting that *E. robusta* only absorbed trace amounts into the roots, whereas *E. camaldulensis* had stunted growth and presence of aluminium in roots only in the 400 ppm treatment. *E. robusta* was amongst tallest in all treatments, whereas *E. camaldulensis* was one of the tallest species in the more dilute treatments. It is concluded that these species are appropriate for soils with up to high levels of aluminium.

Kunzea ericifolia, Melaleuca hamulosa and *E. rudis* appear to be tolerant of only moderate Al^{3+} levels. Seedlings of *K. ericifolia* were only smaller in the highest Al treatment. *Melaleuca hamulosa* demonstrated extremely fast growth, being twice the size of the *Eucalyptus* seedlings in the same treatments, and had shorter and lighter seedlings in the 400 ppm treatment. *E. rudis* was difficult to categorise, as the species was only tolerant of low levels of aluminium, yet demonstrated relatively high growth at high Al levels. Aluminium was also found in roots grown in the high Al treatments. These three species are also thought to be appropriate for revegetating soils with low to moderate levels of aluminium.

E. cladocalyx and *E. diversicolor* were the most sensitive species tested in this experiment. Seedlings grown in any of the Al treatments had reduced growth and aluminium within the roots. However, *E. cladocalyx* was also the shortest and lightest *Eucalyptus* species in all the treatments whereas *E. diversicolor* still proved to be one of the fastest growing eucalypts. These two species are thought to be appropriate for revegetating soils with only low levels of aluminium.

Plants that displayed retarded growth were found to have either accumulated or adsorbed aluminium within the root tips. Aluminium is not easily translocated to the shoots, tending to accumulate in the root tips at sites of cell division and elongation (Horst 1995). Al^{3+} ions can stunt root growth by interfering with the root meristem (Leeper & Uren 1993) and by binding to many important metabolic molecules such as DNA and ATP (De la Feunte-Martinze & Herrara-Estrella 1999). Aluminium toxicity can cause callose formation ((1,3)-β-glucan) in the plant tissue, usually in the root cap cells. This may also reduce root elongation, stunting root growth (Wissemeier *et al.* 1993). Root meristem cell division in the ryegrass *Lolium multiflorum* stopped in as little as 6 hrs after exposure to aluminium (Rengel & Robinson 1989a). It is inferred that aluminium ions interferes with the physiological processes in new roots, retarding root growth and subsequently shoot growth.

Interference with the root physiology reduces potential uptake of water and nutrients. Plants may become deficient in calcium, magnesium and phosphorus. Aluminium has almost 600 times more affinity with the surface of phosphatidychlorine vesicles than calcium so may deprive plants of calcium intake (Haug & Shi 1991; Piñeros & Tester 1993; Plieth *et al* 1999). Aluminium binds to Mg^{2+} specific sites on transport proteins and damages root cell membranes in *L. multiflorum*, reducing net uptake of magnesium (Rengel & Robinson 1989a). Aluminium replaced calcium and magnesium in the root cortex in Norway Spruce (*Picea abies*) seedlings (Godbold, Fritz & Hütterman 1988), in both the roots and the shoots of *Eucalyptus rudis* (Egerton-Warburton, *et al.* 1993) and in roots of *L. multiflorum* (Rengel & Robinson 1989b). Plants may also be deprived of

phosphorus. Aluminium can bind phosphorus in the root surface, in the free spaces of plant roots and in the cell walls (Russell 1980; Lee and Pritchard 1984).

The absence of hematoxylin staining on roots from *E. robusta* seedlings suggests that this species has exclusion mechanisms that lower absorption of heavy metals through the plasmalemma of the root cell. Plants may prevent uptake of metals by either altering the membrane permeability to prevent passive ion passage, increasing the fixed charge of the cell wall or by increasing exudation of metal chelating substances that precipitate metal ions (Verkleij & Schat 1990; Marschner 1991; Ryan, Ditomaso & Kochian 1993; De la Feunte-Martinze & Herrara-Estrella 1999). Exudation of phosphate, malate and citrate into the rhizosphere can occur as early as 15 min after exposure to Al (Rengel 1999). Also, the plant may have some phenotypic tolerance to the metals as a result of general adaptability to metal stress environments (Rengel 1997). It is inferred that species of *Eucalyptus* vary in metal tolerance mechanisms, thus varying in tolerance to metal toxicity.

Several species indicated sensitivity at four weeks but did not fully indicate the particular level of tolerance until six weeks. Measurements taken at 12 weeks only helped confirm variation of tolerance with stronger levels of confidence (P <0.001). This analysis suggests that under the experimental conditions, six weeks is adequate for the plants to show signs of definite aluminium toxicity.

There is difficulty in comparing the results from aluminium toxicity pot trials to actual field conditions. Current work can only recommend whether a species is tolerant of high, moderate or low levels of free aluminium in soil solution, but cannot recommend the maximum level of soil Al within a field site that the species may successfully grow in. Such recommendations involve determining the fraction of soil aluminium that may be absorbed by plant roots (bioavailable Al), not simply total exchangeable Al.

Field soils then need to be tested with appropriate extraction solutions. High-ionic strength extraction solutions, such as 1 M KCl, removes most or all exchangeable aluminium from the surface of clays and organic sites, giving a measurement

considerably higher than the actual Al level that would affect plant growth. A bioavailable fraction of aluminium in soils may be determined by using low ionic strength extraction solutions, such as 0.01 M $CaCl_3$ used in this thesis, to remove only a portion of the bound soil aluminium (Peverill, Sparrow & Reuter 1999). This method is more useful in assessing phytotoxicity of the soil, and allows further categorisation of sites (for example, 50 – 100 ppm bioavailable Al rather than 200 – 300 ppm total extractable Al).

Also, most pot trials measure aluminium as concentration per soil solution, whereas field soils are usually measured in weight of aluminium per weight soil. Compared to the total extractable Al in acid soils, the concentration of aluminium in the soil solution is usually low (<50 ppm) (Edmeades *et al.* 1985, Bruce *et al.* 1989). Therefore in matching appropriate species to soil sites, it is important to measure both the field soil and the pot trial in the amount of bioavailable aluminium that is present in either the soil or soil solution.

There is also difficulty in comparing the results from various aluminium toxicity pot trials. The type of experimental method can greatly affect the level of tolerance displayed by a species. Seedlings of *Picea abies* (Norway Spruce) have been reported to suffer retarded growth at 1350 ppm Al (Fiskesjö 1989), 135 ppm (Göransson and Eldhuset 1991) and 50 ppm Al (Arovaara & Ilvesniemi 1990). A standard toxicity experimental design, using predetermined levels of bioavailable aluminium, is needed to determine the critical toxicity levels of plant species and for effective comparisons to be made between researchers.

In summary, this trial has determined that in terms of tolerance to free aluminium ions and growth rate, *Eucalyptus robusta* is believed to be appropriate for soils with up to toxic levels of aluminium. *E. camaldulensis, Kunzea ericifolia* and *Melaleuca hamulosa* are appropriate for soils with up to high levels of aluminium. *E. rudis* species is only appropriate for soils of moderate aluminium levels or less. *E. cladocalyx* is thought to be only appropriate for low levels of aluminium. More work is needed to further examine the role of *E. diversicolor* for revegetating soils with highly toxic aluminium.

Chapter 4 – Addition of organic matter around seedlings on acidic coal spoils

Introduction

Organic matter is a vital component of soils and consists of living organisms, fresh residues and well-decomposed residues (Magdoff 1992). Fresh residue is the part that decomposes rapidly, being the main supply of food for soil organisms. Some residues break down quickly, while others, like lignin, are more resistant and form humus (Knuti & Korpi 1970).

Chemically, organic matter consists of carbohydrates, proteins and fats, resins, waxes and similar compounds (Knuti & Korpi 1970). These large organic molecules are not available to plants directly. As soil organisms break down these molecules into simpler inorganic forms, major nutrients such as nitrogen, phosphorus and sulphur are released (Vaughan & Ord 1985; Daniels & Zipper 1995). Organic matter, through its buffering properties, may also affect the pH within the soil, causing the release of phosphate ions and trace elements into bioavailable forms. Up to 50% of the total phosphorus in the root medium may be locked up in unavailable organic complexes such as aluminium and iron phosphates (Davey & Krause 1980). Organic matter can also supply nutrients by acting as a reservoir and preventing nutrient loss through leaching by chelation (Vaughan & Ord 1985; Simpson 1986).

In addition, humus can prevent certain minerals, such as aluminium and iron, from bonding with phosphate. This occurs either by coating or by chelating the elements, which prevents any reaction with the phosphate ions (Magdoff 1992). Aluminium chelated with organic ligands is considered less toxic than free Al (Russell 1980). Oxalate, citrate, tartrate and malate all alleviate aluminium toxicity in soybean (*Glycine max*) seedlings (Ginting, Johnson & Wilkins 1998).

The main benefit of organic matter is to improve soil structure and encourage microbial activity (Traynor 1980; Sheat & Schofield 1999). Humic substances can improve soil drainage and aeration of roots. During decomposition, sticky substances are produced that bind the soil particles into granules (called aggregates). This aggregation increases the pore spaces within the soil, improving the tilth. The creation of more pores promotes water penetration and deep root growth and reduces runoff (Knuti & Korpi 1970; Magdoff 1992; Archer, Hodges & LeHunt 1993). Organic material also has a significant role in retaining soil moisture. It may hold three times as much water as will clay. Soils containing organic matter have a higher field capacity than soils without organic matter, thus holding a greater amount of available capillary water (Magdoff 1992; Archer *et al.* 1993).

Organic matter may also provide food for mycorrhiza, and enhance mycorrhiza development (Davey & Krause 1980). Soil with added organic matter, regardless of source, had more microbial biomass than soils given ammonium sulphate (Witter, Martensson & Garcia 1993). Dry bean (*Phaseolus vulgaris*) plants growing in silt loam soils with added manure had a significantly higher percentage colonisation of mycorrhiza and a higher yield than plants grown in untreated soils (Tarkalson *et al.* 1998). *Gastrolobium bilobum* growth in acidic coal overburden was increased when given chicken manure and inoculated with *Pisolithus tinctorius* (Fox & Doronila 1992).

Organic matter has several chemical properties that may indirectly assist plant growth. Materials within humus, such as vitamins and hormones, may directly stimulate plant growth (Vaughan & Ord 1985). Organic matter affects soil acidity, lowering the critical pH value of plant growth than for soils with little or negligible organic matter (Russell 1980). Organic matter can also buffer the soil against changes in pH (Vaughan & Ord 1985). Humus can also bind onto other harmful chemicals such as pesticides and herbicides, preventing further harm to plants (Magdoff 1992; Rouchaud *et al.* 1995; 1996).

Soil organic is of much interest for revegetation because it is the centre of biological activity in the soil; it affects the physical and chemical properties of the soil and the nutrition levels and availability to plants (Leeper & Uren 1993). Soils with low organic matter are more difficult to grow plants in, because fertility and water availability are reduced while soil compaction, erosion, parasites, disease and insect problems may increase (Magdoff 1992).

It is advantageous to attain a sufficient level of organic matter in acid soils. Acidic soils assist in retaining organic matter by decreasing the rate of decomposition (Handreck & Black 1991). However, coal mining waste is devoid of bacteria, fungi and animals that may contribute organic matter; thus, organic matter may not build up after mining (Burns 1989). Without any external supply, the soil will contain roughly the same amount of organic material each year (Handreck & Black 1991).

One source of organic matter is animal manure. Sources of animal wastes include bovine, chicken, sheep and swine. The concentration of nutrients in these wastes varies greatly, depending on ration, collection, storage and handling. The chemical characteristics of manures may vary, depending on feed, bedding materials and system of storage (Bickelhaupt 1980). Manure may contain the full range of nutrients needed by a plant, but not necessarily in desirable proportions. Not all of the nutrients in manures are immediately available to plants. The nutrients initially unavailable form part of the nutrient reserve in the soil (Simpson 1986). On average, every tonne of fresh cow manure contains 5 kg nitrogen, 2.5 kg of phosphate, 5 kg of potash and small quantities of other essential nutrients (Magdoff 1992).

Another source of organic matter is potting mix, which contains considerable levels of peat or sawdust. It does not readily compact, allowing good drainage, aeration and water retention. Fertilisers are also present, but differ in amount according to the quality of the mix. Most potting mixes are pH neutral (Better Homes and Gardens 2000).

Application of lime may enhance organic matter properties in acid soils. One effect of the combined addition of lime and organic matter is to increase bioavailability of cationic micronutrients (Saha, Adhikari & Biswapati 1999). In the long term, liming soil can increase organic matter content and thus soil aggregation (Haynes & Naidu 1998). Application of lime (1.25 t ha^{-1}) and organic fertiliser (4 t ha^{-1}) increased yield harvest of wheat in the first year, and of soybean crops grown on the same site in the second year (Mishra, Paikaray & Mishra 1999). Applied crushed limestone (1.5 t ha^{-1}) reacted with soil organic matter, increasing the aggregate stability of soil in New South Wales (Chan & Heenan 1999). Application of lime and manure compost on acidic tailings (pH 2.4) in China increase growth of the grasses *Cynodon dactylon* and *Agropyron elongatum* (Ye, Wong & Wong 2000).

It must be noted that adding organic matter to acidic soil can only aid plant growth in the short term. Over time microbes break down the supplied organic matter, lowering the content within the soil. This eventually reduces the benefits, including soil buffering capacity, so the soil gradually declines in pH to the previous acidic level (Allison 1973).

The aim of this experiment was to determine if certain ameliorants placed immediately around seedlings of each of four eucalyptus species improves soil conditions, thus enhancing subsequent seedling survival, establishment and growth in acidic overburden soils.

The null hypotheses are:

H_o1: There is no significant difference in nutrient levels between cow manure and potting mix.

H_o2: There is no significant difference in pH and moisture levels near seedlings between treatments.

H_o3: Substrate addition does not significantly affect growth of *Eucalyptus* seedlings on acidic overburdens.

Methods

Seeds of the eucalyptus species *E. camaldulensis, E. grandis, E. maculata* and *E. rudis* were prepared as for the pot and field trials in Chapter 2. In late May 1997, 120 holes of 5L volume were dug at the Ewington 2 lake site in a grid design with each hole being roughly 1m apart. Five sets of substrate treatments were used to fill the holes in a Latin Square Design. The treatments consisted of potting mix (PM); cow manure (CM); 4:1 potting mix to lime (PM+L); 4:1 cow manure to lime (CM+L); and 4:1 overburden to lime (L) (Appendix 7). The potting mix used was Naturegrow™ general potting mix (Appendix 3). Prior to the experiment, 3 samples of each treatment were chemically analysed by CSBP Plant and Soil Analytical Laboratory for nutrient levels (Appendix 4). Lime used was crushed limestone from Wellard, Western Australia and was 68.7% pure $CaCO_3$ (Appendix 3). Lime particles were less than 2.5 mm in diameter.

Two weeks later (June 1997), three soil samples (5 to 15 cm) were collected from each treatment and tested for pH as in Chapter 2. Seedlings of the 4 eucalypt species were then planted in a Latin Square Design at the same time as the species selection field trial (Chapter 2). Eight seedlings each of *E. grandis* and *E. maculata* and 4 seedlings each of *E. camaldulensis* and *E. rudis* species were planted into each of the five treatments (Appendix 7). Seedlings of the same species in the species selection field trial were used as the control plants. Seedlings in the lime only treatment were given 10 g of 9 mo slow release Osmocote Plus™ fertiliser prills (Appendix 3); the same treatment as the control seedlings. The other seedlings were to rely on the potting mix or manure for nutrients. Plant height and width, basal diameter, survival and percentage weed cover within a 10 cm dia of seedling boles were measured and general observations taken every three months for 24 months. Initial seedling heights did not significanty differ between treatments.

In December 1999 (30 months after planting), half of the treatment seedlings and all control seedlings were harvested by cutting the stems at ground level and placing in calico bags. Harvested plants were dried in a glasshouse (25 – 40^0 C) for 30 days and

then weighed. Three samples of each substrate treatment were collected and measured for pH and soil moisture as in Chapter 2.

Data were tested for homogeneity with boxplots and scatterplots. Statistical analysis was performed with one-way ANOVAs and General Linear Models where appropriate and with Fisher`s LSD post-hoc test.

Results

Chemical analysis of organic matter

There was great variation in nutrients between the two organic matter treatments (Table 4.1). CM had significantly more total nitrogen, nitrate, phosphorus, potassium and iron but did not differ in ammonium or sulphur. Therefore H_o1 is rejected: CM has significantly more nutrients than PM.

Table 4.1: Chemical analysis of organic matter substrates (n=3)

Nutrient	Cow manure	Potting mix	F	*P*
Total Nitrogen (mg/kg)	92.5 (17.7)	21.0 (9.9)	24.9	0.038 (*)
Ammonium (mg/kg)	7.5 (3.5)	2.5 (0.7)	3.8	0.189 (NS)
Nitrate (mg/kg)	85 (14.1)	18.5 (9.2)	31.1	0.031 (*)
Phosphorus (mg/kg)	1342 (89.1)	129.5 (13.4)	362.2	0.003 (**)
Potassium (mg/kg)	11100 (990)	604 (52)	224.2	0.004 (**)
Sulphur (mg/kg)	360.5 (67.2)	497.0 (118.8)	2.0	0.293 (NS)
Iron (mg/kg)	1765.0 (36.8)	947.5 (68.6)	220.7	0.005 (**)

Treatment parameters

At the time of transplanting (June 1997), all treatments had significantly higher pH than the control ($P < 0.001$; Figure 4.1). The CM and CM+L treatments were slightly alkaline and had significantly higher pH levels than any of the other treatments. Addition of lime only slightly increased the pH of the cow manure, potting mix and the acidic spoil.

By 30 months (December 1999), the soil pH drifted down in all treatments while there was no significant change in control soil pH (Figure 4.1). The CM and CM+L treatments significantly decreased in pH ($P = 0.003$ and > 0.001 respectively) becoming acidic, but still remained significantly greater than the other treatments and control soil ($P = 0.001$). The PM, PM+L and L treatment did not significantly decrease from the original pH values, but were no longer significantly different from the control.

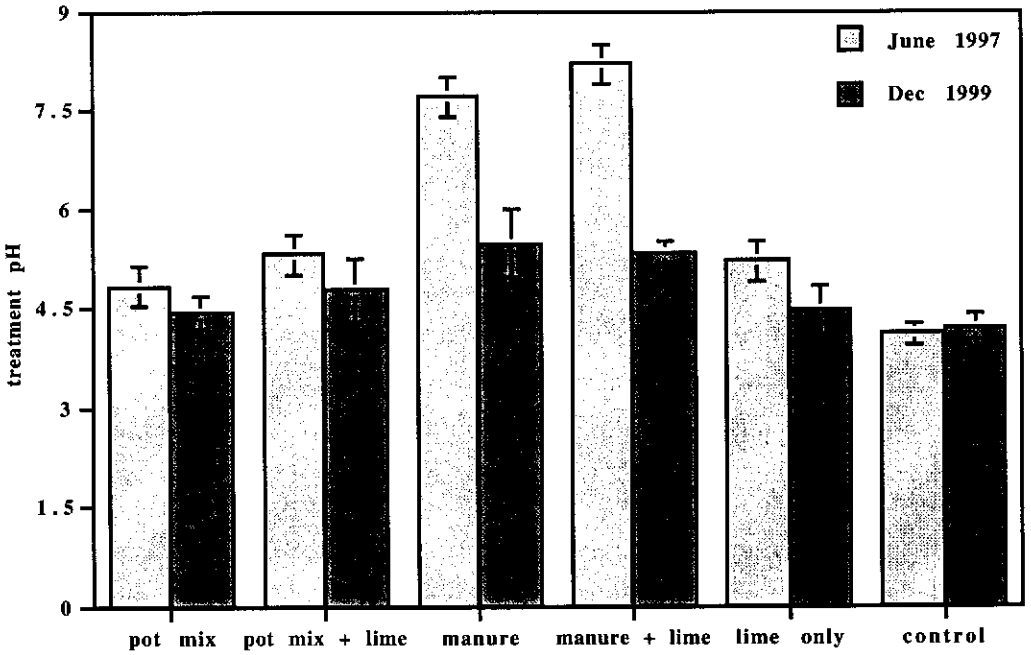

Figure 4.1: Mean pH and SE of substrate treatments at 0 and 30 months (n=3)

The CM and CM+L treatments were also found to have higher levels of moisture than the other treatments and the control soil at 30 months ($P > 0.001$; Figure 4.2). The PM, PM+L and the L treatments did not differ significantly from the control soil. Lime did not affect moisture content of cow manure, potting mix or the overburden material. The high moisture levels at the end of the experiment suggest that cow manure had increased the moisture content of the soil around the seedlings throughout the entire experiment, especially during the summer months. Thus H_o2 is rejected: the pH and moisture levels near seedlings in the cow manure treatments are significantly greater in than the other treatments.

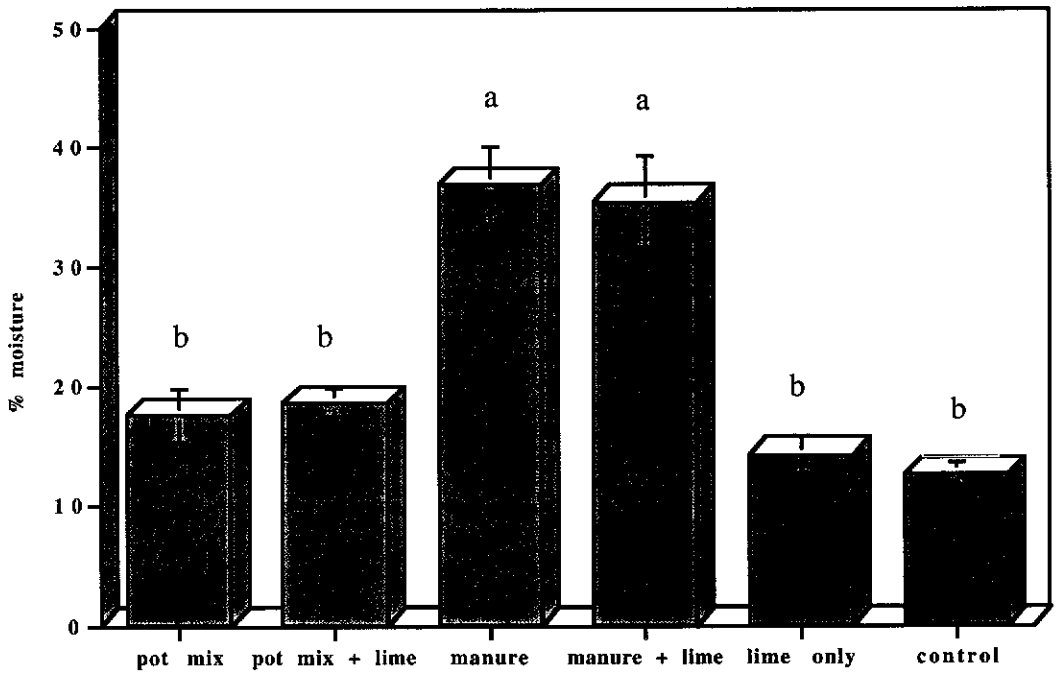

Figure 4.2: Mean soil moisture % and SE of substrate treatments at 30 months (n=3) Treatment with same letter indicate no significant difference

Seedling growth

At the beginning of the experiment (June 1997), no species differed significantly in height or stem diameter between treatments (Figures 4.3 to 4.5). *E. camaldulensis* control seedlings had significantly larger crown diameters than seedlings in the CM and CM+L treatments, but were not significantly different to the PM, PM+L or L treatments (P = 0.004; Figure 4.4). None of the other three species showed any significant differences in crown diameters between treatments.

Seedlings grew fast in the first twelve months, especially over the summer period September 1997 to March 1998. At June 1998, after 12 months, *E. grandis* plants growing in the CM and CM+L treatments were significantly taller (P = 0.014) and had significantly larger crown diameter (P = 0.047) and stem diameter (P = 0.008) than those in the PM, PM+L and L treatments (Figures 4.3 to 4.5). Both *E. maculata* cow manure treatments produced significantly taller plants (P = 0.007) and crown diameters (P =

0.008) than the two potting mix treatments but no significant difference in stem diameter (Figures 4.6 and 4.7). *E. rudis* and *E. camaldulensis* seedlings did not differ in height and crown diameters between treatments due to the two species having fewer replicates, but both species showed similar trends *E. grandis* and *E. maculata.*

The seedlings then slowed in growth and hardly increased in size over the next 15 months. Measurements taken in June 1999 (24 months) revealed similar results to June 1998. *E. grandis* seedlings grown in the CM and CM+L treatments were still significantly taller ($P = 0.002$) and had significantly larger crown diameters ($P = 0.014$) and stem diameters ($P > 0.001$) than the PM, PM+L and L treatments ($P = 0.002$). Both *E. maculata* cow manure treatments had seedlings that were significantly taller ($P = 0.002$) and with larger crown diameters ($P = 0.020$) than the two potting mix treatments and also the lime treatment, but there was no significant difference in stem diameters. *E. camaldulensis* and *E. rudis* again did not show any significant differences. Thus, cow manure remained the best growing medium for over 12 months. Growth conditions were only favourable for the first year.

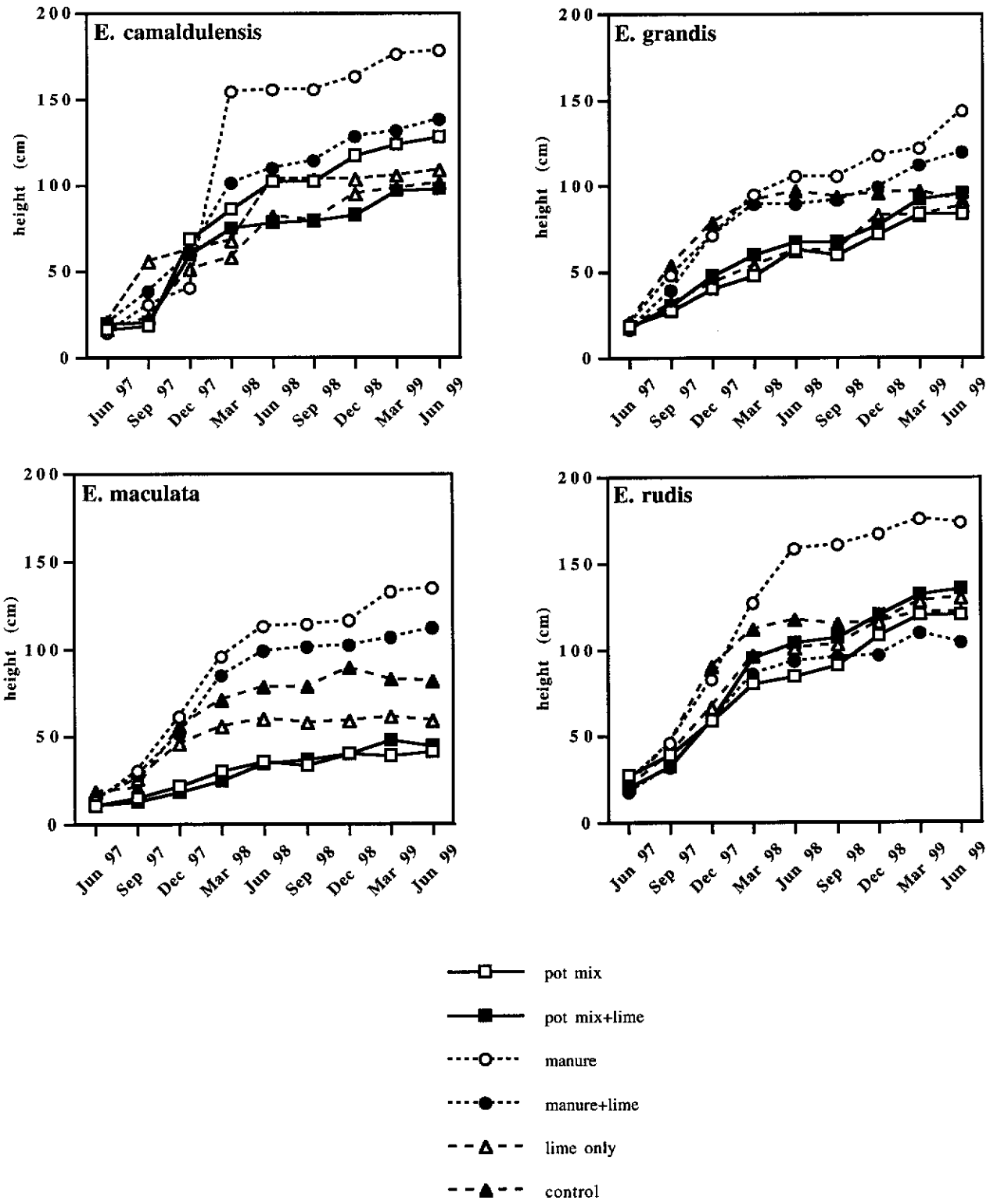

Figure 4.3: Mean hts (cm) of eucalypts in different substrate treatments June 1997-June 1999 (*E. grandis* & *E. maculata* n = 8, *E. camaldulensis* & *E. rudis* n = 4)

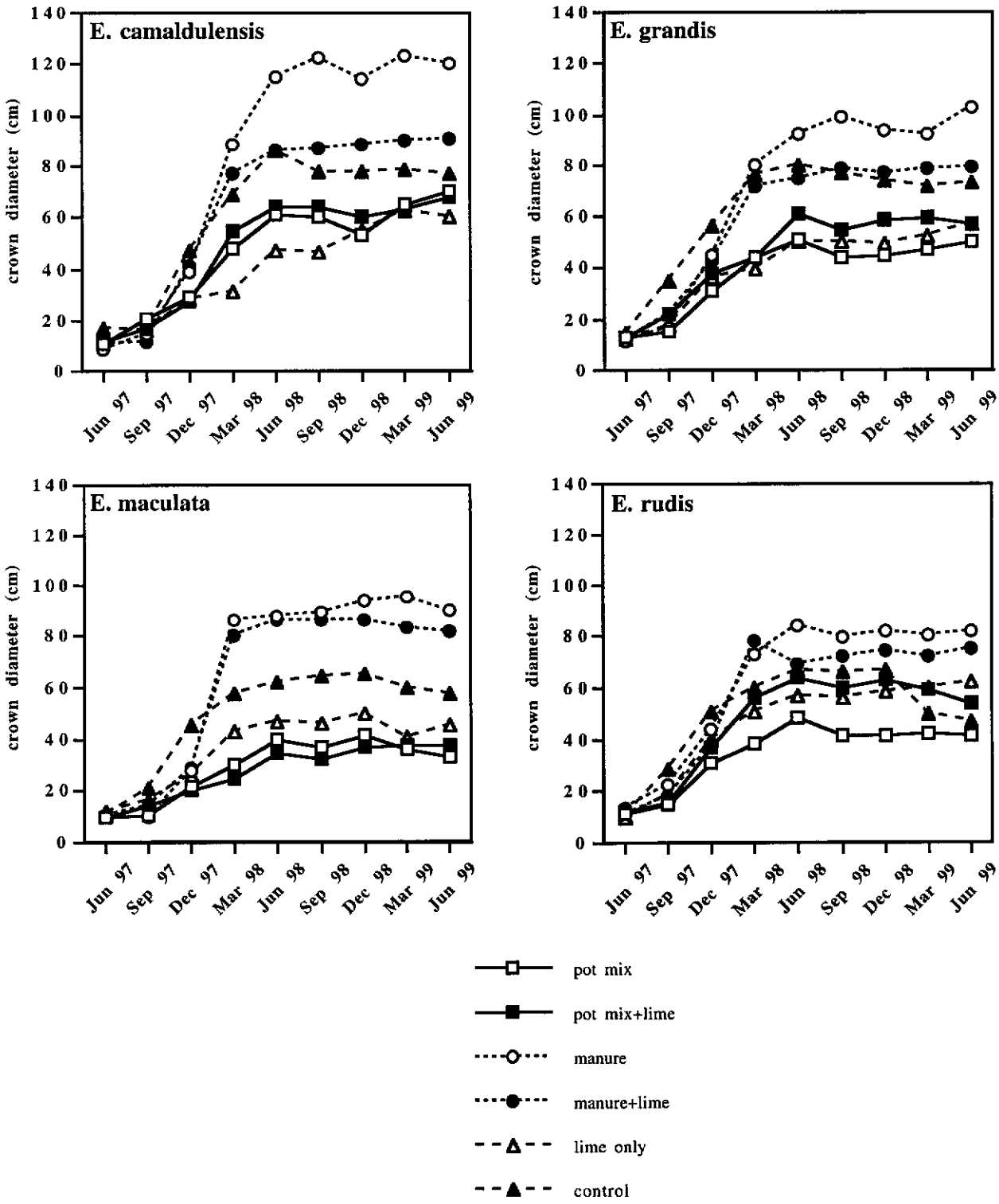

Figure 4.4: Mean crown dia (cm) of eucalypts in different substrate treatments June 1997-June 1999(*E. grandis* & *E. maculata* n = 8, *E. camaldulensis* & *E. rudis* n = 4)

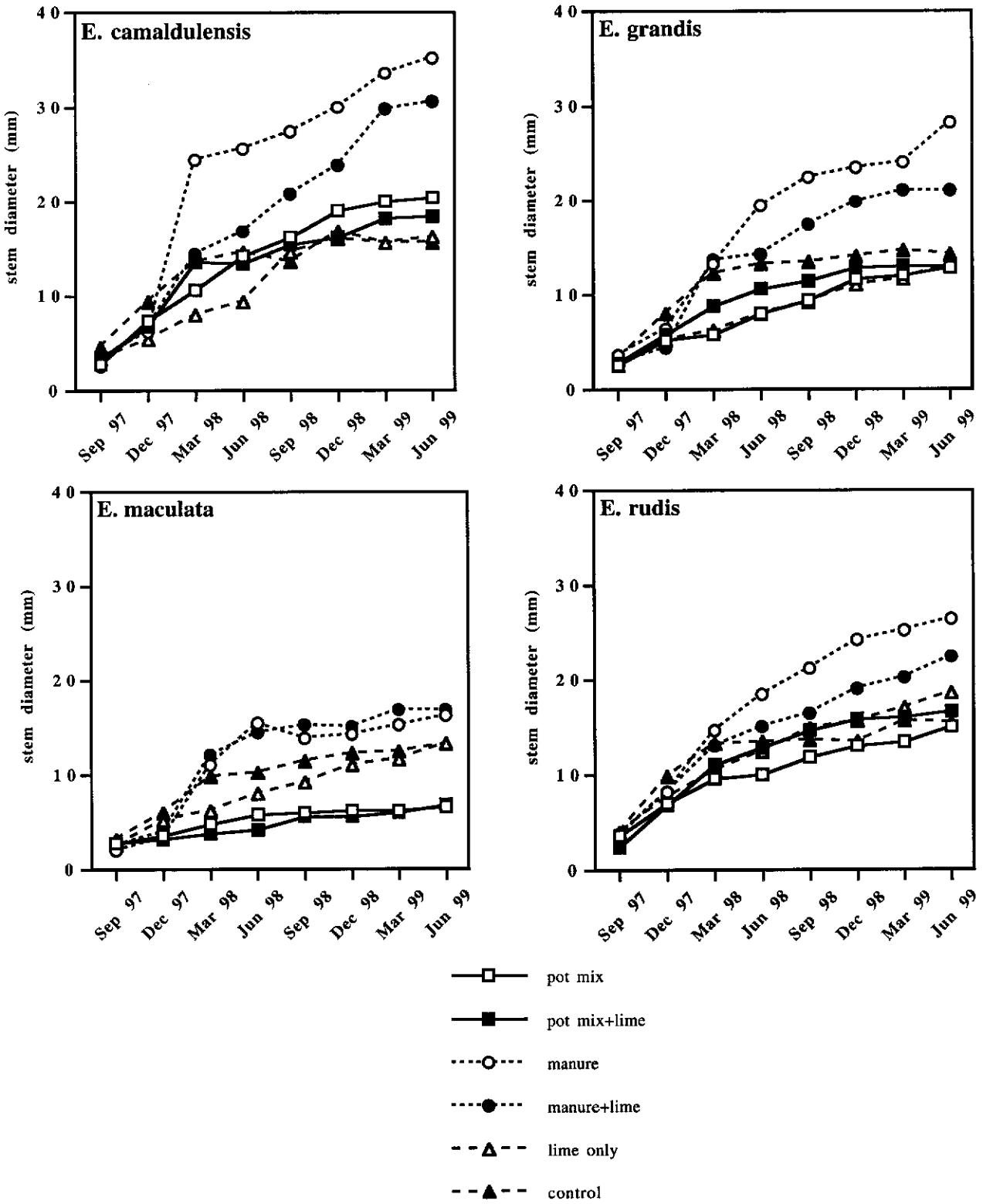

Figure 4.5: Mean stem dia (cm) of eucalypts in different substrate treatments June 1997-June 1999 (*E. grandis* & *E. maculata* n = 8, *E. camaldulensis* & *E. rudis* n = 4)

Figure 4.7: *E. maculata* seedlings given CM+L (left) and CM only (right) at 12 months (heights over 200 cm)

Figure 4.6: *E. maculata* control seedling at 12 months (height under 80 cm)

General Linear Model (GLM) analysis was performed on the dry weights of the seedlings of all 4 species harvested at December 1999 (Table 4.2). Dry weight was significantly affected by ameliorant treatment. There was no interaction found between the two treatments; lime did not significantly affect dry weights of the seedlings singly or with the ameliorant.

Table 4.2: GLM of mean dry weights of eucalypts grown in various substrates

Source	DF	F	*P*
Ameliorant	2	9.03	<0.001 (***)
Lime	1	0.27	0.602 (NS)
Substrate*Lime	2	1.43	0.247 (NS)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

The lime treatment groups were then combined into the ameliorant treatment groups and an oneway analysis of variance performed (Table 4.3). Seedlings grown in the CM treatment were twice the weight of the controls and three times the weight of the seedlings grown in PM. Cow manure increased seedling dry weight; potting mix did not. H_o3 is rejected: organic matter, in the form of cow manure, significantly improves growth of *Eucalyptus* seedlings on acidic overburdens.

Table 4.3: ANOVA of mean dry weights of eucalypts grown in substrates

Substrate	n	Mean Weight (g)	St Dev	F	*P*
Potting mix	26	97.6 b	165.9	9.20	<0.001 (***)
Cow manure	27	303.0 a	236.5		
Control	23	151.4 b	151.4		

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Weeds

In the first year, there was a significantly higher weed cover in the cow manure treatments than in the controls or other treatments. The weeds grew mostly in the winter to spring months and died back in the summer months (Figure 4.8). In September 1998 the CM and CM+L treatments had a mean weed cover over 35%, while all other treatments were less than 10%. By March 1999, mean weed percentage was 4.3% in the CM treatment and 12.3% in the CM+L treatment and less than 3.5% in the other treatments.

Weed percentages were significantly higher in at least one of the cow manure treatments at all measurement times for all eucalypt species. Treatments with potting mix did not have significantly more percentage weed cover at any measurement time. Lime did not increase weed cover, whether applied singly or with the manure or potting mix treatments.

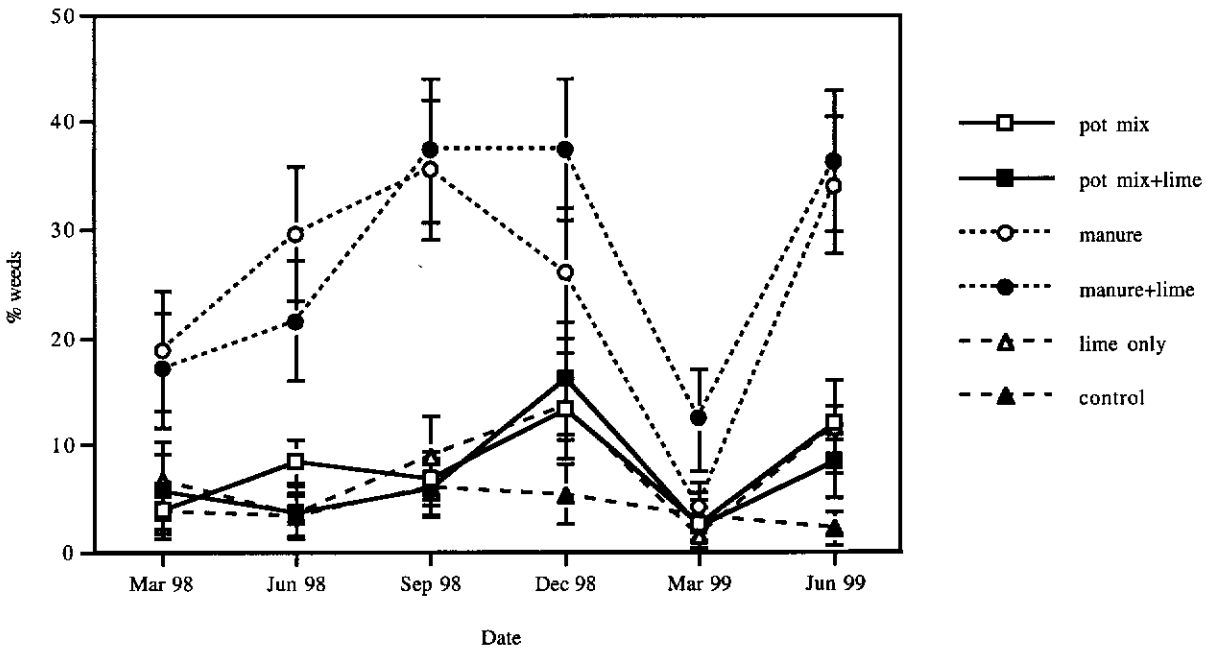

Figure 4.8: Mean weed covers (%) and SE in ameliorants March 98-July 99

There were more deaths of seedlings planted in the manure treatments than the other treatments. By July 1999, 2 of the 24 seedlings had died in the PM treatment, 3 of the 24 had seedlings died in each of the PM+L treatment, 7 of the 24 seedlings died in the CM treatment, 9 of the 24 seedlings died in the CM+L treatment, 4 of the 24 seedlings died in the L treatment while none of the 30 control seedlings died.

In July 1999, seedlings that died in the cow manure treatments had over 70% weed cover within a 20 cm radius of the seedling. This was significantly greater than in the other treatments ($P < 0.001$; Figure 4.9). Weeds are thought to be the main factor responsible for seedling death in the manure treatments (Figure 4.10).

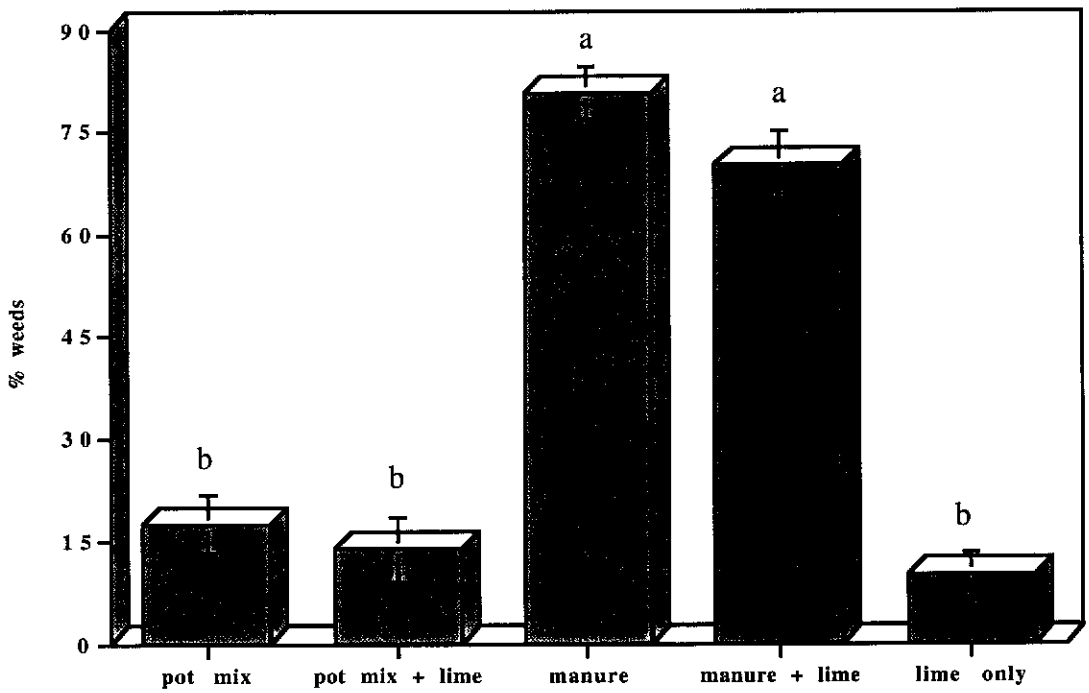

Figure 4.9: % weed cover and SE around dead seedlings per treatment at July 1999 (bars with the same letter do not differ significantly)

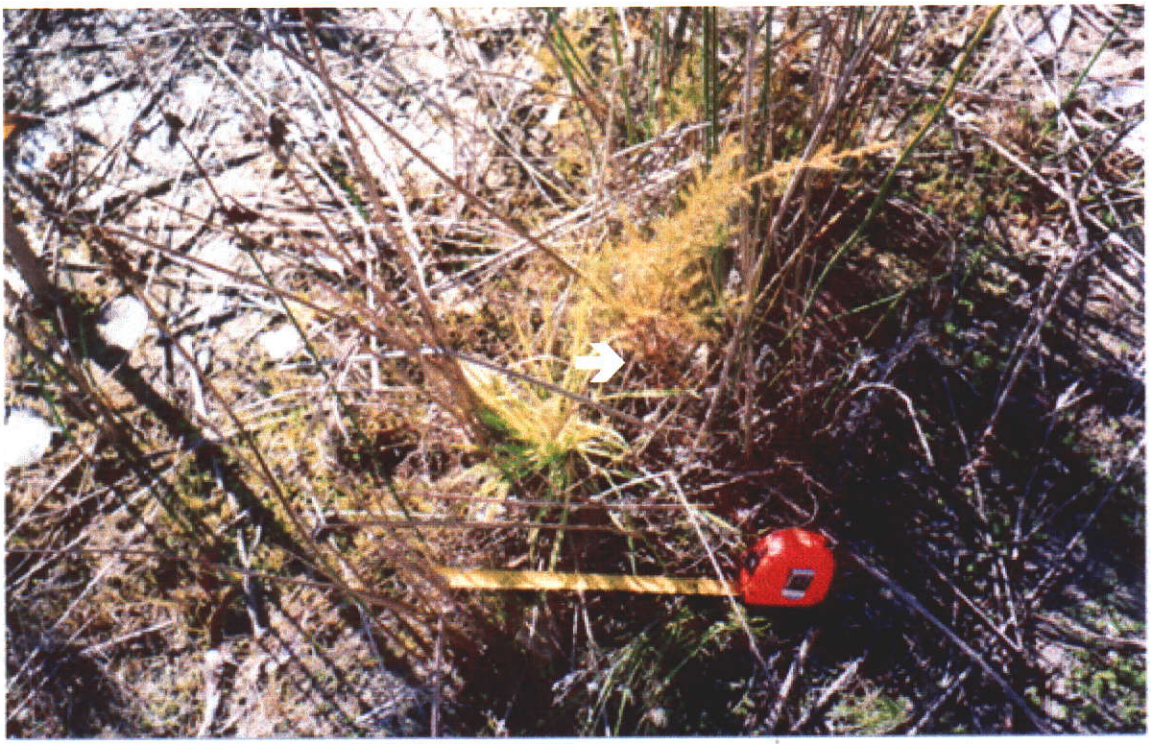

Figure 4.10: Weeds infesting cow manure treatment, killing *E. grandis* seedling (dead seedling indicated by arrow) (tape is set at 30 cm)

Discussion

Cow manure provided higher nutrition, pH and moisture levels than the other treatments. It is believed that the high nutrient levels were exploited by the *Eucalyptus* seedlings allowing faster growth. The pH levels may have rendered nutrients more available, which in turn increased shoot growth. The higher moisture levels in summer may have also increased plant growth by reducing drought stress.

The potting mix treatments and additional lime initially raised soil pH but these treatments became as acidic as the overburden material within 30 months. It is thought that lime reacted with incoming acidic seepage and had been neutralised over time. The materials also had not increased water-holding capacity of the overburdens at the end of the trial period or provided adequate nutrition for the plants. Thus soil conditions were

not improved. Potting mix did not assist plant growth during the entire experiment. Lime, whether applied singly or with the manure or potting mix, did not significantly affect plant growth in any of the tested measurements. It is concluded that neither of these treatments applied in the manner used in this trial are considered appropriate for improving seedling growth on the Collie overburdens.

There have been some successful reports of manure application in improving overburden conditions and plant growth in coal mine revegetation. Chicken manure was applied at Angelsea coal mine in Victoria on acidic overburden at a rate of $50m^3$/ha to increase the organic matter in the soil from the original 1-2% (Tacey, Olsen & Watson 1977). Cow manure increases the pH of metal contaminated soil and lowers the uptake of the metals Cd, Cu, Ni and Zn by plants in alum shale soil (Narwal & Singh 1998). Manure has much potential in mining revegetation.

Plants grew considerably over the 1997-1998 summer months but not the following summer (1998-1999). It is believed that the soils and substrates had been depleted of nutrients, as with the species selection experiment (Chapter 2). Cow manure has a low concentration of nutrients compared to chemical fertilisers. One tonne of typical cow manure contains approximately the same amount of nitrogen, phosphorus and potassium as 50 to 100 kg of modern concentrated fertiliser (Simpson 1986). Application of manure only temporarily increases nutrient and organic content within the soil. The manure rapidly decomposes and releases nutrients; very little is held in the soil over the long term (Magdoff 1992). This experiment concludes that cow manure can only enhance seedling growth over the first year.

Most weeds were Mediterranean annuals. It is believed that some seeds were present in the manure and others may have been wind dispersed from the nearby pasture. Weed growth was most prolific in the manure substrates when receiving adequate moisture from winter and spring rainfall, then died back in the drier summer months. Weeds mainly affect plant growth by competing for light, nutrients, water and space. Young weeds typically exhibit a rapidly spreading and deeply penetrating root system, which

offers an advantage in obtaining water and nutrients (Muzik 1970). Plants respond to competition similarly as to water and nutrient deficiency (Sadanandan & Sands 1993). Many weeds also exude inhibitors, which further reduce plant growth (Muzik 1970). Eucalypts are highly vulnerable to weed competition during the first year (Clemens & Starr 1985; Eldridge *et al.* 1994). Successful production of plantation forests is dependent on sound weed management practices (Sadanandan & Sands 1993). *Pinus radiata* plantations in Australia and New Zealand often carry out removal of non-crop vegetation to enhance tree growth (Richardson 1993).

If cow manure is to be used in revegetating acidic coal overburden, then some form of weed control may be necessary, such as use of sterilisation and herbicides. Sterilisation may prevent weed seeds within the manure germinating, but does not prevent germination of wind borne seeds that enter the revegetating site.

Organic matter can retain herbicides longer in soils, allowing more effective treatment against weeds. Biodegradation of the herbicide metazachlor was slower when adsorbed in soil treated with organic matter than metazachlor in control soil (Rouchaud *et al.* 1995). A single treatment in early autumn may be effective in reducing the growth and propagation of weeds, and therefore reduce the negative impact on eucalypt seedling growth.

To conclude, application of 5L of cow manure but not potting mix around eucalypt seedlings increased growth on acidic coal overburdens. Application of 1 L of lime did not improve plant growth whether applied singly or with cow manure or potting mix. Cow manure aided seedling growth by providing more nutrients, improving soil pH and water holding capacity, but encouraged weed proliferation. If cow manure is to be used in enhancing eucalypt seedling establishment on acidic coal overburdens, some form of weed management is required.

Chapter 5 - Supplementary application of nitrogen and phosphorus fertilisers

Introduction

Mining waste typically lacks nutrients essential for plant growth. Nitrogen, phosphorus and potassium are the most common macronutrients deficient in mine spoils (Burns 1989). The main source of potassium is from soil mineralisation, while the main source of nitrogen and phosphorus is soil organic matter.

Most soil nitrogen is unavailable to plants until converted by micro-organisms into ammonium (NH_4^+) and nitrate (NO_3^-) (Allison 1973), both of which can be taken up by plants (Handreck & Black 1991). Nitrogen is a component of many compounds made by plants, such as amino acids, proteins, nucleic acids, chlorophyll, enzymes, coenzymes, ATP, alkaloids and genes (Dell 1996). Nitrogen is needed in high concentrations in tissues of active growth, such as flowers, fruits, young leaves and root tips. Phosphorus is required in shoot and root elongation, in photosynthesis, protein and cell production and energy transfer (Handreck & Black 1991). It is present in nucleic acids, phospholipids and phosphoproteins and is used in the regulation of some enzymes (Dell 1996).

The most common initial symptom of nutrient deficiency is retarded growth. This is difficult to determine without a well-fed control for comparison (Handreck & Black 1991). Foliar symptoms are often the first signs of nutrient deficiency within eucalypts (Dell 1996). Severe deficiency symptoms are: stunting of growth; off-colour, distorted, mottled leaves; leaves prematurely dying; stems twisted and distorted; and, poor root systems (Handreck & Black 1991).

Nitrogen deficiency reduces protein synthesis and plant growth. As nitrogen is a component of chlorophyll, an inadequate supply of nitrogen causes leaves to appear pale yellow (Allison 1973). Carbohydrates, not being used for protein production, may accumulate in the plant (Dell 1996). Leaves produced may be small and stalks stunted (Handreck & Black 1991). Symptoms of nitrogen deficiency appear initially in older

leaves. Early deficiency symptoms in eucalypts include inter-veinal areas of mature leaves becoming pale green. This spreads to younger leaves and the symptoms become stronger. As deficiency continues, all leaves become uniformly pale yellow in colour (Dell & Robinson 1993). In some species, appearance of red spots appears on older leaves and then abscises (Dell 1996).

Phosphorus deficiency can affect carbohydrate biochemistry and transportation (Dell 1996). Plants may display general stunting and little branching. Leaves may first turn blue-green or purple and then slowly turn yellow (Handreck & Black 1991). Phosphorus deficiency first appears in older leaves as small interveinal reddish spots (for example, *E. diversicolor*, *E. maculata* and *Eucalyptus urophylla* S. T. Blake) or necrotic margins (for example, *Eucalyptus globulus* Labill. subsp. *globulus*). Some leaves become dark bluish green (*E. marginata*). Advanced phosphorus deficiency symptoms include stunted growth and red pigmentation on leaves (Allison 1973). The entire plant may become red (*E. urophylla*). Pigmented patches may occur with pale brown necrotic patches (*E. maculata*); the new leaves are small, the plant is stunted and the older leaves abscise (Dell 1996). Phosphorus deficiency in *E. marginata* decreases the dry weight of root and shoot, causes leaves to be small in size and purple with prominent red veins (Dell, Jones & Wilson 1987).

Fertilisers have been shown to improve numbers, density and growth rates of *Eucalyptus* species in many rehabilitated areas in Australia. Type, amount and rate of fertilisers depend upon the site soil characteristics and future land use. Applications up to 80 kg ha^{-1} of both elemental nitrogen and phosphorus have been used for some Australian minesites using natives for rehabilitation (EPA 1995). It is not always known how long initial fertilisers may last in the soil. Initial fertilisers may only last for one to two years. After initial nutrients are exhausted, a steady decline in biomass and ground cover may be seen. Long-term productivity of the plant/soil system is dependent on: 1) accumulation of organic matter and nitrogen; and, 2) the establishment of an organic phosphorus pool. Both N and P requirements are dependent on introduction and functioning of microbiological communities, such as mycorrhiza (Daniels & Zipper 1995). In the context of acidic soils, the question of repeating fertilisation depends

partly on the complex soil chemistry of acid sulphide soils as pH affects nutrient availability and plant respiration processes (Farmer, Richardson & Brown 1976).

Ammonium nitrate is one of the most common nitrogen fertilisers used because of its high N content (33%). It is of relatively low cost (Allen 1987); has no residue; half the nitrogen is immediately available and the other half available over the longer term; and, there is a minimal loss to volatilisation (Traynor 1980). Superphosphate has been found to be the best phosphorus fertiliser for use in Australia. This is due to two reasons. The grains of superphosphate are up to 2 mm across, and each acts as a depot of soluble phosphate, which may last for several weeks. Secondly, the precipitates formed between the soluble phosphate and the soil are far more finely dispersed into the surface soil than any powder that could be prepared (Leeper & Uren 1993).

Eucalypt seedlings were planted at the Ewington 2 lake site in June 1997 in a species selection field trial (Chapter 2). Seedling growth rate declined after the first year and leaves turned red in colour. It was postulated that the lack of growth and change in leaf colour were due to the plants having exhausted available nutrients in the overburden. This chapter describes a fertiliser trial which was superimposed on the original experiment to determine whether the lack of either nitrogen or phosphorus, or both, was responsible for the observed plant responses.

The null hypotheses are:

H_o1: Application of fertiliser does not significantly increase growth of *Eucalyptus* seedlings on acidic overburdens.

H_o2: Application of fertiliser does not significantly increase growth of weeds on acidic overburdens.

H_o3: Application of fertiliser does not significantly improve foliage colour of *Eucalyptus* seedlings on acidic overburdens.

Method

On July 15th 1999, 50 g single superphosphate fertiliser (= 9 g elemental P; 4.5 kg P ha^{-1}) was applied to half the eucalypt plants in the Ewington species plot on the soil surface within 10 to 20 cm radius of the bole. Plants were selected randomly for P addition. Plant height, crown diameter, stem diameter and leaf colour and weed cover were recorded.

On August 20th 1999, 20 g ammonium nitrate (= 7 g elemental N; 7 kg N ha^{-1}) was applied to half the plants that received phosphate and to half the eucalypt plants that did not receive phosphate. These selections were made randomly. The balance were taken as controls. Measurements were again recorded. The fertiliser was again applied on the surface soil within a 10 to 20 cm radius of the bole. Measurements of plant height, crown diameter, stem diameter, upper crown leaf colour and weed cover were taken again on October 25th (3 months) and December 15th 1999 (5 months) after fertilising. In addition to the previous measurements, new growth - number of new crown leaves and stems and number of epicormic leaves and stems – were also recorded.

P is less bioavailable than N and may be locked up by calcium and metal ions, so the P fertiliser was applied earlier and in larger amounts than N fertiliser to ensure adequate uptake by the eucalypt seedlings.

Parametric statistics were performed on plant measurements by testing data for homogeneity with boxplots and scatterplots and then using the General Linear Model (GLM). Non-parametric statistics were used for leaf colour with the Kruskal Wallis test statistics and Student-Newman-Keuls test. Leaf colour was measured with RHS charts and ranked from most red to most green.

A map of the fertiliser applications in the Ewington species site trial is located in Appendix 8.

Results

Separating the seedlings of all the eucalypt species into treatment groups, there were 13 control plants, 14 plants received P fertiliser only, 15 plants received N fertiliser only and 16 plants received N+P fertiliser. There were no significant differences in plant dimensions or weed cover between treatments at the start of the experiment in July 1999 (Table 5.1).

Table 5.1: Mean measurements of 58 eucalypts July 1999 (0 months)

Measurement	control	P	N	N+P
n	13	14	15	16
Height (cm)	90.2	90.4	82.1	89.5
Crown diameter (cm)	57.8	65.5	57.8	57.7
Stem diameter (mm)	13.24	14.93	13.20	13.70
Height/stem diameter ratio	69.73	61.86	63.31	64.73
Weed cover around bole (%)	4.4	2.8	3.0	2.6

After 3 months (October 1999), considerable growth was evident and additional dimensions were measured: new and epicormic leaves and stems (Table 5.2). The fertiliser addition did not have any significant effect on the standard plant measurements or the percentage of weeds immediately around the seedlings. N addition significantly increased the numbers of new crown stems and leaves ($P < 0.001$ for both) and epicormic stems and leaves ($P = 0.005$ and 0.002 respectively). There was also an interaction of the two fertilisers – plants receiving both N and P grew more new crown stems and leaves than with either fertiliser alone ($P = 0.014$ and 0.016).

Table 5.2: Mean measurements of 58 eucalypts October 1999 (3 months after treatment)

Measurement	control	P	N	N+P
n	13	14	15	16
Height (cm)	91.9	99.2	87.3	93.7
Crown diameter (cm)	52.3	60.4	58.2	61.2
Stem diameter (mm)	13.38	15.76	14.31	15.43
Height/stem diameter ratio	57.17	47.14	44.14	52.86
0-3 months new growth				
New crown stems (No.)	23.3 c	15.2 c	32.1 b	54.4 a
New crown leaves (No.)	80.3 c	53.8 c	114.8 b	185.8 a
Epicormic stems (No.)	3.0 b	2.8 b	12.2 a	8.4 a
Epicormic leaves (No.)	9.5 b	2.6 b	43.9 a	38.8 a
Weed cover around bole (%)	21.0	16.4	19.0	20.2

Treatments with the same letter in the measurement row do not differ significantly at $P = 0.05$

By December, N and combined N+P treatments had significantly increased mean crown diameters, numbers of new crown stems and leaves (P = 0.032, 0.001 and 0.002 respectively) but not height, stem diameters, height/stem diameters ratio or epicormic stems or leaves (Table 5.3). P alone did not significantly increase growth. The amount of foliage growth is concluded to be dependent on availability of N and not P. Therefore H_o1 is rejected: application of N fertiliser does significantly increase growth of *Eucalyptus* seedlings on acidic overburdens.

Table 5.3: Mean measurements of 58 eucalypts December 1999 (5 months)

Measurement	control	P	N	N+P
n	13	14	15	16
Height (cm)	92.8	106.0	100.5	103.7
Crown diameter (cm)	56.6 b	70.0 b	75.5 a	77.0 a
Stem diameter (mm)	13.32	16.99	15.67	16.32
Height/stem diameter ratio	71.63	63.64	65.37	64.28
0-5 months new growth				
New crown stems (No.)	24.2 b	21.6 b	40.7 a	46.6 a
New crown leaves (No.)	55.8 b	71.1 b	99.4 a	122.0 a
Epicormic stems (No.)	1.5	0.1	3.0	2.9
Epicormic leaves (No.)	8.2	1.2	18.3	12.8
Weed cover around bole (%)	16.3 b	16.1 b	33.9 a	37.8 a

Treatments with the same letter in the measurement row do not differ significantly at $P = 0.05$

N increased the percentage of weeds around the stem of the seedlings by December 1999 (Table 5.3). Plants receiving N fertiliser had significantly more weeds within a 20 cm radius of the bole than plants not receiving fertiliser (P = 0.015). Therefore H_o2 is rejected: application of N fertiliser does significantly increase growth of weeds on acidic overburdens.

Many of the control plants still displayed red foliage in October (3 months after treatment). The plants receiving P and plants with both N+P were significantly greener than plants receiving N alone, the latter being significantly greener than the controls (Figure 5.1).

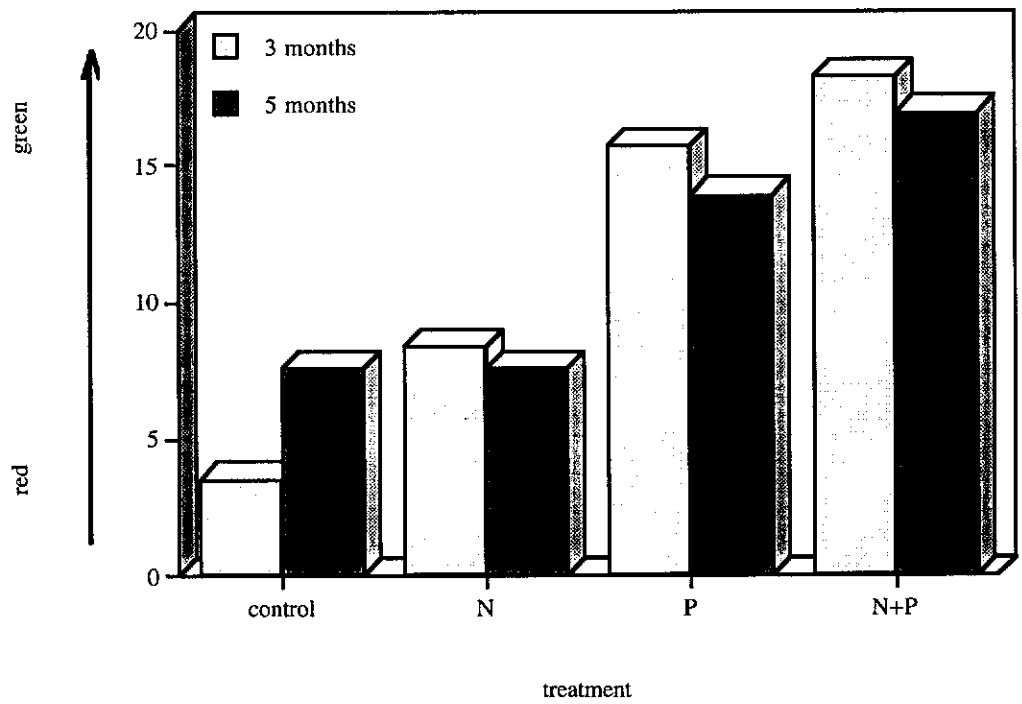

Figure 5.1: Upper crown leaf colour ranking of seedlings at 3 & 5 months (RHS charts)

Most of the eucalypts no longer had red leaves at December 1999, when less variation in leaf colour between the treatments was observed. Analysis revealed that plants given N+P were significantly greener than those given P alone, the latter being significantly greener than the plants given N alone or the controls (Figures 5.1 to 5.3). N alone did not significantly affect the leaf colour. Thus $H_0 3$ is rejected: application of P fertiliser does significantly improve foliage colour of *Eucalyptus* seedlings on acidic overburdens.

Figure 5.3: *E. cladocalyx* seedling after P application (5 months)

Figure 5.2: *E. cladocalyx* seedling before P application (0 months)

Discussion

Under the conditions of this trial, retardation of seedling growth appeared primarily due to N deficiency. Application of N fertiliser resulted in an increase in new foliar growth of the eucalypts within three months. However, N fertiliser also promoted weed growth around seedlings by two times. N application needs to be minimal to reduce weed growth yet adequate for transplanted seedling growth. The recommended level of N fertilisers on rehabilitated bauxite mines to give optimal growth of desired species with least weeds was 72 kg ha^{-1} N (Koch, Sudmeyer & Pickersgell 1988), twice the level applied in this experiment. It is possible that the two sites differ in moisture availability and weed species, affecting weed proliferation. Rehabilitated sites need then to be assessed individually to determine optimal amounts of N fertiliser.

Red foliage is a symptom primarily associated with P deficiency. P fertiliser increased leaf greenness and this was further enhanced with N. N alone did not significantly alter leaf colour. As stated earlier, N and P are both needed for photosynthesis. N is a component of chlorophyll, while P is required for energy transfer. Nitrogen and phosphorus act synergistically in plant growth. Phosphorus adsorption is affected by nitrogen. Plant growth is increased by nitrogen, thus increasing the foraging capacity of roots for phosphorus. Phosphorus can also improve uptake of nitrogen (Allison 1973). Plants displaying red foliage should be treated with fertiliser containing both N and P.

Continued growth of eucalypts on acidic materials requires supplementary fertilisation after initial planting. These findings are supported by other research on revegetating acidic coal overburdens. Application of N and P was found appropriate for early growth and establishment of *Eucalyptus patens* on two acidic overburdens (pH 4.5 and 4.6) from Muja Open Cut in Collie (Fox, Frost & Doronila 1993). Seedlings of *Eucalyptus patens, E. calophylla, E. cladocalyx* and *Melaleuca* species grew better in acidic coal overburden when given N and P fertilisers than when given N or P alone (Fox & Doronila 1992). *E. camaldulensis* and *E. wandoo* grew best in acidic coal interburden

material when supplied with NPK fertiliser than those given incomplete fertilisers (Fox & Colquhoun 1987).

These findings also support research on overburdens from bauxite mining in the northern Jarrah forest. *E. marginata* and *E. calophylla* seedlings growing on rehabilitated bauxite mines significantly increased in height when supplied with N and P (Lockley and Koch 1996). Both *E. maculata* and *E. marginata* seedlings responded better to combined N and P fertilisers than N or P alone (Koch, Sudmeyer, & Pickersgill 1988). *E. globulus, E. microcorys* and *E. resinifera* significantly increased in height and stem diameter when given N and P than either N or P alone (Ward 1983).

Future assessments of N and P deficiency within vegetation on acidic overburdens should be done with chemical analysis of the plant tissue. Analysis of foliage is commonly used in determining any nutrient deficiency (Burns 1989). Leaves collected from the upper crown branch, amongst the innermost leaves, can provide accurate nutrient levels. Fast growing plants are light demanding and shoot growth is greatest in the upper crown. As existing branches are covered by the new upper branches and become shaded, the leaf production and branch growth of the former is reduced and becomes erratic. Also, leaf age and growth rate is difficult to determine amongst the lower branches, so nutrient contents would be difficult to compare (Lamb 1976).

Chemical testing need not only be based on leaf samples. It is thought the bark may be more appropriate to test for phosphorus deficiency in eucalypts such as *Eucalyptus marginata*. Phosphorus levels in the bark have been shown to be higher than in leaves and to be sensitive to age and phosphate bioavailability (Dell, Jones & Wilson 1987). Samples of twigs are also thought to be better than leaves for diagnosing nitrogen and phosphorus deficiencies in eucalypts (Grove 1990). Stems from five tested *Eucalyptus* species all increased strongly in phosphorus content to according levels of phosphate addition. Phosphate concentrations in leaves were lower and were weakly correlated to phosphate addition (Dell, Loneragan & Plaskett 1983).

If fertiliser is to be applied to acidic overburdens, the level needs to be sufficient to ensure an adequate proportion is available for plant absorption. Fertiliser applied to spoils may be less available to plant growth than if the same amount was applied to normal soil. Barley (*Hordeum vulgare*) is not able to attain as much N fertiliser from coal mine spoils in Colorado USA than from topsoil from the same mine site (Reeder & Berg 1977). Certain nutrients, like P, may be bound in the upper surface, reducing bioavailability. Much of the phosphate fertiliser applied on four acidic sites in Otago New Zealand was locked up into iron and aluminium phosphates over five years (Floate & Enright 1991).

To maximise growth, eucalypts should be pre-tested to determine the preferred form or combination of forms of N and P fertiliser. Woodland eucalypts *E. meliodora* Cunn ex Shuer, *E. blakelyi* Maiden and *E. albens* Benth. respond differently to N type than forest eucalypts *E. rossii* Baker & Smith and *E. sideroxylon* Cunn ex Woolls (Moore & Keraitus 1971). Maximum growth of *Eucalyptus maculata* and *E. pilularis* Smith seedlings in pots containing sand was obtained when the NH_4^+:NO_3^- ratio was 3:7 (Halsall, Forrester & Moss 1983). Variation in N source also affects susceptibility of seedlings to Al. Roots of both *Lupinus luteus* and *Secale cereale* seedlings were inhibited more when given NO_3^- than NH_4^+ (Grauer & Horst 1990). However, NH_4^+ frequently increases P uptake more than NO_3^-. This is thought to reflect ion balance, cells can uptake the cation NH_4^+ and the anion $H_2PO_3^-$ more readily than both NO_3^- and $H_2PO_4^-$, which would require balancing cations which may not be available in sufficient amounts. Plants also usually absorb more P as primary orthophosphate ($H_2PO_4^-$) than secondary orthophosphate (HPO_4^{2-}), especially in low pH soils (Allison 1973). Therefore fertilisers containing NH_4^+ and $H_2PO_4^-$ may be most suitable for supplying N and P to plants on acid soils, provided the revegetating species are to highly tolerant to the Al levels present in the overburden.

It is concluded that if mined areas are to be revegetated efficiently, eucalypt seedlings may require sufficient supplementary fertiliser until plants have reached a state of nutrient recycling. The role of fertilisers is further discussed in Chapters 6 and 9.

Chapter 6 – Applying lime and fertiliser to seedlings

Introduction

Application of lime and fertiliser has been found to be necessary for plant survival on coal overburden in Collie (Koch 1984). Plants are unlikely to survive on acidic spoils without an amendment or a covering (Bartle & Riches 1978). Soil amendments may be required to increase the supply of nutrients, and to improve the physical, chemical and water-holding properties of the soil. This amendment may be with fertilisers, lime, or waste products (Jackson 1991).

Previous chapters have indicated positive plant responses from receiving fertilisers in the form of cow manure (Chapter 4) and as straight nitrogen or phosphorus fertilisers (Chapter 5). Another method of supplying plant nutrition is with slow release fertilisers. This may be useful with winter establishment, where waterlogging may occur (Fox, Colquhoun & Leone 1987). Slow release fertilisers have the advantage of regulating the release of nitrogen, which is highly soluble. Application of straight nitrogen fertilisers may initially burn plant roots and then be leached away before the plant can take up much of the nutrient. The release of nitrogen is controlled by temperature. Nitrogen is commonly supplied in slow release fertiliser as urea formaldehyde polymers, which are broken down by microbial activity. Since microbial activity is controlled by temperature, the release of ammonia also varies with temperature. Nitrogen is thus released more in times of warmer weather, when plant growth is higher and demand for nitrogen is also high. Conversely, it is released less in times of cooler weather, when plant growth is less (Sheat & Schofield 1999).

Successful vegetation with substantial liming can control the pH of acid soils (Sorenson *et al.*1980). Nutrient uptake of plants may also be improved by reducing the effects of acidity in reducing fine root production, so increasing the ability to take up nutrients, allowing greater shoot mass (Helyar 1991; Bakker *et al.*1999). It is generally agreed that a pH of 6.0 to 6.8 is preferred for most plants (Knuti & Korpi 1970; Cummings & Elliot

1991). On acidic soils, lime application may be the only option for reducing acidity levels.

Lime can affect the elemental balance and chemistry within soils. Liming can enable the soil to both improve in terms of availability of nutrients and by holding more nutrients (Handreck & Black 1991). Liming can free soil phosphorus from iron and aluminium oxides (Leeper & Uren 1993). Liming may supply calcium and magnesium (Ministry of Agriculture, Fisheries & Food 1981) and enhance bioavailability of many trace elements such as manganese, copper, zinc, boron, molybdenum and cobalt (Boynton 1980). Increasing the soil pH may also decrease metal toxicity by reducing ions to non-bioavailable forms (Aitken, Stephenson & Gallagher 1990; Gupta & Singh 1990; Aitken, Dickson & Moody 1998).

Liming can also provide a long-term effect of producing new compounds from organic matter and improve the structure of the soil (Leeper & Uren 1993). Lime also assists in sealing the surface (Tacey, Olsen & Watson 1977). Liming may increase the level of soil organic matter, which in turn increases the amount of soil micro-organisms. The microbial populations can then break down organic matter and fix nitrogen, improving nutrition supply and soil structure (Boynton 1980). Liming may also increase plant resistance to diseases (Ministry of Agriculture, Fisheries & Food 1981).

Liming materials are products that may be used to neutralise acidic soils. Ideal treatment material should contain useful cations, such as calcium, magnesium and potassium. Compounds containing calcium and magnesium are often cheaper than potassium compounds and are therefore more commonly used. Calcium carbonate (as limestone) is the most widely used liming material. This material is not pure and may contain small amounts of magnesium carbonate, silicate minerals and others (Handreck & Black 1991).

It is common practice in mining revegetation to apply both lime and fertilisers. Liming may increase efficiency of fertilisers by increasing bioavailability and by counteracting

the acid properties of certain fertilisers, such as ammonium nitrate and ammonium sulphate (Boynton 1980). A combined treatment holds the potential to increase plant growth on acidic soils further than either single treatments of fertiliser or lime.

An experiment was conducted in 1999 at the WO5H mine site to determine the effects of lime and slow release fertiliser tablets on establishment of seedlings growing on acidic coal overburden and to discover any positive interaction from combining the treatments.

The null hypotheses are:

H_o1: Lime or fertiliser does not significantly increase plant growth on acidic coal overburdens.

H_o2: There is no significant difference between levels of fertiliser treatment on plant growth on acidic coal overburdens.

Method

Seeds of *E. camaldulensis, Eucalyptus calophylla Labill* (syn. *Corymbia calophylla* (Labill.) Hillis & Johnson), *E. diversicolor, E. robusta* and *E. rudis* were collected from Collie dumps in December 1998, germinated in February 1999 and transplanted into Queensland forestry pots (cylinders 17 cm tall, 3 cm diameter) containing a 1:1:1 mixture of fine sand, coarse sand and peat. The seedlings were given half doses of Thrive™ fertiliser (Appendix 3) once every four weeks for 12 weeks. Seeds of *Kunzea ericifolia* and *Callistachys lanceolatum* Ventenat (syn. *Oxylobium lanceolatum*) were germinated in August 1998. In June 1999 the latter plants, then being over 50 cm tall, were trimmed to a height of 30 cm and given 50 mL of full strength Thrive™ fertiliser.

Numbers of similar sized seedlings per species were selected on June 1st 1999. The species numbers were divided by 9 to determine number of sets for that particular species: *E. calophylla* , *E. diversicolor* and *E. rudis* had 9 sets; *E. robusta* 8 sets; *E. camaldulensis* and *Kunzea ericifolia* 6 sets; and, *Callistachys lanceolatum* 4 sets.

Wesfarmers Coal Pty Ltd in Collie completed mining at a void classified as WO-5H in 1998. The void has pierced the water tables and is slowly filling with ground water. The entire void is expected to be full within 8 to 10 years. There are currently benches of exposed acidic overburden (pH 4.3) which will be eventually inundated by the rising water table. The material has similar low levels of nutrients, conductivity and organic carbon as Muja and Ewington, but is very variable in Al content (soil samples at 5 to 15 cm depth - mean 65.0 ppm, SD 47.8).

The seedling trials commenced in early June 1999. Nine blocks of 18 m x 18 m were set out at the WO5H site. Each block was subdivided into nine plots of 4 m x 4 m, arranged in 3 columns and 3 rows, with a 2 metre gap between the plots and a 1 m border surrounding the plots. Each plot allowed a maximum of nine seedlings to be planted in a 3 row by 3 column pattern, with 2 m spacings between plants. The overall configuration allowed a maximum of 9 plant to be planted in a row or column of the block (Figure

6.1). Seedlings were planted in a random design for each block. The random designs were the same for each plot within that block but were different from plots of other blocks. Up to 7 species of seedlings were planted in each block. Those species with 81 seedlings (*E. calophylla, E. diversicolor* and *E. rudis*) had a seedling planted in each plot of every block. Species with fewer seedlings (*E. camaldulensis, E. robusta, C. lanceolatum* and *K. ericifolia)* – had seedlings planted within each plot of randomly chosen blocks.

Each plot was randomly designated a different treatment containing one of the three levels (none, low or high) of lime and fertiliser. Nine combinations were tested: no fertiliser no lime (control); no fertiliser low lime; no fertiliser high lime; low fertiliser no lime; low fertiliser low lime; low fertiliser high lime; high fertiliser no lime; high fertiliser high lime; and, high fertiliser high lime. Lime levels consisted of 0, 100 g (= 1 t ha^{-1}) and 400 g (= 4 t ha^{-1}) per planting spot. At the site of planting, a hole of approximately 1 L volume was dug. The dug overburden was mixed with lime, then placed back into the hole around the seedling roots. Fertiliser treatments consisted of 0, 1 or 2 x 10 g Agriform™ 9 month slow release fertiliser tablets (Appendix 3). The tablets were placed approximately 5 cm adjacent to the roots. The seedlings were planted within each plot at 2 m spacings.

Each plot contained up to seven plants, each of a different species. The plants were measured for height, mean crown diameters, basal stem diameters and leaf colour at the beginning of the experiment, 2 monthly for 6 months and again at 12 months. Initial seedling heights did not significanty differ between treatments.

Data was tested for homogeneity with boxplots and scatterplots. Statistical analysis was performed using General Linear Modelling (GLM), and oneway ANOVAS. Fishers LSD test was used to discriminate between measurements where statistical differences were obtained.

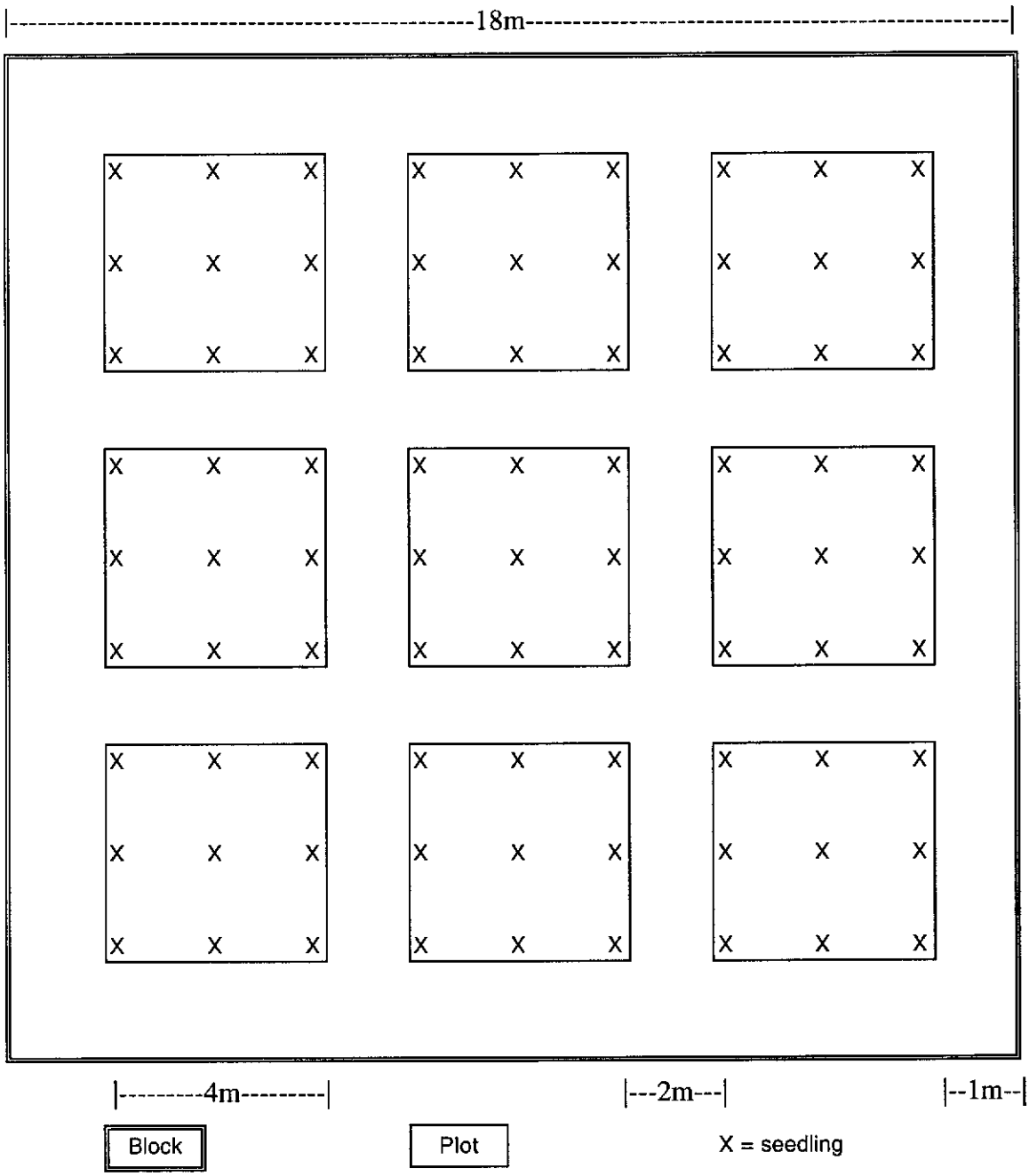

Figure 6.1: Schematic diagram of random block design for lime + fertiliser seedling trial

Results

There was no significant difference between treatment sets for any species at the beginning of the experiment (Tables 6.1 to 6.3). At 2 months, there was again no significant difference between treatments with the exception of *E. calophylla* crown diameters (P = 0.013) and *E. robusta* stem diameters (P = 0.012) when given only fertiliser.

By 4 months, fertiliser significantly increased heights, crown and stem diameters of all seedlings except *E. camaldulensis, C. lanceolatum* and *K. ericifolia* (Tables 6.1 to 6.3). No significant difference was found to be associated with lime for any of the tested species. No significant fertiliser/lime interactions were found. Results at 6 months were similar and indicated greater significant differences due to fertiliser treatment but none for lime or the interaction. Significant differences in height, crown diameters and stem diameters with *E. camaldulensis* were found due to the fertiliser treatment (P = 0.026, 0.003 and 0.024 respectively). *C. lanceolatum* and *K. ericifolia* continued to show no significant differences due to either fertiliser or lime treatment. There was no significant differences in leaf colour between treatments for any of the species.

At 12 months, all eucalypt seedlings indicated a highly significant positive growth response to fertiliser ($P < 0.001$) for all three measurements. However, leaf colour did not vary over time or between treatments. *C. lanceolatum* seedlings did not significantly differ in height with fertiliser treatment but did vary in crown and stem diameters (P = 0.018 and 0.011 respectively). Only crown diameter in *K. ericifolia* gave a response to fertiliser (P = 0.004). There were again no significant differences due to lime or lime and fertiliser interaction in any measurement for any species. Plants grew more when given fertiliser (Figure 6.2). Lime alone did not stimulate plant growth (Figure 6.3) nor did it further enhance growth of plants given fertiliser. Again, there was no significant differences in leaf colour between treatments for any of the species.

Therefore H_o1 is rejected : fertiliser, but not lime, does significantly increase plant growth on acidic coal overburdens.

Table 6.1: GLM of mean seedling heights given lime and fertiliser at WO5H

Species	Treatment		
	Fertiliser	Lime	F*L interaction
0 months			
Eucalyptus calophylla	0.759 (NS)	0.795 (NS)	0.503 (NS)
E. camaldulensis	0.808 (NS)	0.411 (NS)	0.155 (NS)
E. diversicolor	0.659 (NS)	0.121 (NS)	0.910 (NS)
E. robusta	0.963 (NS)	0.334 (NS)	0.495 (NS)
E. rudis	0.077 (NS)	0.604 (NS)	0.531 (NS)
C. lanceolatum	0.539 (NS)	0.351 (NS)	0.115 (NS)
K. ericifolia	1.000 (NS)	1.000 (NS)	1.000 (NS)
2 months			
Eucalyptus calophylla	0.757 (NS)	0.874 (NS)	0.833 (NS)
E. camaldulensis	0.678 (NS)	0.779 (NS)	0.808 (NS)
E. diversicolor	0.935 (NS)	0.205 (NS)	0.587 (NS)
E. robusta	0.163 (NS)	0.804 (NS)	0.560 (NS)
E. rudis	0.048 (NS)	0.233 (NS)	0.663 (NS)
C. lanceolatum	0.169 (NS)	0.802 (NS)	0.847 (NS)
K. ericifolia	0.679 (NS)	0.722 (NS)	0.326 (NS)
4 months			
Eucalyptus calophylla	0.032 (*)	0.923 (NS)	0.546 (NS)
E. camaldulensis	0.240 (NS)	0.175 (NS)	0.377 (NS)
E. diversicolor	<0.001 (***)	0.565 (NS)	0.829 (NS)
E. robusta	<0.001 (***)	0.611 (NS)	0.832 (NS)
E. rudis	<0.001 (***)	0.635 (NS)	0.944 (NS)
C. lanceolatum	0.530 (NS)	0.653 (NS)	0.946 (NS)
K. ericifolia	0.314 (NS)	0.885 (NS)	0.801 (NS)
6 months			
Eucalyptus calophylla	<0.001 (***)	0.970 (NS)	0.631 (NS)
E. camaldulensis	0.026 (*)	0.491 (NS)	0.919 (NS)
E. diversicolor	0.183 (NS)	0.377 (NS)	0.455 (NS)
E. robusta	<0.001 (***)	0.372 (NS)	0.834 (NS)
E. rudis	<0.001 (***)	0.903 (NS)	0.632 (NS)
C. lanceolatum	0.721 (NS)	0.649 (NS)	0.828 (NS)
K. ericifolia	0.505 (NS)	0.299 (NS)	0.689 (NS)
12 months			
Eucalyptus calophylla	<0.001 (***)	0.779 (NS)	0.926 (NS)
E. camaldulensis	<0.001 (***)	0.133 (NS)	0.837 (NS)
E. diversicolor	<0.001 (***)	0.866 (NS)	0.910 (NS)
E. robusta	<0.001 (***)	0.125 (NS)	0.898 (NS)
E. rudis	<0.001 (***)	0.459 (NS)	0.397 (NS)
C. lanceolatum	0.211 (NS)	0.657 (NS)	0.614 (NS)
K. ericifolia	0.252 (NS)	0.398 (NS)	0.972 (NS)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table 6.2: GLM of mean seedling crown diameters given lime and fertiliser at WO5H

Species	Treatment		
	Fertiliser	Lime	F*L interaction
0 months			
Eucalyptus calophylla	0.270 (NS)	0.784 (NS)	0.583 (NS)
E. camaldulensis	0.678 (NS)	0.923 (NS)	0.988 (NS)
E. diversicolor	0.576 (NS)	0.551 (NS)	0.720 (NS)
E. robusta	0.430 (NS)	0.208 (NS)	0.703 (NS)
E. rudis	0.301 (NS)	0.683 (NS)	0.276 (NS)
C. lanceolatum	0.397 (NS)	0.483 (NS)	0.952 (NS)
K. ericifolia	0.311 (NS)	0.486 (NS)	0.648 (NS)
2 months			
Eucalyptus calophylla	0.013 (*)	0.323 (NS)	0.244 (NS)
E. camaldulensis	0.631 (NS)	0.503 (NS)	0.844 (NS)
E. diversicolor	0.484 (NS)	0.088 (NS)	0.691 (NS)
E. robusta	0.122 (NS)	0.112 (NS)	0.249 (NS)
E. rudis	0.057 (NS)	0.847 (NS)	0.188 (NS)
C. lanceolatum	0.248 (NS)	0.477 (NS)	0.865 (NS)
K. ericifolia	0.852 (NS)	0.200 (NS)	0.492 (NS)
4 months			
Eucalyptus calophylla	<0.001 (***)	0.426 (NS)	0.865 (NS)
E. camaldulensis	0.085 (NS)	0.206 (NS)	0.396 (NS)
E. diversicolor	<0.001 (***)	0.242 (NS)	0.377 (NS)
E. robusta	<0.001 (***)	0.132 (NS)	0.242 (NS)
E. rudis	<0.001 (***)	0.883 (NS)	0.360 (NS)
C. lanceolatum	0.781 (NS)	0.921 (NS)	0.644 (NS)
K. ericifolia	0.956 (NS)	0.073 (NS)	0.899 (NS)
6 months			
Eucalyptus calophylla	0.001 (**)	0.348 (NS)	0.893 (NS)
E. camaldulensis	0.003 (*)	0.086 (NS)	0.764 (NS)
E. diversicolor	<0.001 (***)	0.089 (NS)	0.616 (NS)
E. robusta	<0.001 (***)	0.156 (NS)	0.594 (NS)
E. rudis	<0.001 (***)	0.449 (NS)	0.548 (NS)
C. lanceolatum	0.687 (NS)	0.371 (NS)	0.358 (NS)
K. ericifolia	0.214 (NS)	0.350 (NS)	0.731 (NS)
12 months			
Eucalyptus calophylla	<0.001 (***)	0.735 (NS)	0.926 (NS)
E. camaldulensis	<0.001 (***)	0.392 (NS)	0.780 (NS)
E. diversicolor	<0.001 (***)	0.607 (NS)	0.888 (NS)
E. robusta	<0.001 (***)	0.151 (NS)	0.916 (NS)
E. rudis	<0.001 (***)	0.459 (NS)	0.397 (NS)
C. lanceolatum	0.018 (*)	0.505 (NS)	0.935 (NS)
K. ericifolia	0.004 (**)	0.901 (NS)	0.411 (NS)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table 6.3: GLM of mean seedling stem diameters given lime and fertiliser at WO5H

Species	Treatment		
	Fertiliser	Lime	F*L interaction
0 months			
Eucalyptus calophylla	0.320 (NS)	0.471 (NS)	0.926 (NS)
E. camaldulensis	0.062 (NS)	0.235 (NS)	0.700 (NS)
E. diversicolor	0.507 (NS)	0.472 (NS)	0.935 (NS)
E. robusta	0.937 (NS)	0.494 (NS)	0.839 (NS)
E. rudis	0.163 (NS)	0.237 (NS)	0.289 (NS)
C. lanceolatum	0.591 (NS)	0.804 (NS)	0.467 (NS)
K. ericifolia	0.963 (NS)	0.405 (NS)	0.886 (NS)
2 months			
Eucalyptus calophylla	0.172 (NS)	0.273 (NS)	0.257 (NS)
E. camaldulensis	0.551 (NS)	0.168 (NS)	0.574 (NS)
E. diversicolor	0.768 (NS)	0.866 (NS)	0.395 (NS)
E. robusta	0.012 (*)	0.727 (NS)	0.953 (NS)
E. rudis	0.069 (NS)	0.675 (NS)	0.801 (NS)
C. lanceolatum	0.294 (NS)	0.775 (NS)	0.616 (NS)
K. ericifolia	0.458 (NS)	0.523 (NS)	0.853 (NS)
4 months			
Eucalyptus calophylla	0.010 (*)	0.729 (NS)	0.762 (NS)
E. camaldulensis	0.296 (NS)	0.088 (NS)	0.341 (NS)
E. diversicolor	0.001 (**)	0.349 (NS)	0.777 (NS)
E. robusta	<0.001 (***)	0.231 (NS)	0.356 (NS)
E. rudis	<0.001 (***)	0.296 (NS)	0.803 (NS)
C. lanceolatum	0.902 (NS)	0.683 (NS)	0.431 (NS)
K. ericifolia	0.592 (NS)	0.163 (NS)	0.872 (NS)
6 months			
Eucalyptus calophylla	<0.001 (***)	0.781 (NS)	0.699 (NS)
E. camaldulensis	0.024 (*)	0.177 (NS)	0.962 (NS)
E. diversicolor	<0.001 (***)	0.070 (NS)	0.457 (NS)
E. robusta	<0.001 (***)	0.194 (NS)	0.319 (NS)
E. rudis	<0.001 (***)	0.770 (NS)	0.585 (NS)
C. lanceolatum	0.779 (NS)	0.382 (NS)	0.885 (NS)
K. ericifolia	0.910 (NS)	0.780 (NS)	0.875 (NS)
12 months			
Eucalyptus calophylla	<0.001 (***)	0.491 (NS)	0.505 (NS)
E. camaldulensis	<0.001 (***)	0.126 (NS)	0.620 (NS)
E. diversicolor	<0.001 (***)	0.526 (NS)	0.879 (NS)
E. robusta	<0.001 (***)	0.041 (NS)	0.449 (NS)
E. rudis	<0.001 (***)	0.696 (NS)	0.675 (NS)
C. lanceolatum	0.011 (*)	0.212 (NS)	0.523 (NS)
K. ericifolia	0.368 (NS)	0.423 (NS)	0.769 (NS)

Analysis of variance: ***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Figure 6.3: *Callistachys lanceolatum* given 400 g lime at 12 months

Figure 6.2: *Callistachys lanceolatum* given 20 g fertiliser at 12 months

As lime appeared to have no affect on plant growth, each of the lime data sets at 12 months was then combined with the three fertiliser treatments for each species. Oneway analysis of variance was then conducted to determine if any variation could be detected among the fertiliser treatments.

Fertiliser significantly increased height and crown and stem diameters of most species when given either fertiliser treatment (Figures 6.4 to 6.6). *K. ericifolia* had significantly larger crown diameters when given 20 g but not 10 g fertiliser ($P = 0.002$). *Callistachys lanceolatum* did not differ significantly in height between treatments. Most species did not differ significantly in height, crown and stem diameter between fertiliser treatments. *E. robusta* seedlings given 20 g fertiliser were significantly larger in height and crown diameters than those given 10 g fertiliser, the latter being significantly larger than the control plants ($P < 0.001$ for both). *E. diversicolor* seedlings given the high fertiliser treatment had significantly larger stem diameters than the low fertiliser treatment ($P < 0.001$). In general, plant growth was increased similarly with either fertiliser treatment. H_o2 is accepted: there is no significant difference between level of fertiliser treatment on plant growth on acidic coal overburdens.

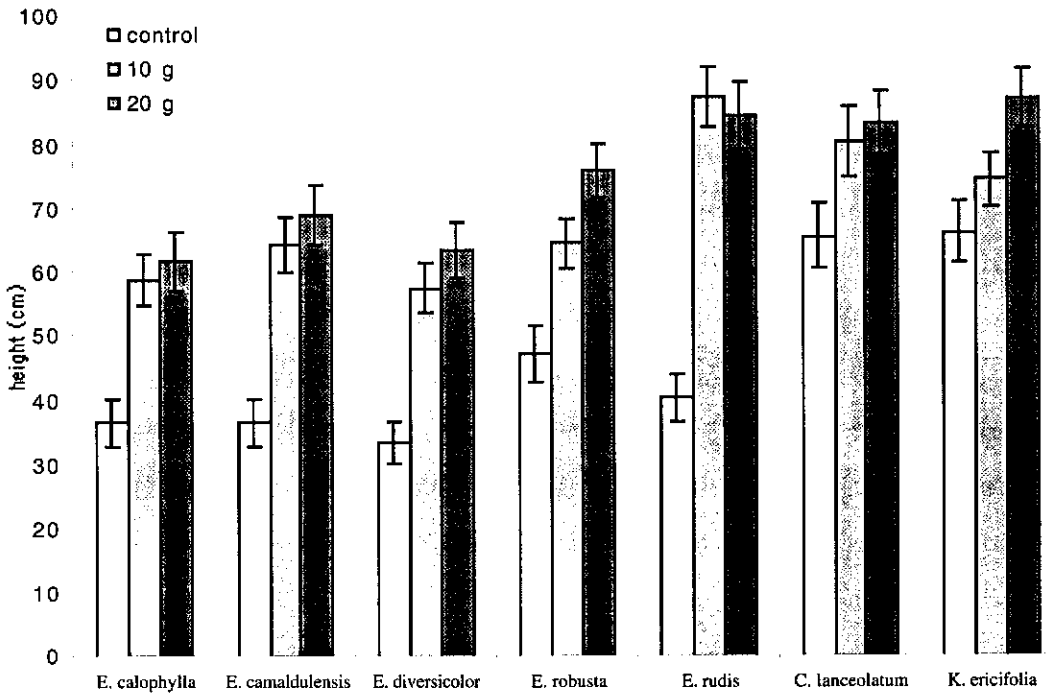

Figure 6.4: Mean heights (cm) and SE of seedlings given fertiliser at WO5H

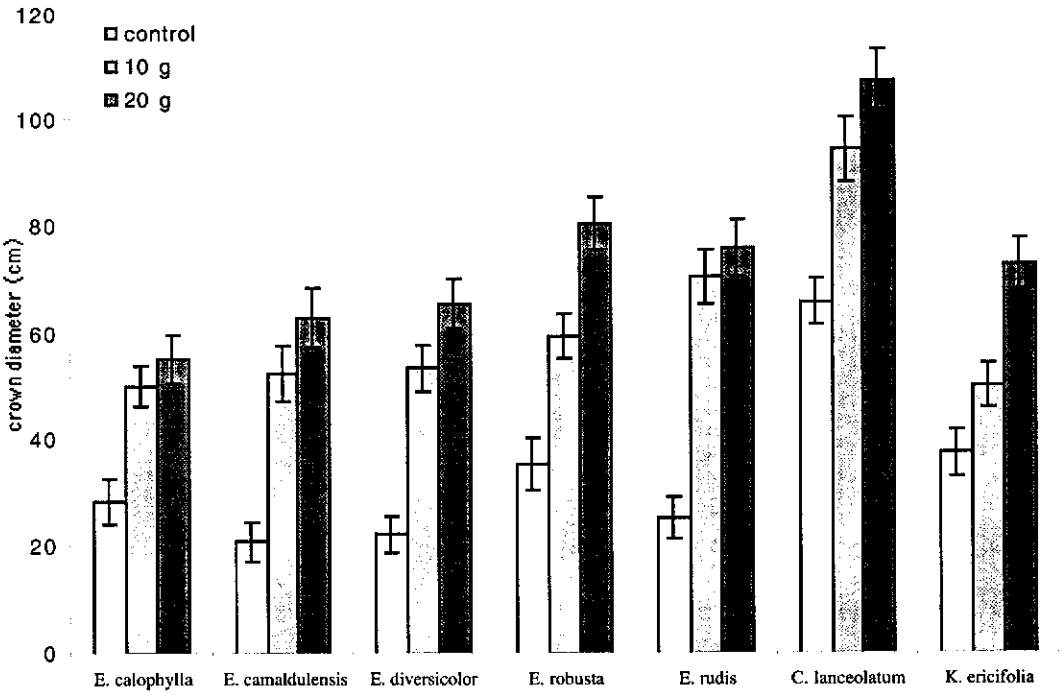

Figure 6.5: Mean crown diameters (cm) and SE of seedlings given fertiliser at WO5H

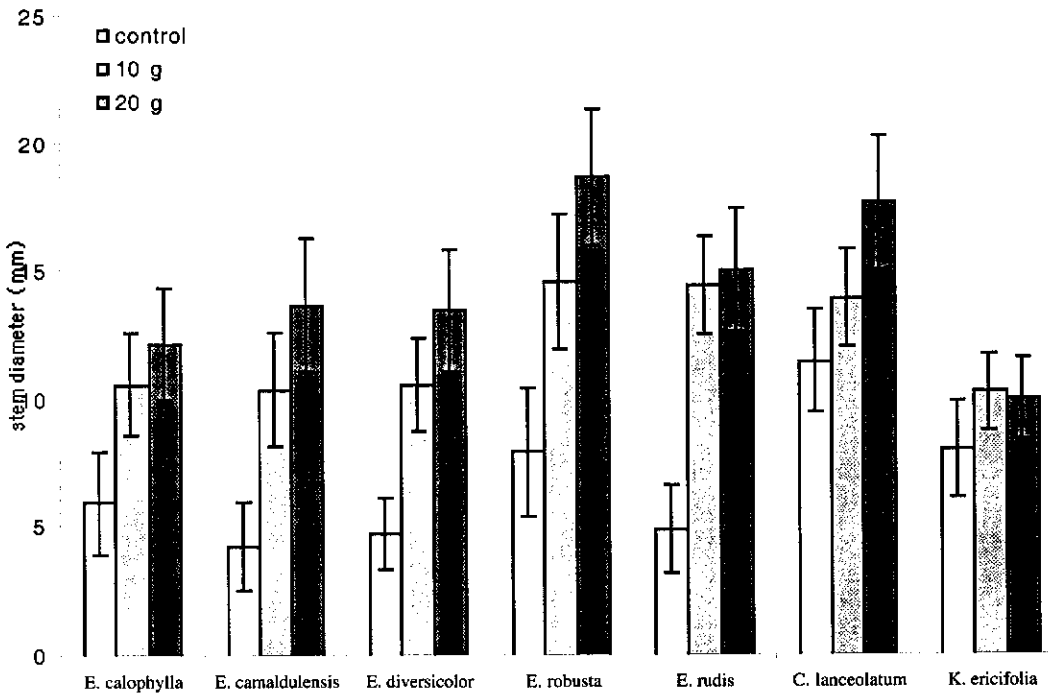

Figure 6.6: Mean basal diameters (mm) and SE of seedlings given fertiliser at WO5H

Discussion

Nutrition is a major factor limiting plant growth on acidic spoil, as indicated in Chapter 5. There have been many other studies showing the benefits of fertiliser on native plant growth on acidic soils (Leggate 1980; Koch 1984; Fox, Colquhoun & Leone 1987; Egerton-Warburton 1989; Fox & Doronila 1992; Fox, Frost & Doronila 1993). If a soil contains an abundant supply of a nutrient, it may be adequate for plant growth despite the unfavourable pH (Handreck & Black 1991; Leeper & Uren 1993). It is then clearly important to add fertilisers to seedlings to enhance revegetation efficiency of acidic spoils.

Seedlings appeared to grow more with the 20 g treatment than the 10 g treatment, but this was not statistically significant due to the soil heterogeneity of the site. However, the fertiliser used was a slow release tablet, so it is as yet unknown whether the single tablet treatment is sufficient for longer term plant survival and establishment, or whether a treatment with a higher amount of fertiliser released will be desirable to improve growth over a longer period. At four years, *E. marginata* seedlings in a mined bauxite area were significantly taller when given 100 and 200 g slow release fertiliser than 50 g (Ward & Koch 1994) and at 8 years, seedlings given 200 g were significantly taller than those given 100 g (Koch *pers comm.*).

This study reveals that liming, whether applied singly or with fertiliser, did not improve plant growth. Liming was also shown in Chapter 4 to not improve seedling growth on acidic soils when combined with manure or potting mix. There are many other instances where liming acid soil does not clearly improve soil conditions or plant growth (Owens & Fox 1988; Wang *et al.* 1999) nor increase growth of plants receiving fertiliser (Dell, Loneragon & Plaskett 1983; Myers, Lispsett & Kirchner 1983; Mason 1989; Dravid & Goswami 1991; Ingerslev & Hallbacken 1999). However, there have been cases where plant growth on acid soil was improved when lime was added alone (Koch & Bell 1983; Longhurst, O'Conner & Toxopeus 1999) and when included with fertiliser treatment

(Davison & Jefferies 1966; Richardson 1980). Liming may be a successful treatment only in certain site conditions but not in others.

It is important to consider the various interactions between soil and plants when recommending liming of acidic soils (Haynes 1982). It is difficult to predict how a plant will react to liming, regardless of how acidic the soil may be. It is also difficult to conclude with certainty that any response of the plant was from liming the soil (Leeper & Uren 1993). The effects of liming may also vary from time to time. Agricultural crops on the same acidic soils are known to sometimes respond well to liming, but other times respond weakly or even give no response (Lightfoot *pers comm.*). Caution must be taken in predicting the outcome of revegetating an acidic site where the soil has been treated with lime.

Lime has only limited benefits to soil conditions. Liming may only reverse the metal toxicity, not the soil weathering caused by the acidity (Goulding & Blake 1998). It is not an equal substitute to topsoil replacement (Koch 1984). Liming generally does not ameliorate subsoil acidity (Campbell 1999). Changing the pH cannot increase the availability of nutrients if the soil does not initially contain those nutrients (Handreck and Black 1991). Liming cannot increase microbial activities if there are negligible amounts of organic matter (Lightfoot *pers comm.*). Acidic coal overburdens in Collie contain virtually no organic matter, nutrients, nor microbial life, so liming cannot assist in improving these conditions for plant growth.

It is difficult to determine how much lime is required to treat a site. Mine spoils are typically heterogenous in potential acidity, so each area requires different liming treatment. Laboratory samples of Collie soils determined that the amount of lime needed to bring the spoils to a pH of 6.5 varied from 12.5 t ha^{-1} to 208 t ha^{-1} (Collie Coal Mines Rehabilitation Committee 1981). Whether the amount of lime applied was adequate may have varied within the site.

It is possible that the size of the lime particles were too large for optimally raising the soil pH. Particles of less than 0.15 mm in diameter dissolve quickly in the soil and may be used up within four weeks whereas particles of 1.5 mm dissolve and react over several years (Boynton 1980; Scott *et al.* 1992). Lime diffuses through the soil slowly, so only the soil immediately surrounding the particle can be neutralised. Finer lime particles may ameliorate more of the soil volume by decreasing the distance between particles (Scott *et al.* 1992). The larger particles may also remain unreactive in the soil (Whitten *pers comm*). The coarse texture of the limestone makes limestone less effective in ameliorating pyritic material than flyash or hydrated lime (Hossner & Porter 2000). The lime particles used in this experiment were less than 2.5 mm in diameter. Most of the lime was of coarse size (>1.5 mm) so only a small amount of the lime used, resulting in the soils only being slightly raised in pH.

It must also be noted that this trial only ran for 12 months and seedlings were planted immediately after liming. Plots should be limed for a considerable period (perhaps a year) before planting seedlings, for the soil to be adequately conditioned (Lightfoot *pers comm.*). Liming acid soils may not be seen as beneficial within the first year of application, but over a ten year period (Sandison 1994). The effect of liming on plant growth may not be noticed for several years, as it takes time for the neutralising process to occur, for microbes to change soil conditions and for lime to work deeper down into the soil profile.

In agriculture, paddocks are monitored for up to 5 years to evaluate success of liming on both soil pH and plant growth (Lightfoot *pers comm.*). Tang & Rengel (2001) showed that acidic paddock soil had reduced in acidity and extractable Al levels 16 yrs after liming. Once the soil is raised to a neutral pH, it may buffer any increase in hydrogen ions for up to several years (Scott *et al.* 1992; Whitten *pers comm.*). Though this experiment found no short-term benefits of liming, this method should not be dismissed but it is recommended that this trial be monitored over a longer period to fully assess the potential value of lime addition in revegetating these acidic spoils.

However, the effect of liming soils may not last for an appreciable period. Liming soils that undergo acidic seepage is difficult, as the accumulating influx of metals, especially aluminium, may lock up the carbonate, rendering the lime inactive (Whitten *pers comm.*). In addition, subsoil acidity may nullify the effects of liming surface soil (Lightfoot *pers comm.*). The constant influx of hydrogen ions will also be continuously reacidifying the soil, eventually exhausting the applied lime. Lime can only raise the pH of such soils for a limited period. Many species were able to survive for only a year on coal overburdens in Collie (pH <4.0) when the surface was amended with 10 t ha^{-1} lime (Bartle & Riches 1978). Liming coal overburdens (pH 3.75 to 4.50) at Blackbird minesite in Idaho, USA, only kept the soil neutral for less than two years (Farmer, Richardson & Brown 1976).

In conclusion, fertiliser is vital for successful revegetation of acidic spoils, though the amount required is still unknown. It is also postulated that the small liming ability of the limestone was only able to adjust the soil pH to a small fraction and was not able to permanently improve soil conditions.

Chapter 7 – Mycorrhizal symbioses on acidic coal overburdens

Introduction

Mycorrhizal fungi and plants formed a symbiotic relationship from the Ordovicia era, some 460 million years BP (Redecker *et al.* 2000). These fungi function as an extension to the plant root system, and explore soil pore spaces for nutrient elements and water, and transfer these to the plant. In return, the fungus gains carbohydrates from the host (Smith & Read 1997). From a functional perspective, mycorrhizal plants are more resistant to drought, high temperatures, toxins, high acidity and pathogens than non-mycorrhizal plants (Marx & Cordell 1988; Smith & Read 1997). For example, mycorrhizae enhance the drought tolerance of loblolly pine (*Pinus taeda*) and rose (*Rosea hybrida*) by altering root morphology and carbon allocation patterns of shoots and roots, thereby maintaining high transpiration rates (Davies *et al.* 1996).

Mycorrhizae improve plant growth by increasing the uptake of essential nutrients, such as nitrogen, phosphorus, potassium, calcium, iron and manganese (Archer, Hodges & LeHunt 1993). Trees with abundant mycorrhizae have been found to collect more nutrients and water than their non-mycorrhizal counterparts. For example, mycorrhizal fungi can increase uptake of phosphorus (Barrow 1977; Harley & Smith 1983; Abbot & Robson 1984; Bougher, Grove & Malajczuk 1990) and nitrogen (Adams & Attiwell 1986). Root nodulation in legume plants may be enhanced, improving nitrogen fixation. Soils with mycorrhizae have been shown to have more beneficial soil organisms than non-mycorrhizal soils (Archer, Hodges & LeHunt 1993). This symbiosis compensates for the lower amount of fine roots and absorption capacities of eucalypts, in comparison to agricultural crops (Grove, Thomson & Malajczuk 1996).

Mycorrhizae may also improve plant tolerance to toxic metals. There are three main mechanisms for metal toxicity by mycorrhiza: 1) accumulation of metals; 2) exclusion of metals; and, 3) production of extracellular polysaccharides or mucilage (Denny & Wilkins 1987). *Eucalyptus rudis* seedlings inoculated with *Pisolithus tinctorius* and

growing in pots containing aluminium accumulated lower levels of aluminium, while the mycorrhiza had aluminium concentrated within the sheaths around the roots. It is thought that the mycorrhiza was more efficient in reducing aluminium absorption by seedlings than the seedling roots (Egerton-Warburton, *et al.* 1993). Seedlings of *Pinus rigida* increased in growth due to *P. tinctorius* inoculation overriding the response of the seedlings to aluminium toxicity (Schier & McQuattie 1996). *Pisolithus tinctorius* and *Scleroderma* species grown in liquid cultures are able to withstand high concentrations of aluminium, iron, copper and zinc (Egerton-Warburton & Griffin 1995; Tam 1995).

Importantly, mycorrhizae may affect the success of a plant growing on in reclaimed land by providing nutrients and water and protection against toxic metals (Allen & Friese 1990). In addition, perennial plants that can form mycorrhizal associations may require less long-term fertiliser management (Bougher, Grove & Malajczuk 1990). The Ranger uranium mine in the Northern Territory includes mycorrhizal fungi instead of fertiliser in revegetation procedures, as fertiliser application tends to encourage dense grass cover, thus inhibiting tree growth (Environmental Protection Agency 1995).

Seven types of mycorrhizal associations are recognised. Of these, two of the main types are ectotrophic (EM) and arbuscular (AM) mycorrhizae. Ectomycorrhizae are typically formed by Basidiomycetes and Ascomycetes and form a mantle around the roots and a Hartig net between root cells. In contrast, arbuscular mycorrhizae are formed by fungi from the order Zygomycetes and produce arbuscles, vesicles and hyphal coils within cortical cells of the roots. More than 95% of all plant families are mycorrhizal (Smith & Read 1997). Australian plant genera that can be infected with AM and EM include *Acacia, Agonis, Allocasuarina, Callistachys, Gompholobium, Eucalyptus, Melaleuca* and *Oxylobium* (Brundett *et al.* 1996).

However, not all mycorrhiza exert the same effect on host growth (Bougher *et al.* 1991). It is important that selected strains of fungi can be demonstrated to form mycorrhiza with the host in site-specific conditions before being used in mining revegetation (Thompson & Medve 1984). Plants infected with strains of mycorrhiza grown from

Collie mine sites are more able to acquire nutrients than the same plants infected with strains collected from adjacent forest sites (Egerton-Warburton 1993). Inoculation of seedlings with specific ectomycorrhiza has been observed to increase plant growth in countries such as the Philippines, the Congo and Australia (Grove, Thomson & Malajczuk 1996).

Pisolithus tinctorius is a common coloniser of coal mine dumps and often forms ectomycorrhizal relationships with *Eucalyptus* species. The fungus also occurs in soils with low organic matter, similar to mine wastes (Bougher & Malajczuk 1990). It has been shown to establish quickly through acid coal spoils in Kentucky and Virginia, initiating mycorrhizae with the plants and producing many fruiting bodies (Marx & Artman 1979). The fungus has been shown to increase growth of *Eucalyptus* species in difficult soil conditions (Dixon & Hiol-Hoil 1992). *P. tinctorius* can also tolerate extreme soil conditions such as high acidity and high temperature found in coal spoils. The fungus has been found as a wide range of host strains (Chilvers 1972). These features give it a competitive edge over many other mycorrhizae (Marx, Bryan & Cordel 1977).

This chapter demonstrates the extent of mycorrhizal colonisation by EM and AM on mine soils, and the role of the EM fungus *P. tinctorius* on growth and establishment of selected plant species in acidic coal overburdens. The null hypotheses are:

H_o1: Plants do not differ significantly in mycorrhizal infections between acidic overburden sites or over time.

H_o2: Plants on acidic overburdens do not differ significantly in mycorrhizal infection type.

H_o3: Plants inoculated with *P. tinctorius* do not significantly increase in growth or in mycorrhizal infection.

Method

Experiment 1: Natural colonisation of roots by mycorrhizal fungi

Fine roots at 2 to 10 cm depth were collected at 5 to 15 cm distance from seedling stems from species selection plots at the two acidic overburdens Muja and Ewington 2 (Chapter 2) in July 1998 and July 2000. Samples from three plants of eight species were taken – *E. cladocalyx, E. gomphocephala, E. maculata, E. rudis, E. wandoo, C. rupestris, K. ericifolia* and *M. hamulosa.* Roots were preserved in formic acid and alcohol solution (FAA).

Experiment 2: Inoculation trial

Sporocarps of *Pisolithus tinctorius* were collected from coal overburden sites in Collie in September 1998. Cultures were isolated on full strength potato dextrose agar (PDA). All cultures were grown at 25^0 C in the dark. Seedlings of *Eucalyptus calophylla, E. camaldulensis, E. diversicolor, Eucalyptus patens* Benth., *E. robusta, E. rudis* and *Allocasuarina fraseriana* (Miq.) Johnson (syn. *Casuarina fraseriana*) were germinated in February 1999 in addition to seedlings used in the fertiliser and lime experiment at WO5H (Chapter 6). Five seedlings of *Callistachys lanceolatum* and *Kunzea ericifolia* were also included in this experiment. Seedlings of the latter two species were also trimmed to 30 cm height and given 50mL of full strength Thrive™ (Appendix 3) four days before transplanting, because the seedlings were larger and older.

Nine separate 4 m by 4 m plots were laid out between blocks used in the lime and fertiliser experiment (Figure 7.1). Seedlings were transplanted in the same week as the lime and fertiliser experiment (June 1999) at 2 m spacing, with a 1 m border around the edge of the plot. One seedling of *A. fraseriana* and of each *Eucalyptus* species were planted into each plot. *C. lanceolatum* and *K. ericifolia* seedlings were randomly assigned among the plots. None of the seedlings received lime or fertiliser. Initial seedling heights did not significanty differ between treatments.

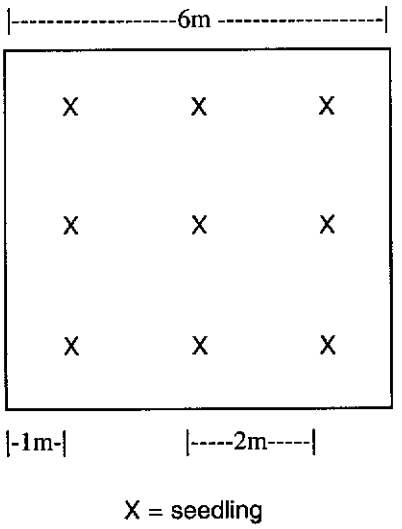

Figure 7.1: Random block design for mycorrhiza trial

One week after transplanting, twenty plates with agar covered in *P. tinctorius* hyphae were blended in 10 L sterilised distilled water. 50 mL of the solution was injected with a syringe into the soil within a 3 cm radius of the bole of each of the seedlings, with the needle tip at 3 cm depth. The seedlings were measured as in the experiment in Chapter 6, at the beginning of the experiment and every 2-mo for 6 mo and again at 12 mo. Control plants were taken as the control plants in the experiment in Chapter 6.

Collecting and staining of roots

Roots from both experiments were stained using the procedure of Brundett *et al.* (1996). Roots were carefully washed with deionised water to remove soil and then cut into 2-4 cm long pieces. Samples were then placed in McCartney bottles, filled halfway with 10% KOH and autoclaved at 121°C for 15 minutes. Roots were then rinsed again with deionised water, placed back into the bottles, filled halfway with 0.03% Chlorazol Black E stain (1:1:1 lactic acid, glycerol and deionised water) and waterbathed at 90°C for

several hours. Roots were again rinsed, returned to the bottles, filled with 50% glycerol solution and left for several days to allow removal of excess stain.

The colonisation measurement method employed was a modification of Brundett *et al.* (1996). A grid of 1.27 cm spacing was drawn on the bottom of a 9 cm diameter petri dish. Each sample was placed into the dish, which was in turn placed under a dissecting microscope and viewed at 4X magnification. Each vertical and horizontal grid line was scanned to quantify the number of intersections between grid lines and roots. Each root counted was assessed for infection, any root part crossing the grid that was infected with either arbuscular or ectomycorrhiza was tallied. The final numbers of arbuscular and ectomycorrhizal infected roots were divided by the total number of intersected roots to determine the percentage of root length colonised by either type of mycorrhiza.

Statistical analysis

Data was tested for homogeneity with boxplots and scatterplots. Statistical analysis was performed by transforming the percentage values to obtain normal distributions, then testing with Oneway ANOVAs and Fishers LSD post-hoc test. Transformation involved dividing the percentage value by 100 to obtain a proportional value between <0.001 and 1.000, which was square rooted then arc sined (Sokal & Rolf 1981). Net field growth of height, crown and stem diameters were calculated by subtracting the initial growth measurements (0 mo) from the final measurements (12 mo) of all surviving seedlings.

Results

Experiment 1: Natural colonisation of roots by mycorrhizal fungi

Fourteen months after inoculation, almost all of the seedlings sampled had roots colonised by mycorrhizal fungi (Table 7.1). The amount of infection varied between sites and was dependant on the plant species. AM infections were absent in *E. maculata* and *E. wandoo* roots collected at Muja, while EM infections were not detected in *C. rupestris* and *M. hamulosa* seedlings collected at Ewington. No significant difference in infection was found between sites for *E. cladocalyx, E. gomphocephala, E. maculata, E. rudis* and *Kunzea ericifolia.* Roots of *E. wandoo* had significantly more AM and EM at the Muja site. *C. rupestris* had higher infections of AM at the Ewington site. In total, roots taken from the Muja site had higher incidence of EM infection and roots taken from the Ewington site had higher percentage of AM infection.

Table 7.1: Mean % infection and SD of mycorrhiza in seedling roots at 14 mo (n=3)

Species	Muja	Ewington 2	*P*
Arbuscular mycorrhiza			
E. cladocalyx	4.0 (4.0)	10.7 (5.0)	0.167 (NS)
E. gomphocephala	17.7 (13.0)	16.0 (14.2)	0.877 (NS)
E. maculata	0	7.0 (9.6)	0.184 (NS)
E. rudis	14.0 (22.5)	16.3 (1.5)	0.539 (NS)
E. wandoo	0	17.3 (3.0)	<0.001 (***)
C. rupestris	0.7 (1.2)	21.3 (3.0)	0.001 (**)
K. ericifolia	8.3 (11.9)	8.7 (9.9)	0.795 (NS)
M. hamulosa	9.3 (7.8)	8.3 (2.5)	0.976 (NS)
Total (n=24)	6.8 (10.9)	13.2 (8.0)	0.002 (**)
Ectomycorrhiza			
E. cladocalyx	13.3 (7.6)	5.0 (8.7)	0.201 (NS)
E. gomphocephala	11.3 (8.6)	1.3 (0.6)	0.092 (NS)
E. maculata	11.7 (7.2)	2.3 (3.2)	0.079 (NS)
E. rudis	28.0 (21.9)	5.7 (5.1)	0.126 (NS)
E. wandoo	30.7 (4.9)	7.7 (5.5)	0.012 (*)
C. rupestris	12.7 (10.8)	0	0.019 (*)
K. ericifolia	1.7 (2.9)	1.3 (2.3)	0.940 (NS)
M. hamulosa	22.3 (11.7)	0	0.004 (**)
Total (n=24)	16.5 (13.0)	2.9 (4.5)	<0.001 (***)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

By 38 months, all roots sampled from both sites had infections of both types of mycorrhiza (Table 7.2, Figures 7.2-7.3). Most species had higher infections at the Muja site. Roots of *E. maculata* and *C. rupestris* from Muja overburden had significantly more AM and EM than those from the Ewington overburden. Roots of *K. ericifolia* from Muja had significantly more AM than roots from Ewington 2 but did not differ significantly in EM infection. Roots of *E. wandoo* and *M. hamulosa* from Muja had significantly more EM than roots from Ewington 2 but did not significantly differ in AM infection. *E. cladocalyx, E. gomphocephala* and *E. rudis* did not significantly differ in amount of either AM or EM infection between sites. In total, Muja had higher percentage infections of both AM and EM than Ewington 2.

Table 7.2: Mean % infection & SD of mycorrhiza in roots between sites at 38 mo (n=3)

Species (n=3)	Muja	Ewington 2	*P*
Arbuscular mycorrhiza			
E. cladocalyx	19.0 (2.9)	18.0 (3.5)	0.484 (NS)
E. gomphocephala	14.6 (5.6)	11.6 (1.2)	0.417 (NS)
E. maculata	16.8 (2.8)	9.2 (3.6)	0.049 (*)
E. rudis	24.2 (2.4)	16.6 (2.5)	0.301 (NS)
E. wandoo	16.4 (12.7)	10.7 (9.4)	0.532 (NS)
C. rupestris	26.3 (9.2)	9.9 (3.6)	0.037 (*)
K. ericifolia	31.7 (3.9)	8.5 (4.2)	0.003 (**)
M. hamulosa	12.6 (7.7)	17.6 (4.3)	0.345 (NS)
Total (n=24)	20.3 (8.4)	13.1 (6.0)	0.002 (**)
Ectomycorrhiza			
E. cladocalyx	22.0 (9.2)	17.4 (5.7)	0.537 (NS)
E. gomphocephala	20.9 (6.4)	20.5 (11.2)	0.889 (NS)
E. maculata	39.1 (5.4)	21.7 (3.8)	0.010 (*)
E. rudis	19.4 (10.0)	15.0 (5.4)	0.601 (NS)
E. wandoo	37.2 (5.7)	13.2 (8.3)	0.028 (*)
C. rupestris	34.3 (10.4)	11.5 (1.4)	0.014 (*)
K. ericifolia	7.2 (4.1)	7.9 (3.7)	0.814 (NS)
M. hamulosa	39.9 (3.2)	23.6 (5.9)	0.018 (*)
Total (n=24)	27.5 (12.8)	16.4 (7.4)	0.002 (**)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

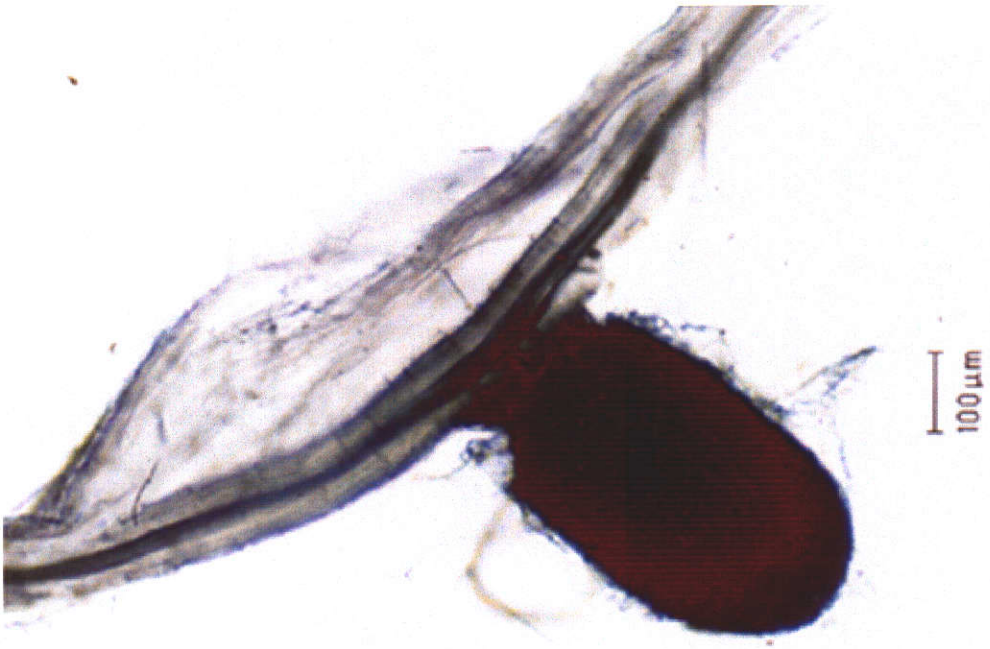

Figure 7.3: Root tip of *C. rupestris* seedling from Muja covered with ectomycorrhizal sheath (38 months)

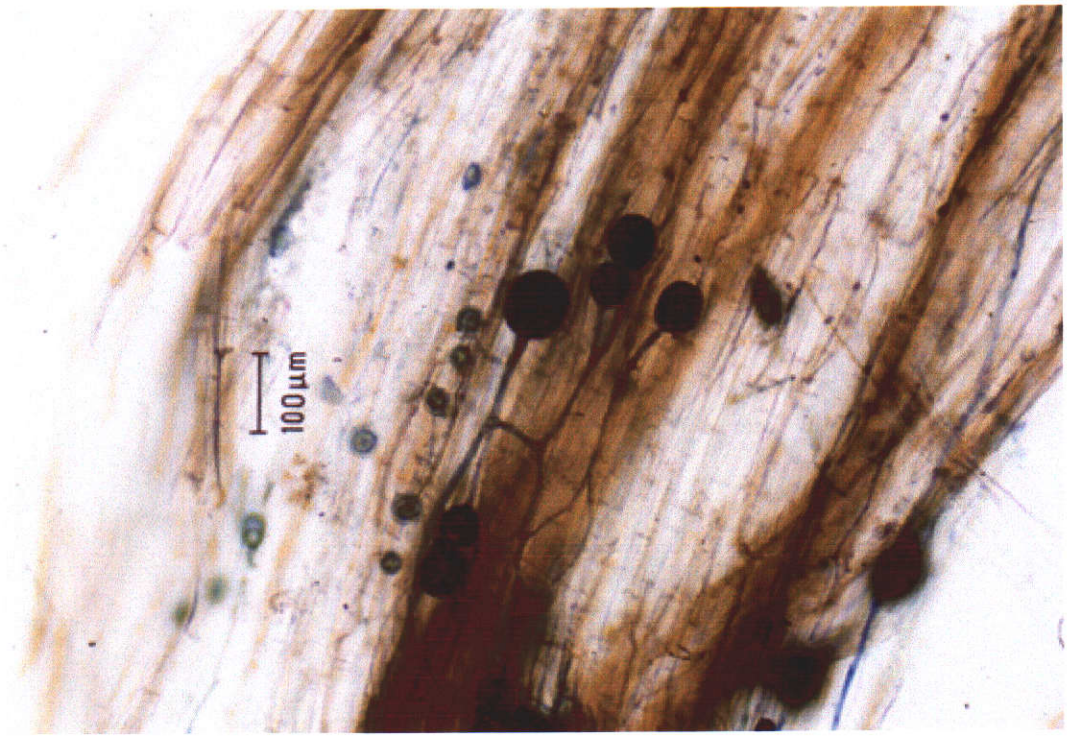

Figure 7.2: Root of *E. cladocayx* seedling from Ewington containing arbuscles (38 months)

The species results for each site were then grouped into sets according to year and type of mycorrhizal infection. The amount of root infection greatly increased over time (Figure 7.4). There were significantly more infections of both AM and EM at 38 months than at 12 months ($P < 0.001$ for both). Seedlings growing at both sites were increasing in infection of both forms of mycorrhiza.

The type of infection was then compared at both times of sampling (Figure 7.4). There was no significant difference in type of infection at 12 months, however there were significantly higher percentages of EM than AM at 38 months. ($P = 0.024$). Plants were able to form more ectomycorrhizal infections than arbuscular infections on the acidic coal overburdens over the 3 year period. Therefore $H_0 1$ is rejected: Plants on acidic overburden sites do significantly differ in mycorrhizal infections between acidic overburden sites and over time.

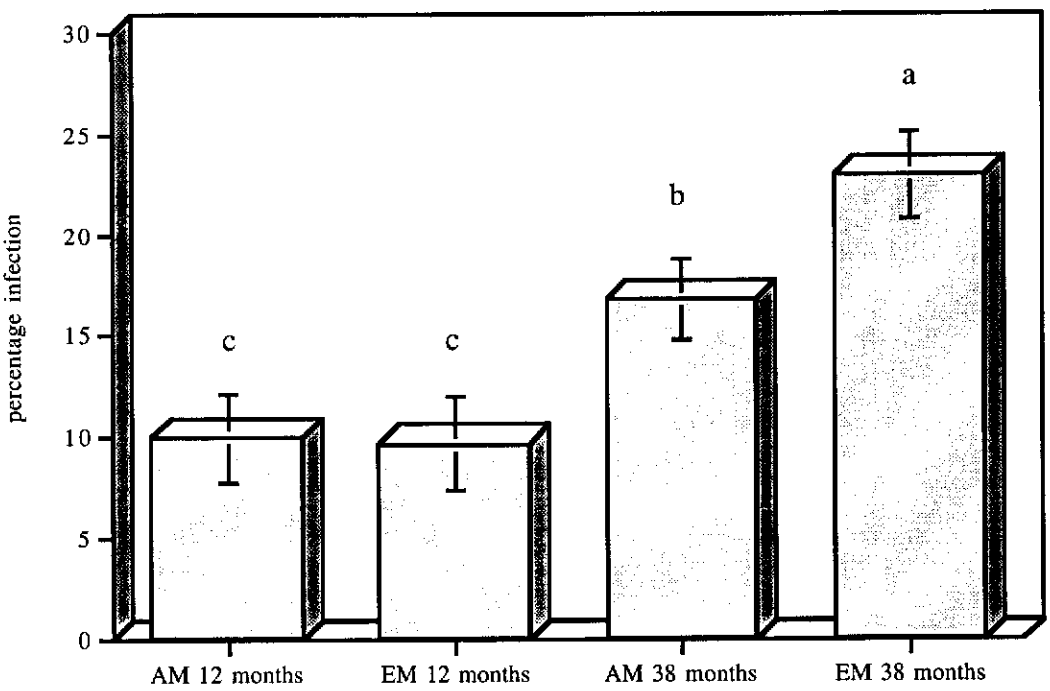

Figure 7.4: Mean root infections % and SE of AM and EM at 12 and 38 months
Bars with same letter show no significant difference

By 38 months, the amount of infections on roots varied between individual plant genera and species. EM infections in the genus *Eucalyptus* were significantly greater than AM (P = 0.009). At the species level, *E. maculata* and *M. hamulosa* roots had significantly higher EM infections than AM infections (P = 0.003 and 0.006 respectively). Roots of *E. gomphocephala* and *E. wandoo* also tended to have higher infections of EM than AM, although not significant. *K. ericifolia* roots also had significantly higher infections of AM than EM (P = 0.048). Therefore $H_0 2$ is rejected: Plants on acidic overburden sites do significantly differ in type of mycorrhizal infection.

Table 7.3: Mean % infection of AM and EM mycorrhiza in roots 38 months

Species	n	AM	EM	F	P
Eucalyptus total	30	16.1 (6.8)	22.6 (10.4)	8.25	0.009 (**)
E. cladocalyx	6	19.0 (3.0)	19.7 (7.3)	0.05	0.915 (NS)
E. gomphocephala	6	13.1 (4.0)	20.7 (8.1)	4.21	0.080 (NS)
E. maculata	6	13.0 (5.0)	30.4 (10.4)	13.55	0.003 (**)
E. rudis	6	21.9 (5.0)	17.2 (7.5)	1.61	0.219 (NS)
E. wandoo	6	13.5 (10.5)	25.2 (14.6)	2.54	0.162 (NS)
C. rupestris	6	18.1 (10.9)	23.0 (14.2)	0.55	0.526 (NS)
K. ericifolia	6	20.1 (13.2)	7.6 (3.5)	5.06	0.048 (*)
M. hamulosa	6	15.1 (6.2)	31.8 (9.9)	12.27	0.006 (**)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Experiment 2: Inoculation trial

None of the nine species showed any significant improvement in height, mean crown diameter or stem diameter with mycorrhiza inoculation either at the beginning of the experiment or at any of the assessment times. Whether inoculated or not, seedlings of these species only approximately doubled in size over the twelve month period (Appendix 9). However net growth of inoculated *E. rudis* and *A. fraseriana* seedlings were significantly larger than control plants (Table 7.4). Only some species significantly increase in growth when inoculated with *P. tinctorius*.

Table 7.4: Mean net growth of inoculated seedlings at WO5H over 12 months

Species	n	Control	n	Inoculated	*P*
Height (cm)					
Eucalyptus calophylla	9	14.0 (15.5)	9	13.8 (6.4)	0.969 (NS)
E. camaldulensis	5	12.6 (8.0)	7	14.0 (4.5)	0.706 (NS)
E. diversicolor	8	11.9 (4.0)	6	10.8 (4.6)	0.659 (NS)
E. patens	4	29.0 (26.3)	4	17.0 (10.8)	0.432 (NS)
E. robusta	7	23.7 (19.0)	9	16.9 (8.3)	0.347 (NS)
E. rudis	7	13.6 (5.7)	7	20.3 (5.3)	0.041 (*)
Allocasuarina fraseriana	3	2.3 (2.5)	4	8.8 (2.8)	0.028 (*)
Kunzea ericifolia	3	17.3 (14.4)	4	14.2 (14.2)	0.188 (NS)
Callistachys bilobum	5	41.0 (35.6)	3	35.5 (9.2)	0.846 (NS)
Crown diameter (cm)					
Eucalyptus calophylla	9	11.7 (15.6)	9	15.8 (32.6)	0.734 (NS)
E. camaldulensis	5	3.8 (4.7)	7	5.0 (5.1)	0.680 (NS)
E. diversicolor	8	4.2 (2.5)	6	3.8 (2.5)	0.766 (NS)
E. patens	4	19.0 (24.8)	4	8.8 (5.1)	0.449 (NS)
E. robusta	7	15.4 (27.9)	9	6.7 (6.8)	0.379 (NS)
E. rudis	7	4.1 (2.5)	7	8.0 (3.8)	0.044 (*)
Allocasuarina fraseriana	3	1.3 (0.8)	4	6.1 (1.5)	0.005 (**)
Kunzea ericifolia	3	14.5 (7.8)	4	18.9 (2.8)	0.191 (NS)
Callistachys bilobum	5	42.6 (25.2)	3	33.5 (5.7)	0.652 (NS)
Basal stem diameter (mm)					
Eucalyptus calophylla	9	3.73 (4.36)	9	3.25 (1.11)	0.751 (NS)
E. camaldulensis	5	1.26 (0.73)	7	1.60 (0.50)	0.354 (NS)
E. diversicolor	8	2.46 (1.37)	6	2.28 (0.55)	0.768 (NS)
E. patens	4	5.20 (5.91)	4	3.47 (0.53)	0.581 (NS)
E. robusta	7	4.97 (6.92)	9	2.75 (1.42)	0.359 (NS)
E. rudis	7	1.08 (0.52)	7	2.21 (0.48)	0.001 (**)
Allocasuarina fraseriana		NA			
Kunzea ericifolia	3	4.85 (3.5)	4	3.01 (0.60)	0.146 (NS)
Callistachys bilobum	5	2.59 (4.77)	3	1.35 (1.43)	0.558 (NS)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

There was a high percent of survival for seedlings over the experimental period (Table 7.5). The amount of survival was similar between treatments; there was an 87.9% survival of inoculated seedlings and a 90.9% survival of control seedlings. There was no significant loss of seedlings in any species; only *A. fraseriana* and *K. ericifolia* seedlings died in both treatments. Inoculation does not improve seedling survival on acid overburdens.

Table 7.5: Survival of seedlings at WO5H over 12 months

Species	Control	Inoculated
Eucalyptus calophylla	9/9	9/9
E. camaldulensis	5/5	7/9
E. diversicolor	7/9	7/9
E. patens	4/4	4/5
E. robusta	7/7	9/9
E. rudis	7/7	7/9
Allocasuarina fraseriana	3/4	4/6
Kunzea ericifolia	3/5	4/6
Callistachys lanceolatum	5/5	3/4
Total (% survival)	50/55 (90.9%)	58/66 (87.9%)

Roots taken from control plants had AM and EM infections (Figure 7.3). Despite whether given the inoculation treatment, the amount of EM infection in all the sampled roots was significantly larger than AM infection (P = <0.001). At the individual species level, roots of *E. robusta, C. lanceolatum* and especially *K. ericifolia* all had significantly larger infections of EM than AM (P = <0.001, 0.006 and <0.001 respectively). The P values of *E. camaldulensis* and *E. rudis* were almost significantly different in type of infection (P = 0.072 and 0.094 respectively), also suggesting that there was more EM infection than AM present in the roots.

In total, plants inoculated with *P. tinctorius* also had significantly higher infections of EM within the roots (Figure 7.5; $P = 0.017$). At the individual species level, roots of *E. camaldulensis* had significantly more AM and EM infections than control plants ($P = 0.007$ and 0.029 respectively). *E. robusta* and *K. ericifolia* both had roots significantly more infected with AM than control plants ($P = 0.031$ and 0.036 respectively). *E. rudis* and *C. lanceolatum* were all significantly more infected in EM than control plants ($P = 0.020$ and 0.040 respectively). Therefore $H_0 3$ is rejected: Plants inoculated with *P. tinctorius* do significantly increase in mycorrhizal infection.

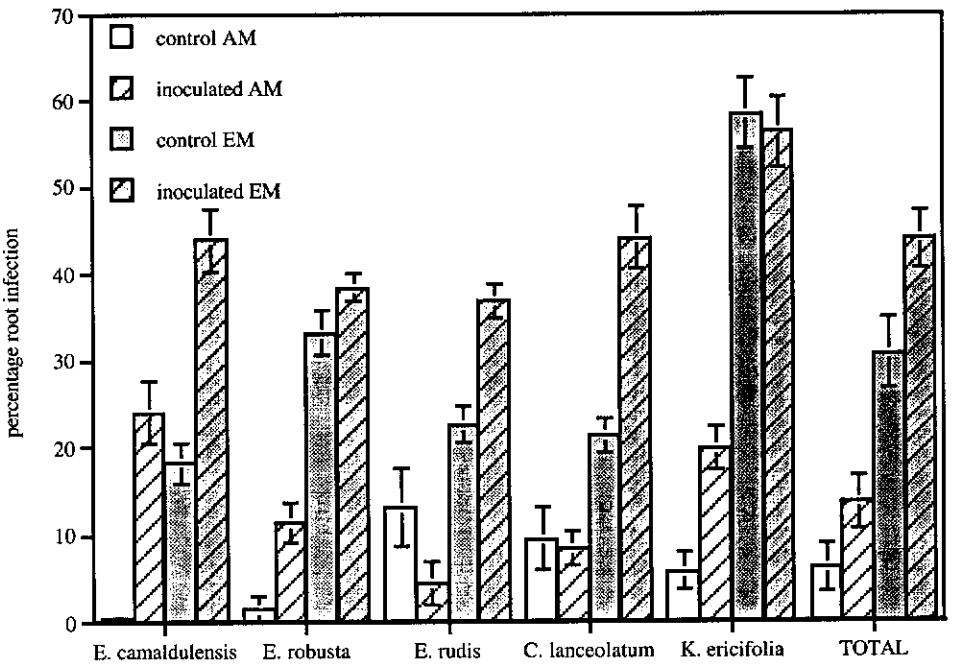

Figure 7.5: Mean % AM and EM mycorrhiza infection of seedlings roots July 2000

Discussion

Plants growing in acidic coal overburdens can be colonised by both ecto- and arbuscular mycorrhizal fungi. All tested plant species at Muja, Ewington and WO5H formed symbioses with both arbuscular and ectomycorrhizal fungi within the first year. However, increasing the amount of infection with inoculum (ectomycorrhiza *P. tinctorius*) only slightly enhanced plant growth of two species within 12 months. It is not known whether the other species benefited from the presence of mycorrhiza within the roots, but the poor growth of these seedlings suggest that the symbiosis is not significantly advantageous for the first 12 months without fertiliser.

In the colonisation trial, the amount of infections on roots varied between plant species. Seedlings of *Eucalyptus* and *M. hamulosa* had higher EM infections while seedlings of *K. ericifolia* had significantly higher AM infections. Mycorrhizas appear to be genus specific, rather than species specific (Allen & Friese 1990). There has been no evidence found for host fungus specifity within the *Eucalyptus* genus (Harvey *et al.* 1986), however, fungi that are able to form mycorrhiza with species within the genus *Eucalyptus* may not be able to form mycorrhizas with members of other genera (Chilvers 1972). It is likely that members of the *Eucalyptus* and *Melaleuca* genera are more adapted to forming mycorrhizas with EM than AM while members of the *Kunzea* genus are more adapted to forming AM than EM.

There was also a difference in amount of infection between sites. In general, there was more of both AM and EM present at Muja than at Ewington. The differences in AM infection symbiosis may be due to the type of fungal propagation. Ectomycorrhiza propagules are wind dispersed, so EM fungi are more likely to colonise more rapidly on open mine sites than can AM fungi (Egerton-Warburton 1993). In Wyoming USA, two and three year old abandoned strip mine sites had around 50% less infection and spores of arbuscular mycorrhiza than on the adjacent prairie levels (Allen & Allen 1980). As the Muja hotspot is closer immediately surrounded by vegetation, this site may receive more colonising AM spores. Ewington, being on a farming property, had little adjacent remnant forest, and presumably was less exposed to AM fungal propagules.

The difference in EM may be explained in terms of soil moisture. Ectomycorrhiza are more prominent in drier soils than in wetter soils. EM fungi require well aerated soil and are inhibited by water logging (Russell 1980). Ewington experiences high water saturation during winter from winter rainfall and runoff (as was discussed in Chapter 2), so the soil may be less appropriate for ectomycorrhizal growth. Muja, having drier soil conditions throughout the year, would be more amenable for ectomycorrhizal fungi.

The roots sampled were found to have significantly higher percentage infections of EM than AM. Ectomycorrhiza may also be more suited than arbuscular mycorrhiza to the hostile overburden conditions. Egerton-Warburton (1993) found fungi of 18 genera that formed ectomycorrhiza with *Eucalyptus* seedlings on various abandoned overburdens at Collie. Ectomycorrhiza are thought to be more important than arbuscular mycorrhiza in improving plant growth. More study is required into examining the role of EM fungi in supplying plants with nutrients on acidic overburdens, and how ectomycorrhiza may be utilised in order to improve plant growth in such hostile conditions.

The limited success of *P. tinctorius* to aid seedling growth is surprising, considering that that there has also been much positive work on symbiosis with *P. tinctorius* and *Eucalyptus* plants. Seedlings of *Eucalyptus tereticornis* inoculated with *P. tinctorius* grow more than non-inoculated seedlings when planted in nursery soil (Reddy & Satyanarayana 1998). This fungus increases the growth of *Eucalyptus diversicolor* in phosphorus deficient sand (Bougher & Malajczuk 1990). Other plant genera have also been shown to benefit from forming mycorrhiza with *P. tinctorius* (Marx, Bryan & Cordel 1977; Marx & Artman 1979; Davies & Call 1990; Yazid, Lee & Lapeyrie 1994; Schier & McQuattie 1996). Other research has shown the fungus did not assist growth of *Eucalyptus* seedlings on acidic coal overburdens. Fox & Doronila (1992) reported that inoculation did not appear to improve the growth of *E. calophylla, E. patens, Gastrolobium bilobum* and *Actinostrobus pyramidalis*. This fungus has also been shown not to increase growth of other plant genera (Cram, Mexal & Souter 1999)

One reason why the inoculations failed to increase plant growth in most of the species may be due to poor nutrient status of the overburden (demonstrated in the lime and fertiliser experiment - Chapter 6). Mycorrhizal fungi may only provide plants with nutrients if there are some nutrients available to exploit. Because the spoils had virtually no nutrients available, plants did not receive any increase in nutrient uptake from the symbiosis, and suffered a net carbon drain to the fungi.

The benefits of mycorrhizal inoculation may then be enhanced in presence of a small amount of fertiliser. Seedlings of four *Eucalyptus* species in acid coal overburdens grew better when infected with *P. tinctorius* and given a trace amount of N+P fertiliser (Egerton-Warburton 1993). Seedlings of *Eucalyptus, Melaleuca, Gastrolobum bilobum* (Fox & Doronila 1992) and *Pinus taedea* (Marx 1990) inoculated with *P. tinctorius* grew better in acid spoils than non-inoculated plants given the same N+P fertiliser treatment. Seedlings that have improved in growth from a combination of mycorrhiza and phosphorus fertiliser include the genera *Acacia, Eucalyptus* and *Melaleuca* (Bougher, Grove & Malajczuk 1990; Burgess, Malajczuk & Grove 1993; Jasper & Davy 1993; Mason *et al.* 2000a). Inoculation may result in less fertiliser being needed to be applied to overburdens, as the fungi may collect the applied nutrients more efficiently than the plant roots.

It is important to select the most suitable type of fertiliser to enhance growth of inoculated plants. Fertilising inoculated plants with NH_4^+ fertiliser may be more beneficial than NO_3^- fertiliser. Mycorrhizas absorb more N in the form of NH_4^+ than NO_3^-, supplying more N to the plant host. Also, the cation nature of NH_4^+ may result in reduced uptake of Al, thus reducing Al toxicity (Cummings 1990). However, plants may release protons when uptaking NH_4^+, making the rhizosphere more acidic, thus increasing the bioavailability of Al.

However, care must be taken to not oversupply the fertiliser treatment. Increasing the amount of nutrients to a level where it is no longer limiting, reduces the positive response to mycorrhiza, through a reduction in function and growth of mycorrhiza. A

combination of mycorrhizal inoculation and fertilisation can therefore only be most effective when the amount of fertiliser applied is below the critical level for the plant to extract enough nutrients effectively without need for mycorrhizal symbiosis.

Absence of mycorrhizal symbiosis is not a problem for coal spoils. Ectomycorrhiza appear superior to arbuscular mycorrhiza in both colonising overburdens and in infecting plant roots. It is concluded that plants did not appear to benefit from the symbiosis with the ectomycorrhizal fungus *P. tinctorius* within the first year because overburdens were nutrient deficient. More work is required into examining whether other ectomycorrhizal species may be appropriate for inoculating plants. A more detailed study is also required into whether a combination of small applications of fertiliser and ectomycorrhizal infection may assist seedling growth on acidic coal overburdens.

Chapter 8 – Using *Kunzea ericifolia* branches to seed overburden

Introduction

Much of this thesis (Chapters 2, 4 to 7) has dealt with the use of seedlings transplanted onto the overburden material, because this enables definite results to be obtained for selection of appropriate species, fertilisers, liming and mycorrhiza. Seedlings are more beneficial in areas more difficult to seed (such as slopes), have the ability to overcome understorey growth, allow for the numbers, spacing and location of the trees to be controlled and have a higher survival rate than direct seeding (Koch 1984).

However, the transplanting method is only practiced on a small scale in WA mining revegetation operations, as it is generally more expensive than broadcast seeding. Seeding is usually a more economical, practical and reliable method of establishing most species. Further, seeding may result in a random distribution of species, allowing a more natural appearance to revegetated sites (Ward & Koch 1994; Simcock & Ross 1995).

There are two main components in broadcast seeding that need to be examined. Firstly, direct seeding must be shown as a successful method for revegetating a site, as climate and site conditions may affect the outcome of seeding (Handreck & Black 1991). Secondly, particular species selected must also be appropriate for the particular site. The most useful species for seeding are generally those that produce large numbers of easily collected, viable seeds that have high germination and seedling survival rates in the field (EPA 1995). That species for Collie overburdens must be able to germinate and survive on the hostile spoils is an overriding consideration (Chapter 2).

One problem in using broadcast seeding in mining revegetation is the limited choice of species to collect seeds from. Species that retain unopened fruit for over a year (bradysporous species) are rarely used in seed mixtures as such because of the difficulty of collection. One potential method of direct seeding such species is by spreading foliage with the capsules directly onto the dump. The seeds are then shed when the capsules dry out (Nicholls 1983; Environmental Protection Agency 1995). This method has been given many names: fascining; forest slashing; mulching; and branch (or brush) layering. Fascining may prove to be a relatively cheap and easy method of revegetating spoils using Myrtaceae genera with capsule fruits, such as *Agonis, Baeckea, Callistemon, Calothamnus, Kunzea, Leptospermum* and *Melaleuca.*

Fascining is used successfully in Western Australia, England and especially New Zealand. Mulch of stripped native vegetation provides 85% of the germinable seeds that occur on rehabilitated sand mine soils at Eneabba (Peterson & Herpich 1996). Thirty one taxa, from Myrtaceae, Casuarinaceae and Proteaceae, including species of *Melaleuca, Leptospermum* and *Eucalyptus*, established from seeds in the applied mulch (Bellairs 1990). Application of harvested shoots from heathland to previously cultivated farmland in England increases the number of heathland plant species (Pywell, Webb & Putwain 1995). Fascining has been used successfully with manuka (*Leptospermum scoparium*) and to a lesser extent kanuka (*Kunzea ericoides*) in New Zealand on forest soils of pH between 4 and 5.5 (Nicholls 1983; Simcock *pers comm.*).

In addition to seed provision, fascining is valuable in rehabilitation for restoring insects and other biota; some nutrient recycling; and, formation of micro-sites (Pywell, Webb & Putwain 1995; Ross, Simcock & Gregg 1995). When the branches dry, the seeds are released into a secondary mulch of fallen leaves, which may assist in forming a seedbed, allowing long-term germination of up to five years (Nicholls 1983). The branches may also improve the growing environment by increasing humidity and temperature (Porteous 1993), providing organic matter and protection from wind and water erosion (Bell, Carter & Hetherington 1986) and reducing the entry of grasses and other weeds to the site. Some consider this method potentially more economical than broadcast seeding (Pywell, Webb & Putwain 1995).

There are several steps for fascining to be successful. Firstly, the site of harvesting must be easily accessible to transport the branches, and also must be near the revegetation site, so the species are ecologically suited to the new site (Nicholls 1983). Any other vegetation at the site, such as weeds and grass, should be removed by cultivation; burning or spraying otherwise the germinating seedlings may not survive in competition with the weeds (Porteous 1993).

The method of harvesting and applying the branches is also important. Dense, brush like branches are ideal for fascining, as the material is easy to cut, transport and lay (Nicholls 1983). If multiple harvests are wanted from one site, only branch tips with capsules should be harvested because manuka and kanuka do not coppice readily (Simcock *pers comm.*) The branches should be laid thickly enough to break the impact of rain, but not so thickly as to prevent germination (Porteous 1993). Fifty to sixty percent cover is usually ideal (Nicholls 1983), lowering to thirty percent cover on gentle slopes (Simcock

pers comm.). The branches should be laid across the slope to capture falling debris and reduce run off. Material can be tied down with stakes or biodegradable netting on sites with steep slopes or wind problems (Nicholls 1983; Porteous 1993).

One concern about using fascining is whether germination is inhibited or promoted by sunlight (photoblastism) (Mott & Groves 1981; Whalley 1987). Although not common to the Australian flora, the germination of seeds from some species may be affected by either light availability or restriction. Light may enhance the amount of germination of many species such as *Eucalyptus*, though this may only affect certain seeds within the seed lot, and is dependant on temperature and moisture levels (Cremer, Cromer & Florence 1978; Boland, Brooker & Turnbull 1980; Mott & Groves 1981). Harty and McDonald (1972) demonstrated that the seed of *Spinifex hirsutus* will not germinate in light. Clifford (1953) found only 2 of 41 eucalypt species required light to germinate, while several other species only required light if seeds were immature. Presence or absence of light affected germination in seeds of 17 of 43 tested species native to the south-west of Western Australia (Bell *et al.* 1995). Since fascining causes seed to fall from capsules onto overburden surfaces and be exposed to sunlight, the nature of germination, and thus the outcome of fascining, may be affected by the light sensitivity of the seeds.

The general aim of the experiments reported in this chapter was to determine whether slash layering with *K. ericifolia* onto Collie acidic overburden dumps will result in adequate seedling establishment. There were three null hypotheses:

H_o1: Exposure to light does not affect the germination of *K. ericifolia* seeds.

H_o2: Placing capsule laden branches of *K. ericifolia* onto acidic coal overburden does not produce seedlings.

H_o3: Applying fertiliser and lime does not enhance the survival and establishment of any seedlings that originated from this seeding treatment.

Method

Experiment 1: Germination of *Kunzea ericifolia*

A germination trial commenced in January 1998. Branch ends of *K. ericifolia* bearing semi-ripe capsules were harvested from the periphery of Ewington 2 lake. The branchlets were divided into 5 groups holding 10 capsules each and placed into 5 calico bags, which were then dried to a constant weight in a 40^0 C oven for 3 days. Seed numbers were counted from each bag to obtain mean number of seeds per capsule. Ten sets of 100 seeds were then counted out and surface sterilised by soaking in 5% bleach solution for 10 seconds. Ten petri dishes were prepared by placing an 11 cm diameter filter paper on top of a 13 cm diameter filter paper over moist vermiculite that had been sterilised by standing for 24 hours in a 100^0 C oven. Enough distilled water was added to make the filter paper and vermiculite moist. The seeds were placed evenly on the top filter paper.

Five sets were wrapped in alfoil to exclude light. The dishes were then placed in a 25^0 C germination cabinet. Five dishes were taken out each day and placed in the sunlight for 6 hours while the other five sets were kept inside the germination cabinet. Germinations were recorded daily in shade for 35 days. Fungal growth was minimised through use of the fungicide Previcur™. Moisture was added when necessary.

Experiment 2: Field trial

A field trial was set out in early March 1999. Four 4 x 4 m plots were laid out at WO5H after the soil surface had been broken open by raking to a depth of approximately 2 cm. Branches of four mature shrubs with capsules were harvested at Ewington 2 and laid out into the plots. Chicken wire (5 cm mesh dia) was placed over the branches and held down with rocks to hold them in place. Numbers of seedlings per square metre were estimated for each of the plots at three months after fascining (June 1999). The plots were then subdivided into 4 subplots of 2 m x 2 m. Three treatments were applied individually to a subplot within each plot: One treatment was 400 g NPK Blue Special™ granular fertiliser (Appendix 3), one was 800 g ground limestone (Appendix 3), one was equivalent rates of both NPK Blue Special™ granular fertiliser and ground limestone treatments and the fourth was a control. Lime and fertiliser was applied by sprinkling evenly over the subplots.

One plot had been completely buried by erosion during winter rainfall (July 1999) so was discounted from the experiment. Between 20 and 30% of two plots were also buried by eroded material (Appendix 10). Seedlings in the buried sections were completely covered and perished. None of the seedlings in the erosion sections were completely or partly covered by erosion. Nine 0.125m^2 square quadrats were randomly placed within the unburied parts of each subplot and the number of established seedlings was counted at 2-monthly intervals for six months. Mean heights of the tallest 10 seedlings in each subplot were obtained at the end of the experiment (September 1999).

Analysis of data

Data was tested for homogeneity with boxplots and scatterplots. Statistical analysis was performed using oneway and twoway ANOVAs. The final percentage germination values were transformed by dividing all values by 100 to obtain a proportion value between <0.001 and 1.000. The new values were square rooted and then arc sined to obtain a normal distribution (Sokal & Rolf 1981).

Results

Experiment 1: Germination of *Kunzea ericifolia*

The mean number of seeds per capsule in *K. ericifolia* was 50.5 (SD = 25.6). The fresh seeds had no apparent dormancy mechanism and were of high viability, (> 80% seeds germinating). Initial germination had started after just 7 days; 50% had germinated by 10 days. The final germinations were obtained in under 30 days. Harvesting semi ripe capsules does not appear to affect the number of viable *K. ericifolia* seeds.

There was no difference in germination performance of *K. ericifolia* seeds, whether exposed to or deprived of sunlight (Table 8.1). There was no significant difference in days to first, 50% and final germination or percentage germination, though seeds given the light treatment did tend to germinate quicker by one to two days. The first null hypothesis was accepted; light does not affect germination of *K. ericifolia* seeds.

Table 8.1: Effect of light/dark on germination parameters in *K. ericifolia* seeds

Treatment	Dark (n=5)	Light (n=5)	*F*	P
Days to first germination	8.2 (0.4)	7.8 (0.4)	2.00	0.194 (NS)
Days to 50% germination	11.2 (0.9)	10.3 (0.6)	3.52	0.097 (NS)
Days to final germination	29.0 (2.6)	26.6 (1.7)	2.55	0.897 (NS)
Mean final germination (%)	82.6 (6.3)	81.8 (11.8)	0.00	0.960 (NS)

Experiment 2: Field trial

Seedlings appeared in the remaining three plots by 3 months (June 1999) and continued to persist for the rest of the experiment. However, no seedlings emerged from the buried branches for the entire experimental period. Most, if not all, seedlings were thought to have germinated during the first three months (winter rainfall). Provided that erosion can be controlled, the second hypothesis is rejected: Placing capsule laden branches of *K. ericifolia* onto acidic coal overburden does produce seedlings.

Seedling density remained significantly higher in the lime and fertiliser subplots than the other treatments for the entire experiment (Figures 8.1 to 8.2). The number of germinants in the fertiliser-alone subplots was significantly greater than the control and lime-only subplots at both 5 and 7 months ($P = <0.001$ and 0.002 respectively) but not at 9 months. There was no significant difference in number of seedlings between control and lime-only subplots during the experiment. Seedling numbers were increased by fertiliser and were enhanced further with lime.

There were also severe decreases in seedling numbers over time in each treatment (Figure 8.1). Over 4 months, there was a mean of 62.5% loss of seedlings in the control subplots, 53.1% loss in the lime subplots, 66.0% loss in the fertiliser plot, and 55.9% loss in the lime and fertiliser plot. Regardless of treatment, over half of the seedlings died during the experimental period.

Figure 8.1: Mean number of *K. ericifolia* germinants on acidic overburdens over time. The arrow indicates time of lime and fertiliser treatments (bars are standard errors).

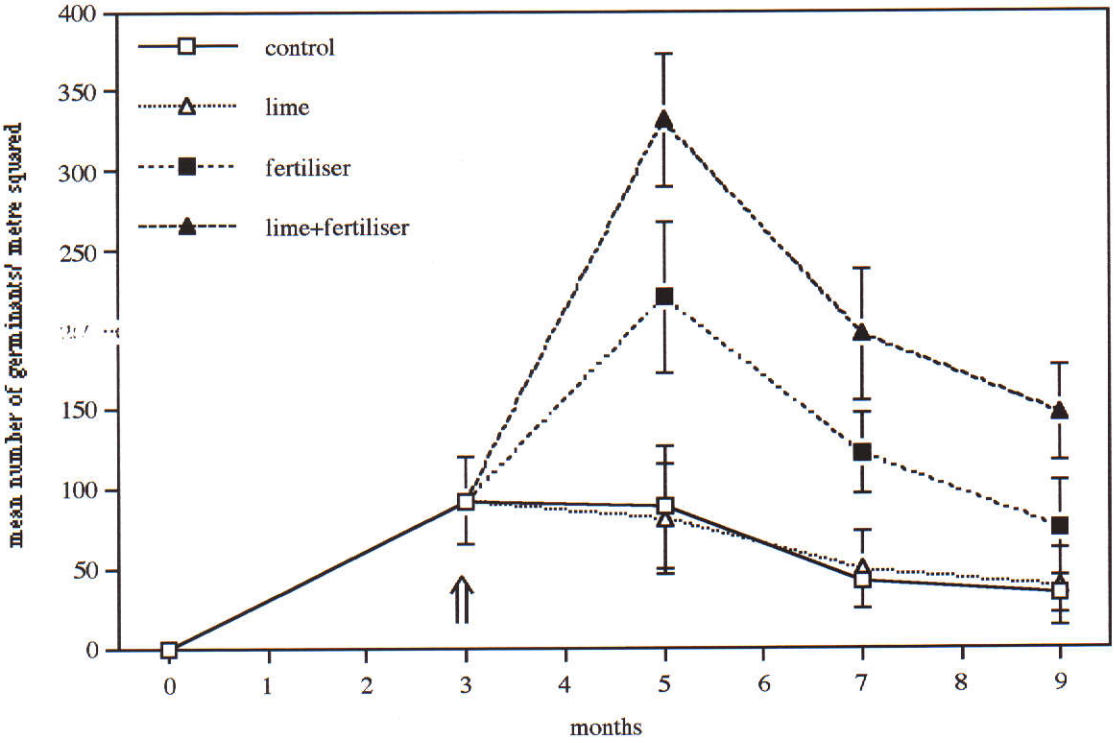

Figure 8.2: High density of *K. ericifolia* seedlings in a lime and fertiliser subplot (chicken wire mesh is 5 cm diameter)

Mean height ranking of the tallest ten seedlings by subplot at six months after lime and fertiliser application follow the same pattern as density (P = < 0.001; Figure 8.3). Seedling height was increased by fertiliser and was further increased with additional application of lime. The third hypothesis was thus rejected: applying fertiliser alone and with lime does enhance the survival and establishment of any seedlings that originated from this seeding treatment.

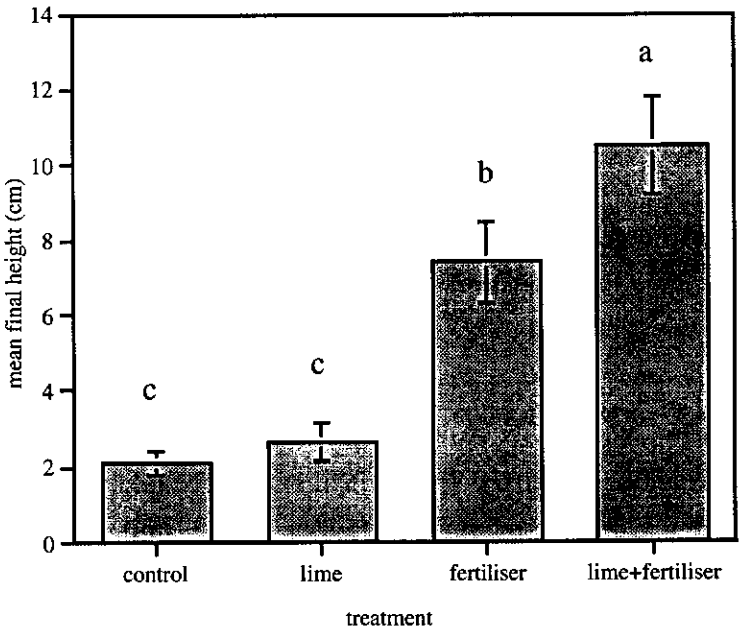

Figure 8.3: Mean final heights and SE of the ten tallest *K. ericifolia* seedlings per subplot on acidic overburden at 6 months after fertiliser and lime treatment (bars with same letters to do differ significantly)

Discussion

Kunzea ericifolia is an ideal species for the fascining method of establishment on acidic coal overburden. The seed has demonstrated germination and establishment properties in fascining that are similar or greater than the New Zealand *Leptospermum scoparium.* In both the germination and field trials, the seed capsules produced large numbers of viable seeds, despite being harvested when semi-ripe. This germination percentage is greater than the bradysporous *L. scoparium* (Clemens *pers comm.*), as most of the seed of the latter species is still immature at the time of branch harvest, so does not germinate. Initial germination of *K. ericifolia* is similar to *L. scoparium;* both species germinate quickly, allowing fast initial establishment.

Other plant species should be trialed to determine which species can successfully produce high numbers of seedlings by fascining. Even if large amounts of seed laden branches are laid out onto the field site to ensure high numbers of released seed, the number of seeds germinating may be heavily reduced by several factors. Seeds of some plant species may be harvested by ants (Ashton 1979, Anderson 1991) from the overburdens and moved to nests in adjacent forest areas. Light seeds laying on the soil surface may be blown away by the wind. The overburden surface may heat up from sunlight, drying the exposed seeds too quickly resulting in fractures that injure the embryo (Bewley & Black 1982). All this may result in few or no seedlings establishing on the site.

The choice of species also determines the timing of branch harvesting. Timing is important to provide a moist sheltered seedbed and to reduce competition by herbaceous weeds (Simcock & Ross 1995). The time window for fascining is limited by how long the selected species retain seeds within the foliage. Fascining with species such as *L. scoparium* that retain seeds within the capsules for a long period may be done within a period of several months, whereas in species such as *K. ericoides,* that release seeds as soon as capsules are ripe, can only be done within a few weeks. However, regardless of the seed-retaining period, layering must be done quickly after harvesting as the seed capsules quickly dry out and release the seed (Nicholls 1983).

The use of either light sensitive or non-sensitive species in fascining may affect the amount of initial and long-term germination. Some seeds falling from capsules may lie directly on the soil surface, thus exposed to sunlight, while the rest will be buried in the leaf mulch, and hidden from sunlight. Provided there are no other dormancy mechanisms present, all viable non-light sensitive seeds may germinate in the first growing season, while only the non inhibited fraction of the light sensitive seeds (on the surface if requiring light or in the mulch if inhibited by light) will germinate in the first growing season. Non-light sensitive seeds such as *K. ericifolia* are capable of fast establishment, but the revegetation success is vulnerable to climatic conditions of that growing season. In contrast, light sensitive seeds such as *L. scoparium* may germinate less in the first season, and allow more germination gradually over the following years, provided that the seeds can persist in the soil bank.

Half-lives of *K. ericoides* seed in the soil seed bank in south-eastern Australia are thought to be greater than two years (Auld, Keith & Bradstock 2000). However, investigations of soils seed banks in New Zealand forests revealed that no *Kunzea ericoides* seeds

germinated in the soil seed bank after 3 months (Flynn *pers comm.*). Since *Kunzea ericifolia* is closely related to *K. ericoides*, it is unlikely that seed of *K. ericifolia* can last long in the soil seed bank. Germination success of this species therefore depends on seeds germinating and surviving the first year.

Light sensitivity in seeds has been proposed as an evolutionary adaptation to ensure seeds germinated into an ecology where the seedlings could establish successfully – whether in forest shade or in open ground (Bell *et al.* 1995; Rokich & Bell 1995; Herron, Clemens & Greer 2000). Most negatively photoblastic species of the jarrah forest are understorey legume species (*Acacia, Bossiaea* and *Gompholobium*) although *Paraserianthes lophantha* (a species that normally occurs in open areas) responds positively to light stimulation (Bell *et al.* 1995). *Leptospermum scoparium* occurs mainly in open habitats and has seed that operates on a phytochrome mechanism, resulting in germination only in light (Herron, Clemens & Greer 2000). Seeds of *Trachyandra divaricata*, four *Acacia* species, *Bossiaea aquifolium*, *Gompholobium marginatum* and *Sphaerolobium vimineum* are negatively photoblastic while *Oenothera stricta* is positively photoblastic (Bell, King & Plummer 1999). Seeds of *Kunzea ericoides* germinate well in both dark and light conditions (Burrows 1996). This study supports the theory that the seed of *K. ericifolia* are not light sensitive; seedlings were observed in Collie, both in open areas and under tree canopies.

Combined application of fertiliser and lime improves the seedling number and height. Germination and initial establishment stages of *K. ericifolia* seedlings may be vulnerable to hostile spoil conditions. Egerton-Warburton (1989) found that when seeds of Fabaceae, Mimosaceae, Proteaceae and Myrtaceae (including *K. ericifolia*) are exposed to low pH, the time to initial germination, the period of germination and the amount of germinants are all adversely affected. Koch (1984) demonstrated that early seedling growth of several *Acacia* and *Eucalyptus* species was extremely sensitive to low pH and high aluminium levels. The application of lime and fertiliser presumably aids seedling survival by increasing soil pH and decreasing concentration of aluminium ions. However the applied lime is thought to be exhausted from constant acidic seepage and runoff from the upslope dumps, so seedlings would not have a lower soil pH during establishment.

Despite more than 50% of the seedlings perishing, a considerable proportion of seedlings still survived the experimental period. Bellairs (1990) reported that competition between seedlings reduced seedling numbers in mulching trials at mineral sand mining at

Eneabba. It is thought that this also happened in this experiment; the high number of seedlings were in a close proximity to each other, and were in competition for nutrients, moisture and root space. Application of fertiliser and lime may also have reduced competition for nutrients, allowing more seedlings to survive and grow.

The effectiveness of fascining was reduced by erosion burying one of the plots. Bellairs (1990) also reported that wind erosion killed many seedlings that emerged from mulching trials on mineral sand mines at Eneabba. In New Zealand, seed and small branches have been washed away by high intensity rainfalls (>100 mm day^{-1}) at one site where the angle of repose was 35 degrees. At many sites, eroded material collects against manuka branches laid across the slope, inferring that some seed is buried (Simcock *pers comm.*). Seeds of *Kunzea ericoides* buried at more than 5 cm deep germinated but died underground. (Burrows 1996). If fascining is to be undertaken in erosion prone areas, then some protection against erosion may be required, such as barricades placed up-slope from the branches.

In conclusion, fascining demonstrates considerable promise for seeding of acidic overburdens. Factors that still need to be considered are control of erosion, determining the best amount of lime, fertiliser and branching to be applied per unit area, timing of branch application and what other species may be used. *Kunzea ericifolia* has proven to be suitable for fascining, provided that a considerable amount of seeds can germinate and survive the year after the branches are laid. Further work is required to determine how long seeds of this species can survive in the soil seed bank.

Chapter 9 – General Discussion

Species selection

Plant species differ greatly in their tolerance to acidic soil conditions around coal-mine dumps. Species able to grow faster in acidic overburdens will transpire more water, thus reducing the amount of acidic run-off entering the voids. Survivors that grow well are able to tolerate physiological drought, free aluminium in soil solution and to co-habit in symbiosis with acid-tolerant mycorrhizal fungi (*Pisolithus tinctorius, Scleroderma* sp.). Choice of species examined in this thesis for performance on the more intractable sites (moist or dry acidic materials), was limited to the general range of large tree species (plus some shrubs and the grass *Neurachne alopecuroidea*) conventionally grown on dumps around the Muja void. As tree growth and response to stress may change as trees grow older, care must be taken in extrapolating long-term trends from early performance (Bartle & Shea 1978; Burns 1989).

It must be noted that most of the species tested in this thesis, although not necessarily natural to the area, had seeds collected from plants growing in the Collie region, resulting in some provenance selection occurring. Egerton-Warburton (1995) determined there was no ecotypic variation in three local *Eucalyptus* species between those growing on bare acidic coal mine spoils and those growing in adjacent forest. This infers that local provenance species may not be subjected to further selection when growing on overburdens, so may have had an advantage over foreign species which have not undergone selection pressure to local conditions. Care must be taken then to interpret the performance of following taxa as provenances, than simply as species. In this account, candidate species (Chapters 2 to 3) are discussed in relation to suitability for revegetation of acidic overburdens.

Of all species tested, *Eucalyptus robusta* is the most adapted to acidic conditions. It has proven to be the most non-demanding species of those trialed and can tolerate very high levels of free aluminium. *E. camaldulensis* is the second most adapted species, also indicating fast growth in acid spoils and tolerance to high levels of free aluminium. It is interesting to note that whereas *E. robusta* has a limited natural range, *E. camaldulensis* has the widest natural distribution of all the eucalypt species (Boland *et al.* 1992). In other countries, both species are successfully grown in a range of latitudes and climates, even in plantations in the sub-Himalayan zone of India (Gogate 1988). The key similarity is thought to be that both species develop deep, penetrating roots and can grow rapidly when

moisture is available, allowing plants to be tolerant of both flooding and drought (Turnbull & Pryor 1978; FAO 1979; Eldridge *et al.* 1994).

The remaining species indicated at least some unsuitability to the overall spoil conditions. Some species may still be considered for revegetating acidic overburdens, but only in specific circumstances where limiting factors, such as aluminium toxicity and lack of soil moisture are not problems. High moisture sites are likely to be more toxic in respect to aluminium as high soil moisture would allow more ions to enter the soil solution in the vicinity of tree roots. Roots may also be exposed to a higher degree of Al ions, as the amount of seepage is also greater. As a general rule, species planted on moist sites need to be more tolerant of Al than species planted on drier sites.

Eucalyptus cladocalyx is known for its fast growth and drought resistance. It normally grows in low rainfall areas in South Australia where it can reach over 6 m in height within 5 years (Lord 1964). At Collie it grows well in both dry and wet acidic soils (pH 3.3-4.0) and has been observed to form mycorrhizal associations with *Pisolithus tinctorius* and *Scleroderma* species. It is a high water-using species on saline soils (Greenwood *et al.* 1985; 1994; 1995) but this dissertation has shown seedlings tolerate only low levels of free aluminium. Thus *E. cladocalyx* may only be appropriate for revegetating drier acidic sites with low levels of free aluminium.

Aluminium ions can damage tree roots, inducing physiological drought. Moisture dependant species may still fail in high Al sites if they are Al sensitive. *Eucalyptus resinifera, E. rudis,* and the shrubs *Callistemon speciosus* and *Melaleuca hamulosa* are all dependant on adequate soil moisture for efficient growth. These species are naturally restricted in distribution to moist sites: *E resinifera* in areas above 1000 mm annual rainfall (Colquhoun *et al.* 1984); *E. rudis* near stream beds (Boland *et al.* 1992); *C. speciosus* in swampy heathlands; and, *M. hamulosa* in saline depressions and swampy areas (Corrick & Fuhrer 1997). These species are only recommended for sites of high moisture.

The local species *E. rudis* is closely related to *E. camaldulensis*, yet was seen to be less tolerant of dry acidic sites and aluminium toxicity. As it is geographically restricted in distribution to the relatively high rainfall area of the south-western corner of Western Australia, *E. rudis* is less tolerant to drought conditions, so may also be more vulnerable to physiological drought as a result of aluminium damage to roots. This may explain why, despite demonstrating good growth on acid soils, *E. rudis* can tolerate only moderate levels

of aluminium. Therefore this species should only be used on moist sites that are not high in Al ions.

M. hamulosa also demonstrated good growth on acidic spoils, but only moderate tolerance to aluminium. Fox & Doronila (1992) reported that this species appeared tolerant of acidic coal overburden. However, *M. hamulosa* appears to be less tolerant than members of the tropical *Melaleuca leucodendra* complex, recommended for revegetating wet acid sulphate soils in Malaysia (Brinkman & Xuan 1991). As with *E. rudis*, this species is only appropriate for revegetation of moist sites with moderate levels of free aluminium.

E. resinifera grew only moderately in comparison with the other candidate species. This species is reported as growing well in areas of high moisture on rehabilitated bauxite mine dumps in the northern jarrah forest (Mazanec 1994). Slower growth of *E. resinifera* and dependence on adequate soil moisture make it not particularly useful.

The shrub species *C. speciosus* was inhibited in growth on dry acidic overburdens. This species had been reported by Fox & Doronila (1992) as appearing tolerant of acidic coal overburdens at Collie. *C. speciosus* may be appropriate for revegetating moist sites of pH > 4 but not on the more hostile sites.

Nutrient deficiency is a key factor preventing plant establishment and survival on acidic spoils. Species selected must demonstrate efficient use of limited resources during short-term establishment to ensure a strong chance of long-term survival on the acidic material. *Eucalyptus gomphocephala, E. grandis, E. maculata* and *Calothamnus rupestris* all indicated nutrient deficiencies in the short-term when growing in acidic material, so these species are not considered suitable for revegetating acidic spoils.

Apart from early nutrient deficiency, *E. gomphocephala* did not appear adversely affected by other site factors. The species has a deep taproot and extensive feeder roots, allowing access to limited soil moisture. *E. gomphocephala* also tolerates a wide range of soil acidity, despite its natural distribution on sand over limestone (alkaline soils) and is successfully grown in calcareous soils of the Mediterranean region (FAO 1979). Despite these broad adaptations, this species is not considered to be as useful as others mentioned for revegetating acidic overburdens around voids in Collie.

E. grandis and *E. maculata* have been reported to demonstrate good growth in similar environments. *E. grandis* grows very well in acidic soils in Brazil (Marcar 1995). *E. maculata* grows successfully in rehabilitated bauxite mine sites in the northern jarrah forest in areas of low moisture (Colquhoun *et al.* 1984; Mazanec 1994). These responses are probably due to fertiliser application, as both species grew only moderately in both acidic spoils in Collie.

C. rupestris suffered severe nutrient deficiency within 6 months growth in Ewington overburden and plants shed many leaves at both overburden sites within 12 months. The species was also observed to have grown poorly on hotspots south of Stockton lake. Consequently, this species is deemed not appropriate for revegetating mine spoils.

Eucalyptus wandoo grew very poorly in both wet and dry acidic overburdens. This species has been reported to grow poorly in Collie coal overburdens (Fox and Rhodes 1981; Koch 1984; Fox, Colquhoun & Leone 1987) and also in overseas plantation trials (FAO 1979). The species is a low water user, having lower transpiration rates than other tested *Eucalyptus* species from the jarrah forest (Colquhoun *et al.* 1984) and in a saline catchment (Greenwood *et al.* 1985). This species is not appropriate for revegetating mine overburdens

Seedling establishment of *Neurachne alopecuroidea* was poor on the acidic soils, with extremely high mortality rates and little growth. The species had been observed on disturbed soils adjacent to the overburden sites. It is possible that seedlings may not transplant easily and could be sown directly onto overburden sites. The species is not an ideal ground cover plant, as it is tussock forming, and has a poor density in the northern jarrah forest (~ 0.5 to 1 plant per square metre). The species is also a poor coloniser; single plants on rehabilitated bauxite sites only occur at a density of 1 per 20 m^2 (Koch *pers comm.*). This species is not recommended for transplanting onto acidic overburdens, but may prove appropriate if it can be established from broadcast seeding.

Additional species

Other species were included in follow up experiments (Chapters 3 to 8) to provide broader range of supplementary data for a suitable species for acidic sites. A preliminary assessment was made of early growth of these species. These included several local tree species for which seeds are accessible from individuals/stands already growing on interburden dumps.

In the first year of growth at WO5H, *E. calophylla* indicated high tolerance to acidic overburden. Elsewhere, seedlings have been observed growing and forming mycorrhiza with *Scleroderma* species in hotspots on 1-3 yr old dumps in Collie (pH 3.3 to 3.5). However, surviving *E. calophylla* trees observed in hotspots on a 10-yr old dump in Collie (pH 3.3) had stunted growth and marked stem taper, which suggests that aluminium was sequestered. *E. calophylla* seeds are the largest of the eucalypts, being as long as 2 cm (FAO 1979). Greater seed size provides an advantage over other species, in both survival and establishment. Embryos and cotyledons can access considerable food reserves compared to most eucalypt species. The large cotyledons also allow an early effective start to photosynthesis. Preliminary results suggest this species could be appropriate for revegetating slightly less acidic soils (pH > 4).

Although *Eucalyptus diversicolor* grew well on acidic material, it was particularly sensitive to damage from the presence of free aluminium. The species naturally occurs on mildly acidic soils with very low soil nutrients, and is limited in distribution by soil moisture (Churchill 1967; Boland *et al.* 1992). This species may only be useful in rare conditions where acidic spoils have low aluminium and high soil moisture.

Some tolerance has been shown earlier by *Eucalyptus patens* in a lime and fertiliser trial. The species exhibits moderate to good growth in acidic materials of pH 3.9 to 5.0 (Koch 1984; Fox *et al.* 1987; Doronila & Fox 1990; Fox, Frost & Doronila 1993). However, at some Collie sites it was observed to establish poorly and surviving seedlings were misshapen in form (Fox & Doronila 1992). It naturally colonises acidic spoils (< pH 4.5) (Bartle and Riches 1978) but appears to be successful only if seeds fall into favourable micro-sites where adequate nutrition is available (Fox, Colquhoun & Owens 1988). The species has indicated poor performance when broadcast sown (Koch 1984). This species may prove appropriate if transplanted onto less acidic soils (pH > 4), where the additional costs may be allowable compared with routine waste dump rehabilitation.

Allocasuarina fraseriana is a common colonising species in the Collie area. The species was observed to have high mortality and survivors grew poorly when transplanted on the WO5H overburden. Seedlings transplanted in acidic overburden (pH 4.6) suffer a considerable level of mortality (Alvin 1990). The species forms low levels of mycorrhizal infection with colonising Actinomycetes fungi and is sensitive to phosphorus (Fox & Doronila 1992). Trees growing on Collie overburden were observed to have a distinctive taper, suggesting aluminium sequestration. The low germination of seeds collected from these trees may be a result of sequestered aluminium within the seeds. Seed collected from another species, *E. calophylla,* from acidic, high Al spoils contain Al and have lower final germination values than seed collected from forest sites (Egerton-Warburton 1993). *A. fraseriana* is thought not to be appropriate for revegetating acidic sites.

Kunzea ericifolia demonstrated high tolerance to aluminium and grew relatively well as germinants from fascining and as transplanted seedlings, though suffering considerable mortalities. Walker (2000) reports success in establishing seedlings on acidic overburden (pH 2.4 to 4.6) in Collie by fascining but not by transplanting. The failure of transplanting may be due to water stress, as the species usually occurs around swamps or on banks of creeks or rivers (Toelken 1994). The species may be ideal for revegetating acidic spoils prone to seepage and immediately next to voids.

The pruned *Callistachys lanceolatum* seedlings resprouted surprisingly well at WO5H. This legumious species is a nitrogen fixer, a useful characteristic for these sites. This species is recommended as a possible species for dry and wet acidic sites.

Pruning has proved to be a useful pre-planting technique for establishing seedlings of *C. lanceolatum* and *K. ericifolia* onto overburden dumps. Pruning large seedlings increases the root:shoot ratio, allowing a larger root biomass than with seedlings grown to the same size. This allows the seedling to establish into the soil more efficiently with less stress from evapotranspiration demand. Planting of pruned seedlings may accelerate the revegetation process and lower mortality. However, it is only of value on those species that are not harmed by the pruning process.

Future selection of species

During the course of this study, other species were considered to have potential for revegetating acidic sites and are recommended as candidate species for further studies. The following is a brief introduction to these species and to their favourable characteristics. The selection of provenances and hybrids, and proposals for improving aluminium toxicity experiments are also discussed.

Eucalyptus globulus Labill. subspp. *globulus* (Tasmanian blue gum) can be acclimatised into a wide range of soils and climates (Turnbull & Pryor 1978; FAO 1979). A strain of *E. globulus* subspp. *globulus,* dubbed "Western Blue Gum", has been bred in the southwest of Western Australia to tolerate local climate and soil conditions. This species is currently planted in the southwest as a successful fast growing timber and pulp crop. Studies have also been conducted into selecting more drought tolerant varieties (Brooksbank 1997). Seedlings are currently growing successfully on Griffin coal dumps (Doronila & Fox 2000) and have been observed to tolerate hotspots (pH as low as 3.0) and soil moisture in summer as low as 0.6%, although with reduced growth. This species may have a role in ameliorating acidic seepage sites as well as and a potential timber crop.

Eucalyptus occidentalis Endl. (Swamp yate or Flat-topped yate) is naturally restricted within 150 km of the southern coast of Western Australia (Turnbull & Pryor 1978; Boland *et al.* 1992) but has been demonstrated to tolerate extreme conditions, from drought and flood, high temperatures to frequent frosts, and to be able to grow in saline, clay and alkaline soils (FAO 1979). The wide range of tolerance and fast growth suggests that this species may prove successful in growing on acidic overburden. The species is a high water user (Greenwood *et al.* 1995) so may also act effectively as a biological water pump.

Eucalyptus saligna Smith (Sydney blue gum) is closely related, and sometimes confused with, *E. grandis*. *E. saligna* is similar in growth rate yet is more tolerant to cold climates (Borough *et al.* 1978; Eldridge *et al.* 1994) and low soil moisture (Cremer, Cromer & Florence 1978). The species has indicated good survival and growth on poor sites in southern Western Australia (White 1968). The high durability of this species may render it more tolerant to acidic overburden materials than the moderately tolerant *E. grandis*. The species has demonstrated some transpiration regulation in the jarrah forest during summer (Colquhoun *et al.* 1984), so may do well on drier overburden sites.

Eucalyptus tereticornis Smith (Forest red gum) has the most extensive latitudinal range of the *Eucalyptus* genus and occurs in a wide range of altitudes, thus existing in a wide variety of climatic patterns (FAO 1979; Boland *et al.* 1992). The species is more drought resistant than *E. grandis*, though less so than *E. camaldulensis,* and is highly adaptable to waterlogged conditions (Turnbull & Pryor 1978; FAO 1979; Eldridge *et al.* 1994). Despite being reported not to be suitable for timber production in strongly acidic soils (FAO 1979), this species could be tested for revegetating acidic overburdens, where water pumping may be more important than timber properties.

Few understorey species were included in the dissertation. The legume species *Acacia baileyana* F. Muell., *Acacia celastrifolia* Benth., *A. extensa* Lindl., *A. myrtifolia* (Sm) Wild., *A. polycholia* R. Br., and *Paraserianthes lophantha* (Wild.) Nielson have performed well on revegetated dumps at Collie, quickly growing to maturity, then perishing after several years, providing a large supply of organic matter to the soil (Bartle and Riches 1978; Koch & Bell 1985; Fox, O'Dea & Patroni 1985; Coulson 1996), although Fox, Gazey & Barret (1987) reported that *A. pulchella* and *A. extensa* were not suitable for revegetating overburden material of pH 4.6. Members of the *Melaleuca* genus are well known for tolerance to saline and waterlogged sites (Read 1994; Bicknell 2001). *Melaleuca densa, M. lateritia* and *M. lanceolata* occur near creeks in the Collie region and grow fairly well in acidic spoils from Collie (Fox & Doronila 1992).

The disadvantage of using plants to ameliorate overburden is that plant growth may be slow and requires a long time to achieve results. Most species have broad distribution that allows selection of provenances within a species to be tested for tolerance (Fox & Doronila 1992). The selection of local provenances is of paramount importance in nature conservation (Brown & Hillis 1978, van Leeuwen 1994). As a general principle, seeds of local ecotypes should be used to select varieties more likely to grow well in a particular region (Silcock 1992, van Leeuwen 1994). Seeds for coal mine rehabilitation, either for direct seeding or to grow seedlings for transplanting should be collected from successfully established plants growing on coal mine rehabilitation sites. Seeds of *Acacia decurrens* and *Allocasuarina fraseriana* collected from a coal dump were heavier than those collected from a non-dump source (Alvin 1990; Fox *et al.* 1994). This suggests that the coal dump environment selects out individuals that are more adapted to such conditions. Additional seed of the recommended species should be collected from sites that have been shown to produce superior plants.

Selection need not be confined to pure species. Many members of the genus *Eucalyptus* are well known to readily hybridise, especially within the subgenera *Symphomyrtus* and *Corymbia* (Turnbull & Pryor 1978). Many hybrids have been observed to perform better in drought tolerance and growth rate than the parent species on exotic soils (FAO 1979; Eldridge *et al.* 1994). The four *Eucalyptus* species recommended in this dissertation belong to the subgenus *Symphomyrtus*. Hybrids of these species could be tested against the parent species for performance on acidic soils.

Seedling establishment

Establishing vegetation on coal spoils faces challenges, including compaction, poor water-holding capacity, infertility and high acidity (Bending & Moffat 1999). For plant cover to be sustainable, such limiting conditions must be improved and effective carbon and nutrient cycling in place (Fierro, Angers & Beauchamp 1999). Supplying nitrogen and phosphorus is critical to ensure successful seedling establishment and also the basis for self-sustenance (EPA 1995). This thesis deals mainly with the issue of vegetation establishment on very acidic sites that are nearly or completely bare. The efforts described here are directed to developing long-lasting, artificial ecosystems in a forest environment.

The problem sites arise because localised acidic surfaces develop from sulphides associated with overburden materials between coal seams, either not removed or buried in the initial efforts at modern rehabilitation; or because they are on historical sites that were never treated after the cessation of mining. Tree establishment often fails on these particularly acidic surfaces, largely due to the unavailability of nitrogen and phosphorus. Not only does the presence of acidity reduce availability of major nutrients to plant roots but the over- and inter-burden materials themselves (derived from considerable depths) have little in the way of nutrients present. Historically, topsoils were not conserved. Such tree growth as did occur was often stunted and roots confined to the surface (Bending & Moffat 1999).

There is variation in acidity levels between strata (Koch 1984). Conventional dump revegetation establishment relies on a combination of burial of the more acidic overburdens by ~ 2 m surface placement of the more benign strata (in terms of acid-generating potential); return of previously stored topsoil; ripping to harvest water; direct seeding (Bartle & Slessar 1989), of trees and shrubs, with a high component of *Acacia* species (and other

leguminous shrubs: *Bossiaea etc.*); plus fertiliser dressing. Amelioration with a surface dressing of lime may also be used but unfortunately supplies of economic neutralising materials are scarce in the region (Koch & Bell 1983). Where these conventional procedures are inadequate for tree establishment, the remedial practices trialed and reported in this thesis emphasise tree planting. Transplanted eucalypt seedlings require a relatively benign planting hole and, particularly, an initial input of nutrients, most easily made via fertiliser placement.

Fertiliser and lime

Localised preparation to benefit transplanted seedlings may also stimulate growth of unwanted, non-native plants. The local environment around dumps in the Collie area is now dominated by agricultural land, mainly used for cattle grazing. An extensive suite of introduced annual species comprises the pastures that are based on ryegrass (*Lolium perenne*) and sub-terranean clover (*Trifolium subterranean*). Fertilising mine dump surfaces will stimulate growth of these annual species. Use of cow manure also increases the amount of seed from annual species that will germinate in and around holes used for transplanted eucalypt seedlings. Vigorous growth of grass and broad-leaved weed species provide competition with the newly planted seedlings and may pose as a fire hazard (EPA 1995). Weed proliferation from application of fertiliser in revegetating dumps at the Curragh coal mine in Queensland and mine spoils in New Mexico, resulted in mortality of transplanted seedlings (Fisher, Fancher & Aldon 1990; Orr, Bell & Mulligan 1990).

Weed infestation, then, must be controlled to maximise growth of desired plant species. Successful production of plantation forests is dependent on sound weed management practices (Turnbull & Pryor 1978; Sadanandan & Sands 1993). Weed infestation on rehabilitated mine sites is difficult to control, so attention is best directed to early control of weeds to enhance the growth and survival of seedlings. Fertilisers and manures should be used carefully as both can stimulate weed growth, seed set and spread (Burns 1989; Minerals Council of Australia 1998).

Regardless of which application method was used, seedlings clearly benefited from fertiliser. This is advantageous in that there is a wide range of options in supplying fertiliser to seedlings. Growth of *Eucalyptus* seedlings on rehabilitated bauxite areas in the Darling Ranges is stimulated, irrespective of the fertiliser type, provided high levels for nitrogen and

phosphorus is applied (Ward 1983). Research on fertiliser timing has not been conducted, but as much of the annual uptake of nutrients in south-western Australia is thought to occur during spring and early summer (Grove, Thomson & Malajczuk 1996), it is likely that applying fertiliser at the beginning of this period would allow maximum uptake and minimum loss of nutrients. This also is suitable for straight nitrogen fertiliser, as it must be applied when soil is not too dry, to prevent volatilisation, and not during time of rains, to prevent loss by leaching (Allen 1987).

The natural colonisation of plants is slow but planting or seeding techniques aim to speed up ecosystem development. Some sites abandoned after mining in the past, with no effort at seeding or planting, revegetated rather poorly and single species locally dominated some sites under the founder species rule. Leguminous shrubs should be welcomed in such contexts as they will contribute nitrogen. Kaye *et al.* (2000) demonstrate that interplanting of the leguminous tree *Albizia falcataria* (L.) Fosberg among *Eucalyptus saligna* Sm. in Hawaii, increases availability of soil nitrogen with the proportion of *Albizia* in the mixture.

Heavy organic amendments are sometimes used to accelerate construction of a functional ecosystem. It may be relatively easy to construct simple ecosystems, such as a single grass species (for example: Fierro *et al.* 1999). Revegetation aims to speed up the processes of natural succession and practices that reduce the number of subsequent interventions are to be preferred. Very acidic sites may require a long time span before new vegetation stands become self-sustaining. In a parallel with development of vegetation on abandoned agricultural land it is likely that both carbon accumulation and nitrogen cycling will take many years to attain sustainable levels (Knops & Tilman 2000). The general experience is that tree growth is positively related to foliar nitrogen and phosphorus levels (Bending & Moffat 1999).

The frequency of fertiliser application needs to be addressed. For cropping, annual nutrient maintenance is often necessary. Legume-based pastures need less frequent nutrient maintenance and it is clearly uneconomical to grow tree crops using annual fertiliser applications. The issue of repeating fertilisation depends partly on the complex soil chemistry of acid sulphide soils as pH affects nutrient availability and plant respiration processes (Farmer, Richardson & Brown 1976). A single application is more economical of labour than several reapplications. It is possible that one large application (such as 200 g N+P per plant) may be adequate for several years of optimal growth. A combination of low

and highly soluble phosphorus fertiliser may meet both the initial and long-term demand of *Eucalyptus* seedlings to allow high productivity (Fernandez *et al.* 2000).

For established stands, supply of nitrogen and/or phosphorus fertiliser has been observed to improve growth of established eucalypts seedlings in various trials on plantations and rehabilitated mine sites (Pryor & Clark 1964; Ward 1983; Grove 1988; Koch, Sudmeyer & Pickersgell 1988; Fox & Doronila 1992). Nitrogen inputs may increase soil carbon storage by decreasing decomposition (Kaye *et al.* 2000). However, supplying nitrogen and phosphorus alone may not be adequate as other nutrients may be required for plant growth. Lack of calcium appeared to be a contributing factor in mortality of *E. patens* seedlings on coal mine interburdens (Fox, Colquhoun & Owens 1988). Care must be taken to ensure that the fertilisers applied contain the particular nutrients that the deficient vegetation require. Application of micronutrients does not affect height or establishment densities of seedlings on rehabilitated bauxite mines in Western Australia (Lockley & Koch 1996). Issues of soil fertility must be addressed for each component of constructed ecosystems. For example, whereas members of the Myrtaceae generally respond to added phosphorus, species in the Proteaceae do not and generally appear to grow well with little better phosphorus (Lamont 1978).

A combination of manure and inorganic fertilisers may prove to be more beneficial to plant growth than either alone. Manure improves soil structure and encourages microbial activity but contains fewer nutrients than fertilisers (Sheat & Schofield 1999). If the soil is also supplemented with chemical fertilisers, nutrients can be retained within the organic matrix of the manure, thus improving long-term productivity beyond the immediate growth stimulus provided by fertiliser application (Knuti & Korpi 1970; Simpson 1986; Polgase, Comderford & Jokela 1992). Grass populations growing on zinc and copper slag tips responded to sewerage application but grew significantly more when chemical fertiliser was also applied (Gadgil 1969). A combination of poultry manure and inorganic fertiliser was more effective in maximising plant growth on bauxite and gold mining waste materials than with either treatment alone (Bell *et al.* 2000).

A case may be put that local sources of manure may be used, to avoid unwitting introduction of weedy species that may or may not already be present in the acidic sites. However, under some circumstances non-local manure may lead to reduced fitness of weed species (Keller, Kollman & Edwards 2000).

Slow release fertilisers have potential in providing a long-term nutrient supply and do not encourage weed infestation to the extent that conventional materials do. It is believed that the higher fertiliser application rates may improve growth more than the lower application in the long-term. Tablets made from diammonium phosphate (DAP) contain high levels of nitrogen and phosphorus. DAP improves *Eucalyptus* seedling growth on revegetated bauxite mined areas at the same rate as Agras™ and IBDU™ slow release tablets, yet DAP tablets are more economical. Growth was not affected whether DAP is applied as a single large, or several small applications (Koch 1989). DAP may prove the most appropriate fertiliser for improving seedling establishment on acidic spoils.

It has not been possible to demonstrate a rapid and dramatic change in soil acidity through lime application. This remains a possibility and an important topic for future research. Lime was only able to increase germinant growth in combination with fertiliser, and did not enhance growth of established seedlings given fertiliser. There are several possibilities why lime did not assist plant growth. The most obvious is that the quantity of lime used was insufficient to neutralise the spoil for a reasonably adequate period. The material may also have been too coarsely crushed to neutralise the acidic overburden rapidly. Limestone quality may have affected neutralising capacity; using lime with higher calcium content may assist soil neutralisation. Magnesite is thought to be a more suitable neutralising agent as it is more available and appears to be more environmentally benign than lime (Creagh 1993).

Mycorrhiza

Application of hyphal inoculum of *Pisolithus tinctorius* did not improve seedling growth on acidic overburden materials. In this case there may have been insufficient nutrients within the spoils for the mycorrhizal fungi to collect. However, mycorrhizal research is still in its infancy so this method should not be simply dismissed. Soil plugs are sometimes used to introduce desirable species (Handa & Jefferies 2000). Such procedures may be adapted to incorporate inoculum of beneficial mycorrhizal fungi. The symbiosis may yet prove to assist plants indirectly over the long term, such as resistance to metal toxicity and harmful microbes.

Addition of manure or woody materials may increase the efficiencies of mycorrhizal symbiosis. Plant nutrition may be improved by increasing organic matter and microbial activity (Archer, Hodges & LeHunt 1993). *Pisolithus tinctorius* and chicken manure

increased growth of the shrub *Gastrolobium bilobum* in coal interburden treatment (Fox & Doronila 1992). Decayed wood and humus may serve an important role in supporting ectomycorrhiza. Soil humus is a major substrate for ectomycorrhiza for most of the year, except in mid- to late-summer when it most commonly occurs in decaying wood, where conditions are more moist (Harvey, Jurgenson & Larson 1978).

Two potential genera of ectomycorrhizal fungi are *Scleroderma* and *Laccaria*. Members of the genus *Scleroderma* are common coloniser species of coalmine dumps that may form ectomycorrhizal relationships with *Eucalyptus* species. *Scleroderma flavidum* Pers. is known to increase the tolerance of birch seedlings to nickel (Jones & Hutchinson 1988). Several *Eucalyptus* seedlings were seen to respond better if inoculated with *Scleroderma verrucosum* (Bull.) Pers. rather than *P. tinctorius* (Fox & Doronila 1992; Burgess, Malajczuk & Grove 1993).

Laccaria is a common genus of fungi that occurs on Australian forest floors (Shepherd & Totterdell 1988) that has have been reported to infect plants in acidic conditions (Marx & Bratislav 1965). This genus may be appropriate for inoculating plants in moist sites. *Laccaria lactata* (Scop.) Fr. forms mycorrhiza with *Eucalyptus* seedlings in all but the wettest treatment and can enhance phosphorus levels in seedlings more than *P. tinctorius* (Bougher & Malajczuk 1990; Burgess, Malajczuk & Grove 1993). *Laccaria fraterna* (Cooke & Massee: Saccardo) Pegler infected *Eucalyptus* seedling roots more than *P. tinctorius* in wet conditions and had greater stomatal conductance in dry conditions than non-inoculated seedlings (Mason *et al.* 2000b).

Several arbuscular mycorrhiza species have also improved plant growth in poor soils. Inoculations of *Scutellospora calospora* (Nicolson & Gerdemann) Walker & Sanders and *Glomus fasciculatum* Thaxter & Gerd. increased growth of *Acacia concurrens* seedlings in nutrient deficient mineral sands mine spoils at Stradbroke Island (pH 5) and Eneabba (pH 5.8) by increasing plant uptake of phosphorus (Jasper, Abbott & Robson 1989). *E. marginata, E. diversicolor* and *A. pulchella* were all observed to form mycorrhiza with *Glomus fascilulatus* in pot trials (Malucjzuk *et al.* 1981).

Conclusion

In conclusion, revegetation of acidic areas requires use of tree species that are tolerant of the site conditions. The most important site conditions are lack of nutrients, acidic *per se*, free aluminium ions and physiological drought. Plant roots must have access to adequate nutrient supplies. Liming remains a topic for further investigation. Under the conditions examined, the most suitable species is *E. robusta* for all sites. On some sites, *E. camaldulensis, E. cladocalyx, E. rudis* and *Melaleuca hamulosa* are useful. *Kunzea ericifolia,* and probably a few other myrtaceous species, can be established using fascining. Longer-term sustainability of these artificial ecosystems requires understorey species and appropriate mycorrhizal symbionts be present. A range of *Acacia* and Fabaceae species is already well known to the rehabilitation practitioners. Fungi that deserve further attention include the ectomycorrhizal genera *Scleroderma* and *Lacarria* as well as such arbuscular genera as *Glomus* and *Scutellospora.*

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Personal Communications

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Appendices

Appendix 1 – Rehabilitation Protocol

Mining rehabilitation is site specific; each waste material and climate condition determines the extent and type of revegetation (Fox 1984). Revegetating acidic overburden requires four steps. The first is to assess the site to determine the specific conditions of the overburden and to select areas for appropriate types of revegetation. The second step is to prepare those areas for revegetation and to secure adequate amounts of seed or seedlings. The third step is to then revegetate the site. The final step is to maintain good seedling growth over the first 12 to 18 months.

1. Site assessment

Areas scheduled for treatment must be measured to determine the size and location of bare sites: to distinguish extant natural vegetation and weedy areas. Each site must be sampled for pH; level of fertility; soil moisture status; Al levels; and amount and types of weeds present. Appropriate methods of revegetation will be designated for each site. Sowing is preferred for large bare or weedy areas. Transplanting of seedlings is recommended for smaller areas or sites with native vegetation.

It is vital to thoroughly test the overburden to determine the soils with high Al. These areas should be mapped out and revegetated with plants known to be tolerant of these levels. If species prescriptions are to be effective to revegetate overburden with high bioavailable aluminium, the species used must have a known high Al tolerance. By testing certain plant species to known Al toxicities levels, appropriate species can be determined to revegetate the more metal toxic spoils. These surveys should not be done in a grid design but should be focused on soil type, colour and topography to determine pH status of each area (Tacey, Olsen & Watson 1977; Sorenson *et al.*1980). Samples should be taken at least 30 cm depth as surface soil pH and extractable aluminium may not be ideal indicators for sub-surface pH and aluminium extractability (Dolling, Porter & Robson 1990; Campbell 1999).

2. Site preparation

Weeds need to be sprayed with herbicides in early March. Myrtaceous vegetation with semi-ripe seed capsules should be found and the location noted. Numbers of tube stock seedlings needed for transplanting should be estimated in October/November. An ideal spacing between seedlings is 3 x 3 m. This maximises the number of seedlings within a given area without compromising growth. 1108 seedlings/ha can be planted at 3 x 3 m spacing. Long narrow areas may require high totals per hectare due to edge effects. Seedlings should be at least 6 months old and approximately 25 cm tall for optimum establishment. Orders should be placed at wholesale nurseries by December to ensure adequate supplies of seedlings.

Direct seeding may be a successful method and is cheaper and faster than planting seedlings but are vulnerable to weather conditions. Species most suitable for seeding must be able to produce large numbers or easy collected viable seeds with high germination and survival rates in the field. Local trials need to be executed to determine if seeding may be a successful and efficient method of revegetation (Handreck & Black 1991). Seedlings can be more expensive to establish than direct seeding so are only appropriate when the species cannot be established by seeding or by topsoil return (Environmental Protection Agency 1995).

There are two transplanting methods that have proved to be effective: 1) placing cow manure around the seedlings at time of transplanting; 2) using slow release DAP fertiliser tablets when planting seedlings. The amount of cow manure and fertiliser needed will be calculated and orders placed for delivery in Autumn.

Sites given the cow manure transplanting method must be prepared in May. 5 L holes must be dug at 3 x 3 m spacings, filled with manure, and then covered with overburden. Each hole must be marked to be located at the time of transplanting stage. The amount of manure needed per hectare is 5540 L.

3. Site establishment

All sites will be vegetated with species shown to be tolerant of the estimated soil acidity and Al levels, whether by sowing or transplanting. Revegetation is best commenced under suitable rainfall conditions to water the seeds or seedlings (Environmental Protection Agency 1995). The seed should be sown in early winter to minimise ant and bird predation and to maximise the period for the roots to establish before the dry summer (Bartle, McCormick and Shea 1978). In April, a seed mix of selected species and fertiliser may be directly sown using a Rally seeder into the selected overburden areas. The mix should consist of 5 to 6 kg seed and 500 kg fertiliser per hectare. A fertiliser high in nitrogen and phosphorus is required to continue plant growth and chlorophyll production. A suitable granular fertiliser is diammonium phosphate (DAP) with 20.5% nitrogen and 22.9% phosphorus. Seed mixes used should consist of the candidate species thought tolerant of the Al levels in the particular sites.

The most appropriate time for transplanting is just before the start of winter rain, approximately May 15th. Seedlings should be planted into all sites with manure holes. Other seedling sites require planting a seedling with a 100 g DAP fertiliser tablet a few centimetres adjacent to the seedling.

All sites should be sparsely covered with branches of Myrtaceous species bearing almost ripe seed capsules directly onto lightly scarified surfaces. Time of planting depends on time of harvesting the branches. The ideal time to harvest *K. ericifolia* branches appears to be in March.

4. Maintenance

Maintenance may be required to allow a site to reach the long-term planning objectives (Tacey, Olsen and Watson 1977). A site should receive maintenance until it reaches a self-sustaining state. Areas of dead seedlings need to be replaced by either seeding or transplanting.

A site poor in nutrition and vegetation will take many years to reach self sustaining and fully vegetative. Repeated treatments of fertiliser may be required until a nutrient recycle commences. Seedlings should be given 100g DAP. The amount of fertiliser needed is 115.6 kg/ha.

Manure has been shown to encourage weed growth. Weeds need to be subdued during the first twelve months as this time is most critical for seedling survival. A method of weed control is to carefully spray herbicide around the seedlings in spring as the weeds appear.

Appendix 2 – Candidate species

Table A.1: Seed source of candidate species

Species	Source	Date of collection
Allocasuarina fraseriana	Ewington 1	November 1996 July 1997
Callistachys lanceolatum	Walpole	September 1998
Callistemon speciosus	Stockton Lake	December 1996
Calothamnus rupestris	Stockton Lake	December 1996
Eucalyptus calophylla	Capel Shire	June 1998
Eucalyptus camaldulensis	Mine Entrance Marron Pool	April 1996 December 1998
Eucalyptus cladocalyx	Marron Pool	December 1996
Eucalyptus diversicolor	Walpole	September 1997
Eucalyptus gomphocephala	Australind	December 1996
Eucalyptus grandis	Marron Pool Stockton Lake	December 1998 December 1996
Eucalyptus maculata	SEC dump Marron Pool	December 1996 December 1998
Eucalyptus patens	Jarrahdale	January 1993
Eucalyptus resinifera	Stockton Lake	December 1996
Eucalyptus robusta	Muja mine entrance	December 1996 December 1998
Eucalyptus rudis	Muja mine entrance	December 1998
Eucalyptus wandoo	Marron Pool Muja mine entrance	December 1996 December 1998
Kunzea ericifolia	Coalfields road	February 1997
Melaleuca hamulosa	Ewington 1	December 1996
Neurachne alopecuroidea	Ewington 2	December 1998

Candidate species

Eucalyptus calophylla Labill (syn. *Corymbia calophylla* (Labill.) Hillis & Johnson) (common name Marri or Redgum) trees reach up to 40 m in height, sometimes forming as a mallee in poor soils. The species is widely distributed throughout the south-western area of Western Australia, and is part of both the jarrah and karri forest. The latitudinal distribution is from 29-36.5° S and altitudinal range from just over sea level to 300m. Trees occur in lateritic sandy gravels of the Darling Range plateau and on slopes and plains from the Range to near sea level. Ideal soils are sandy loam alluvium in valleys, but the species can also tolerate poor soil (Boland *et al.* 1992). *Eucalyptus calophylla* has been successfully grown in Hawaii (FAO 1979). Taxonomically, the species was originally part of the subgenus *Corymbia* (Pryor and Johnson 1971), which has been elevated to genus status (Hill & Johnson 1995). The older taxonomic name is used in this thesis.

Eucalyptus camaldulensis Dehnh. (River red gum) is the most widely distributed and variable of the *Eucalyptus* species. The natural distribution ranges from 12.75° S to 38.25° S (Eldridge *et al.* 1994). Trees normally grow to about 20 m in height, but some may reach up to 45 m. The species occurs in nearly all the seasonal waterways in the arid and semi-arid regions, and along rivers in the south-eastern end of the continent. *Eucalyptus camaldulensis* grows in a wide range of climatic conditions of temperature, humidity and rainfall. Soils are normally sandy alluvial (Boland *et al.* 1992). *Eucalyptus camaldulensis* is one of the two most widely grown species in overseas plantations (Brown and Hillis 1978), grown in South America, south-east Asia, Western Europe, the Middle East and Africa (FAO 1979; Eldridge *et al.* 1994). The species is closely related to *Eucalyptus rudis* and to a lesser extent *Eucalyptus tereticornis*; all three species belong to subgenus *Exsertaria,* series *Tereticornes* (Pryor and Johnson 1971).

Eucalyptus cladocalyx F. Muell. (Sugar gum) grows up to 15m height in poor soils and up to 35 m in higher rainfall areas. The species is endemic to the Eyre Peninsula of South Australia (Cremer, Cromer & Florence 1978). Distribution is discontinuous along

and near the coast, where mean annual rainfall is less than 600 mm (Turnbull & Pryor 1978). Altitude varies from near sea level to 300 m in the southern areas, and from 300 to 600m in the Flinders Ranges. Mean annual rainfall is from 380 to 650 mm with a winter maximum. Soils are usually shallow, sometimes deep sands (Boland *et al.* 1992). *Eucalyptus cladocalyx* is planted in Africa, Western and Mediterranean Europe and the Middle East (Turnbull & Pryor 1978; FAO 1979). The species belongs to subgenus *Symphomyrtus*, series *Reduncae*, and is closely related to *Eucalyptus wandoo* (Pryor and Johnson 1971).

Eucalyptus diversicolor F. Muell. (Karri) is one of the three tallest *Eucalyptus* species, reaching up to 87 metres in height (Cremer, Cromer & Florence 1978), but it is usually between 45 and 70 m (Boland *et al.* 1992). The species occurs in high rainfall areas in the extreme south-west tip of Western Australia, latitude 34 to 35° S. Mean annual rainfall is mainly between 900 and 1300mm, with a winter maximum. *Eucalyptus diversicolor* grows in a variety of soils developed over granite, limestone, laterites and sand. The soils are low to deficient in nutrients, especially in phosphorus and are neutral to slightly acidic. Texture may vary from fine sands to sandy loams (Churchill 1967; Boland *et al.* 1992). The species occurs in mixed stands with *E. calophylla, E. marginata, E. jacksonii* and *E. guilfoylei* (Boland *et al.* 1992). The species is the second most important timber species in Western Australia (Benson & Jacobs 1978; Turnbull & Pryor 1978) and is also grown in plantations in Algeria, Chile and Israel (FAO 1979). Taxonomically, the species is a member of subgenus *Transversaria,* series *Diversicolores* (Pryor & Johnson 1971).

Eucalyptus gomphocephala D.C. (Tuart) trees normally reach between 25 and 40 m height, but those in the northern extreme are only 10 -15 m tall. Latitudinal range is from 31 to 33.75° S, mostly within 1 km of the coast, and up to 30 m in altitude. Mean rainfall is from 800 to 900 mm, with a winter maximum. Soil is neutral to alkaline, weakly podsolized with limestone. The species occurs with other local eucalypt species – *E. marginata, E. calophylla, E. cornuta* and *E. rudis* (Boland *et al.* 1992). The species has been successfully grown as a plantation species in the Mediterranean, south-east

Asia, the Middle East and Africa (FAO 1979). *E. gomphocephala* is placed in the monotypic subseries *Gomphocephalinae* (Pryor & Johnson 1971).

Eucalyptus grandis Hill ex Maiden (Rose gum or Eastern flooded gum) is the chief species of moist hardwoods of New South Wales and Queensland. Trees usually grow between 45-55 m height, but some have reached 75 m (Boland *et al.* 1992). There are two geographic groups of *E. grandis*, both within 100 km of the eastern Australian coast. The latitudinal range of the southern group is from 32.8° S to 26° S and the altitudinal range is up to 600 m. The second group ranges from 18° to 16° S, and altitudinal range is 500 to 1100 m. The temperature and rainfall varies greatly across the populations, from less than -1 to more than 31°C and from 690 to 2480 mm annual rainfall. *E. grandis* can grow in a wide range of soils but the species usually grows in moderately fertile soil in lower valley slopes near rainforests. The species can exist as pure stands and also with other *Eucalyptus* species such as *E. robusta*, *E. saligna* and *E. tereticornis* (Eldridge *et al.* 1994). *E. grandis* is the second most frequently grown *Eucalyptus* species overseas (FAO 1979). It is also grown in plantations in Western Australia, New South Wales and Queensland (Benson & Jacobs 1978; Opie, Curtin & Incoll 1978). Taxonomically, the species belongs to series *Salignae*, within the subgenus *Symphomyrtus,* and is closely related to *E. robusta* (Pryor and Johnson 1971).

Eucalyptus maculata Hook (syn. *Corymbia maculata* (Hook) Hillis and Johnson)(common name Spotted gum) normally grows to 35-45 m tall, but may reach up to 70 m (Boland *et al* 1992). Populations are found within latitudes 38° S to 25° S within 75 to 150 km of the coast of Eastern Australia. In altitude, *E. maculata* occurs between near sea level to 800 m, though is rarely above 300 m in the southern part of the distribution. It grows in either temperate to subtropical and coastal to inland climates. Mean annual rainfall varies from 700 to 1750 m, varying from uniform in the southern areas to summer maximum in northern areas. The species naturally grows in a wide range of soils but grows best in slightly moist but well drained soils that are moderately heavy in texture (Hall, Johnson & Chippendale 1975; Boland *et al* 1992). The species is one of the ten most widely used plantation *Eucalyptus* species overseas (Turnbull &

Pryor 1978) and has been grown in Latin America, Africa and the Mediterranean (FAO 1979). Taxonomically, the species is extremely closely related to *E. citriodora*, both part of the genus *Corymbia* (Hill & Johnson 1995). Again, this *Corymbia* species is referred to by the former name *Eucalyptus maculata* throughout this dissertation.

Eucalyptus patens Benth. (Western blackbutt) grows to 45 m in height and occurs in the south-west corner of Western Australia. Latitudinal distribution ranges from 32 to 35° C and altitude from near sea level to 300m. Climate ranges from warm to sub-humid, mean temperatures are from 5 to 31° C. Mean annual rainfall is 850 to 1250 mm, with a winter maximum. The species grows best in moist, not waterlogged, soils with well developed clay subsoil. *E. patens* is a minor tree species in the karri forest (Benson & Jacobs 1978) and is associated with *E. calophylla, E. diversicolor* and *Eucalyptus rudis* (Boland *et al.* 1992). *E. patens* has had little success overseas (FAO 1979). Taxonomically, the species is within the subgenus *Monocalyptus,* section *Renatheria*, series *Marginatae,* and is closely related to *E. marginata* (Pryor & Johnson 1971).

Eucalyptus resinifera Smith (Red mahogany) reaches 45 m in height (Turnbull & Pryor 1978). The species occurs right along the eastern Australian coast (14 to 35° S). Altitude ranges from sea level to 300 m in the south to 1200 m in the north. Rainfall ranges from 800 to 2500 mm, with a weak summer maximum. The climate is mostly warm-humid. Extreme temperature ranges are 1 to 34° C. The species grows best in light fertile sandy podsols and deep loams (Boland *et al.* 1971). *E. resinifera* is grown in South America, Hawaii, South East Asia and Africa (FAO 1979). The species has also been used in sawlog production on suitable sites in south-western Western Australia (Turnbull & Pryor 1978). *E. resinifera* is in the subgenus *Symphomyrtus*, subseries *Resiniferinae*, and is related to *E. grandis, E. saligna, E robusta* and *E. diversicolor* (Pryor & Johnston 1971).

Eucalyptus robusta Smith (Swamp mahogany) trees grow to 30 m in height. The species occurs in a narrow coastal belt from 23 to 35.5° S. The altitudinal range is small, from near sea level to 90 m. Temperature ranges from 6 to 32° C, and the climate is warm

humid. Mean annual rainfall is from 1000 to 1700 mm, which varies from a summer maximum in the north and even rainfall in the south. The species is restricted in habitat, mainly growing in swamps, edges of saltwater estuaries and lagoons and rarely on slopes in valleys. Soils are usually heavy clays, but it can also grow well on light sandy clays to almost pure sands. The species naturally grows in pure stands or mixed with species such as *E. resinifera* and *E. tereticornis* and *Melaleuca* species (Boland *et al.* 1992). *E. robusta* is one of the most widely planted *Eucalyptus* species overseas (Hillis & Brown 1978; Turnbull & Pryor 1978) and is grown in South America, Africa, Fiji, India, south east Asia and the United States (Turnbull & Pryor 1978; FAO 1979). Taxonomically, the species is in the subgenus *Symphomyrtus* and shares the subseries *Saligninae* with *E. grandis* and *E. saligna* (Pryor & Johnson 1971).

Eucalyptus rudis Endl. (Western flooded gum) usually grows between 10 and 20 m in height. The species mainly occurs within 200 km of the WA coast between latitudes 28 and 35° S, and altitudes of near sea level to 350 m. Mean annual temperatures range from 4 to 36° C and the climate is warm-humid to semi-arid. Mean annual rainfall ranges from 400 to 1000 mm, with a winter to early spring maximum. The species usually occurs near streams and rivers and even in streams that flow intermittently. This pattern resembles that of the closely related *E. camaldulensis*, which it replaces in the southwest. The species grows best in heavier slits and loams but will grow in clays and sands provided that there is enough soil moisture. Clay is usually present with depth and drainage is poor. *E. rudis* is associated with species *E. calophylla, E. wandoo, E. marginata* and various local species of *Melaleuca, Acacia* and *Hakea* (Boland *et al.* 1992). The species has had some success in overseas plantations, being grown in South America, India, the Middle East and Africa (FAO 1979). *E. rudis* belongs to subgenus *Exsertaria,* series *Tereticornes* (Pryor & Johnson 1971).

Eucalyptus wandoo Blakely (Wandoo) grows to 25 m in height. It occurs mainly in the foothills hills of the Darling Range in Western Australia. The latitudinal distribution is from 29.75 to 34.5° S and altitudinal range is from 100 to 300 m. The climate varies from warm sub-humid to semi arid and temperatures vary from 4 to 35° C. Mean annual

rainfall varies from 400 to 700 mm with a winter maximum. The species typically grows on podsols, dark loamy sands and sandy loams with some gravel but sometimes grows in clayey loam with fair surface drainage. Trees occur with species such as *E. marginata* and *E. calophylla* (Boland *et al.* 1992). The species belongs to subgenus *Symphomyrtus*, series *Reduncae*, and is related to *E. cladocalyx, E. gomphocephala* and *E. occidentalis* (Pryor & Johnson 1971).

Allocasuarina fraseriana (Miq.) Johnson (syn. *Casuarina fraseriana*)(Western Sheoak or Forest Sheoak) grows up to 15 m in height and occurs in the south-western corner of Western Australia, between latitudes 31 and 35° S and altitudes of near sea level and 300 m. Mean temperatures range from 4.5 to 35° C and mean annual rainfall is 750-1000 mm, mostly through winter. The species occurs mostly on impoverished lateritic gravels and on heavily leached yellow siliceous sands along the coast. *Allocasuarina fraseriana* occurs frequently with *Banksia* species, either as an understorey in the jarrah forest or in sandy open forest woodlands (Boland *et al.* 1992).

Callistachys lanceolatum Ventenat (syn. *Oxylobium lanceolatum*)(Native willow) is a fast growing shrub species that grows to 4 m in height. This leguminous species is found along the coast of southern Western Australia, in moist clay loams and sandy soils (Corrick & Fuhrer 1997). Plants require full sunlight and well drained soils (Wrigley & Fagg 1996).

Callistemon speciosus (Sims) D. C. (syn. *Callistemon glaucus*)(Albany bottlebrush) is a woody shrub to 5 m height and 3 m diameter (Wrigley & Fagg 1996). The natural habitat is along the south-west coast in WA, in swampy heathlands that are seasonally inundated (Corrick & Fuhrer 1997).

Calothamnus rupestris Shauer (Netbush or Mouse ears) is a hardy WA shrub species to 2 m height by 2 m width. The species grows best in well drained soils and slight shade to full sunlight (Wrigley & Fagg 1996).

Kunzea ericifolia (Sm.) Heynh (Spearwood) is a fast growing Western Australian species that can grow to 3 m tall and 2 m wide. This heath species grows best in full sunlight and on well drained soils (Wrigley & Fagg 1996). *K. ericifolia* is a pioneer species in disturbed areas and has been observed in low pH soils around Collie, particularly at the shore edge of Ewington 2 lake. The species is closely related to the New Zealand species *Kunzea ericioides* (kanuka)(Toelkin 1996).

Melaleuca hamulosa Turcz (Creekline honey myrtle or Broom brush honey myrtle) is a shrub species to 3 m in height and diameter (Wrigley & Fagg 1996). The species grows in kwongan and shrublands, in sandy soils, usually in saline depressions and swampy areas (Corrick & Fuhrer 1997).

Neurachne alopecuroidea R. Br. (Foxtail mulga grass) is an inconspicuous perennial grass that grows to 40 cm height. The species is the only C3 species of the *Neurachne* genus that occurs in the southwest of Western Australia (Prendergast & Hattersley 1985). *N. alopecuroidea* naturally occurs in woodlands, on slopes and shrublands (Marshal 1990). The species has been observed on disturbed soils in the Collie region, on roadsides and bordering overburden dumps.

Appendix 3 - Analysis of fertilisers, lime and potting mix

Table A.1: Nutrient analysis of Thrive™ fertiliser

Nutrient	Percentage %
NO_3	3.0
NH_4	2.6
Soluble P	5.5
Soluble K	9.0
Mg	0.15
SO_4	0.22
Cu	0.005
Zn	0.02
B	0.005
Mn	0.04
Fe	0.18
Mo	0.0002
Biuret binding agent	0.4

(Source: product container)

Table A.2: Contents of Naturegrow™ general purpose potting mix

Component	Amount
Composted organics	40%
Composted pine bark	55%
Horticultural sand	5%
Crop King 88 fertiliser	1.0 kg/ m^3
$FeSO_4$	1.5 kg/ m^3
Magrilime	1.0 kg/ m^3
Waterwell™ deep wetting agent	0.7 kg/ m^3

(Source: Envirogreen Pty Ltd technical data sheet)

Table A.3: Nutrient analysis of Osmocote Plus™ fertiliser prills

Element	Percentage
N	15%
P	9%
K	12%
Ca	2.5 mg/m^3
Cu	1.0 mg/m^3
Fe	1.0 mg/m^3
Mo	5.0 mg/m^3
Zn	10.0 mg/m^3

(Source: product container)

Table A.4: Nutrient analysis of Optimum™ hydroponic solutions A and B

Nutrient	Solution A (%W/V)	Solution B (%W/V)	Total (%W/V)	Full strength mg/L	Full strength mg/20mL
NO_3^-	3.66	0.90	4.56	31.92	0.64
NH_4^+	0.25	0.00	0.25	17.50	0.36
Soluble P	0.00	0.80	0.80	5.60	0.12
K	2.50	3.50	6.00	42.00	0.84
Ca	3.60	0	3.60	25.20	0.50
SO_4^{2-}	0.00	2.00	2.00	14.00	0.28
Mg	0.00	1.50	1.50	10.50	0.20
Fe chelate	0.10	0.00	0.10	0.70	0.02
Mn chelate	0.00	0.02	0.02	0.16	0.002
Zn chelate	0.00	0.004	0.004	0.028	0.0006
Cu chelate	0.00	0.003	0.003	0.020	0.0004
B	0.00	0.007	0.007	0.054	0.0010
Mo	0.00	0.001	0.001	0.008	0.0016

(Source: product container)

Table A.5: Chemical analysis of crushed limestone

Chemical	Percentage
$CaCO_3$	68.7
SiO_2	22.4
P_2O_5	0.08
K_2O	0.42
Mg	0.79
S	0.09
Na	0.14
Fe	0.39
Al	0.94
Mn	<0.01

(Source: Lime Industries Pty Ltd technical data sheet)

Table A.6: Nutrient analysis of Agriform™ fertiliser tablet

Element	Percentage
Nitrogen	20.0
Phosphorus	4.3
Potassium	4.1
Calcium	2.1
Sulphur	1.6
Iron	0.35

(Source: product container)

Table A.7: Nutrient analysis of NPK Blue Special™ granulated fertiliser

Element	Percentage
Nitrogen	12.0
Phosphorus	5.2
Potassium	14.1
Sulphur	7.0
Calcium	3.5
Magnesium	1.2
Iron	0.1
Zinc	0.04
Boron	0.02

(Source: product container)

Appendix 4 – Summary of soil and plant analysis methods

Plant Analysis

Nitrogen

Finely ground plant material is combusted at 950^0 C in oxygen using a Leco FP-428 Nitrogen analyser. The released nitrogen from the sample is measured as it passes through a thermal conductivity cells (Sweeney & Rexroad 1987).

Copper, zinc, manganese, calcium, magnesium, boron, sodium, iron, potassium, phosphorus and sulphur

Plant material is digested in nitric acid using a Milestone microwave. Elements are measured by ICP-AES (McQuaker, Brown & Klucker 1979).

Chloride and Nitrate

Plant material is extracted in deionised water and the chloride and nitrate nitrogen are measured simultaneously using a Lachat Flow Injection Analyser. The nitrate is reduced to nitrite through a copperised cadmium column and the nitrite measured colormetrically at 520 nm. The concentration of chloride is measured colormetrically at 480 nm (Fall, Fisher & Garner 1959).

Soil Analysis

Ammonium and Nitrate

The ammonium and nitrate nitrogen are measured simultaneously using a Lachat Flow Injection Analyser. Soils are tumbled with 2 M potassium chloride for 1 hour at 25 ^{0} C employing a soil: solution ration of 1:5. The concentration of ammonium nitrogen is measured colormetrically at 420 nm using an indo-phenol blue reaction. The nitrate is reduced to nitrate through a copperised cadmium column and measured colormetrically at 520 nm (Searle 1984).

Phosphorus and Potassium

Available phosphorus and potassium are measured using the Colwell (1965) method. Soils are tumbled with 0.5 M sodium bicarbonate solution adjusted to pH 8.5 for 16 hours at 25^0 C employing a soil: solution ration of 1:100. The acidified product is treated with ammonium molybdate/antimony trichloride reagent and the concentration of phosphorus

is measured colorimetrically at 880 nm. The concentration of potassium is determined using a flame atomic absorption spectrophotometer at 766.5 nm (Colwell 1965, Rayment & Higginson 1992).

Extractable Sulphur

Soils are extracted at 40° C for 3 hours with 0.25 potassium chloride and the sulphate sulphur is measured by ICP (Blair *et al.* 1991).

Organic Carbon

Concentrated sulphuric acid is added to soil wetted with dichromate solution. The heat of dilution is used to induce oxidation of soil organic carbon. The amount of chromic ions produced is proportional to the organic carbon oxidised and is measured colormetrically at 600 nm (Walkley & Black 1934).

Reactive Iron

Soils are tumbled with Tamm's reagent (oxalic acid/ ammonium oxalate) for 1 hour employing a soil/solution ratio of 1:33. The concentration of iron is determined using a flame absorption spectrophotometer at 248.3 nm (Walkley & Black 1934).

Electrical Conductivity, pH (calcium chloride) and pH (water)

Soils are stirred in deionised water for 1 hour at 25^0 C employing a soil: solution ratio of 1:5. The pH of the extract is measured using a combination of pH electrode calibrated against 0.01 M KCl. After pH and EC have been measured, calcium chloride solution is added to produce a concentration of 0.01 M $CaCl_2$ and pH is determined using a combination pH electrode (Rayment & Higginson 1992).

Boron

Soils are extracted with boiling 0.01 calcium chloride solutions for 15 minutes and the boron concentration is measured colorimetrically with azomethine-H (Rayment & Higgins 1992).

Extractable Aluminium

Soils are extracted with 0.01 M calcium chloride solution and the aluminium concentration of the extract is measured by ICP-AES (Bromfield 1987).

Appendix 5 – Plot maps for species selection field trial

Muja site

	12	11	10	9	8	7	6	5	4	3	2	1
10	Mel	Gomp *	Clad *	Neur	Rob	Mac *	Calo *	Rud *	Wand	Spec *	Gran *	Res *
9	Res	Mel *	Gomp	Clad	Neur	Rob *	Mac	Calo	Cam	Wand *	Spec	Gran
8	Gran *	Res *	Mel	Gomp *	Clad *	Neur *	Rob	Mac *	Calo *	Cam *	Wand	Spec *
7	Spec	Gran	Res	Mel *	Gomp	Clad	Neur	Rob *	Mac	Calo	Cam	Wand *
6	Wand	Spec *	Gran *	Res *	Mel	Gomp *	Clad *	Neur	Rob	Mac *	Calo *	Rud *
5	Rud	Wand *	Spec	Gran	Res	Mel *	Gomp	Clad	Neur	Rob *	Mac	Calo
4	Calo *	Cam *	Wand	Spec *	Gran *	Res *	Mel	Gomp *	Clad *	Neur	Rob	Mac *
3	Mac	Calo	Rud	Wand *	Spec	Gran	Res	Mel *	Gomp	Clad	Neur	Rob *
2	Rob	Mac *	Calo *	Cam *	Wand	Spec *	Gran *	Res *	Mel	Gomp *	Clad *	Neur
1	Neur	Rob *	Mac	Calo	Rud	Wand *	Spec	Gran	Res	Mel *	Gomp	Clad

|--1m--|

Fenceline ↓ N

* = harvested plants

Species	
Calo	*C. ruprestris*
Cam	*E. camaldulensis*
Clad	*E. cladocalyx*
Gomp	*E. gomphocephala*
Gran	*E. grandis*
Mac	*E. maculata*
Neur	*N. alopecuroidea*
Res	*E. resinifera*
Rob	*E. robusta*
Rud	*E. rudis*
Spec	*C. speciesus*
Wand	*E. wandoo*

Ewington site

lake

	12	11	10	9	8	7	6	5	4	3	2	1
10	Mel	Gomp	Clad	Neur	Rob	Mac	Calo	Cam	Wand	Spec	Gran	Res
9	Res	Mel	Gomp	Clad	Neur	Rob	Mac	Calo	Cam	Wand	Spec	Gran
8	Gran	Res	Mel	Gomp	Clad	Neur	Rob	Mac	Calo	Rud	Wand	Spec
7	Spec	Gran	Res	Mel	Gomp	Clad	Neur	Rob	Mac	Calo	Cam	Wand
6	Wand	Spec	Gran	Res	Mel	Gomp	Clad	Neur	Rob	Mac	Calo	Rud
5	Cam	Wand	Spec	Gran	Res	Mel	Gomp	Clad	Neur	Rob	Mac	Calo
4	Calo	Rud	Wand	Spec	Gran	Res	Mel	Gomp	Clad	Neur	Rob	Mac
3	Mac	Calo	Rud	Wand	Spec	Gran	Res	Mel	Gomp	Clad	Neur	Rob
2	Rob	Mac	Calo	Cam	Wand	Spec	Gran	Res	Mel	Gomp	Clad	Neur
1	Neur	Rob	Mac	Calo	Rud	Wand	Spec	Gran	Res	Mel	Gomp	Clad

|--1m--|

Overburden dump

↑
N

Species	
Calo	*C. ruprestris*
Cam	*E. camaldulensis*
Clad	*E. cladocalyx*
Gomp	*E. gomphocephala*
Gran	*E. grandis*
Mac	*E. maculata*
Neur	*N. alopecuroidea*
Res	*E. resinifera*
Rob	*E. robusta*
Rud	*E. rudis*
Spec	*C. speciesus*
Wand	*E. wandoo*

Appendix 6 – Chemical analysis of plants grown poorly in pot trial (Chapter 2)

Values as % or ppm dry weights (standard deviation in brackets) n=2

Table A.8: Levels of nutrients in *E. gomphocephala* seedlings

Nutrient	control	Muja	Ewington	F	*P*
Leaf					
N (%)	0.89 (0.05)	0.98 (0.10)	1.08 (0.01)	1.79	0.211 (NS)
P (%)	0.08 (0.01)	0.08 (0.01)	0.09 (0.01)	0.75	0.543 (NS)
K (%)	0.64 (0.03)	0.57 (0.16)	0.52 (0.01)	0.73	0.551 (NS)
Ca (%)	0.67 (0.02)	0.57 (0.12)	0.55 (0.08)	1.07	0.445 (NS)
Mg (%)	0.21 (0.02)	0.20 (0.03)	0.23 (0.05)	0.47	0.662 (NS)
Na (%)	0.32 (0.07)	0.36 (0.11)	0.44 (0.07)	0.92	0.488 (NS)
Cl (%)	0.54 (0.24)	0.47 (0.22)	0.56 (0.16)	0.11	0.900 (NS)
Cu (ppm)	5.50 (0.14)	6.15 (0.07)	6.40 (0.99)	0.129	0.395 (NS)
Zn (ppm)	31.7 (5.5)	87.5 (76.9)	148.0 (22.8)	3.14	0.184 (NS)
Mn (ppm)	481.8 (84.5)	444.9 (73.1)	514.1 (48.1)	0.49	0.656 (NS)
Fe (ppm)	37.7 (1.2) a	34.8 (1.2) a	23.5 (2.1) b	11.04	0.041 (*)
NO3 (ppm)	39.5 (2.1)	23.0 (8.5)	42.0	8.36	0.059 (NS)
S (%)	0.15 (0.01)	0.19 (0.06)	0.26 (0.03)	4.06	0.140 (NS)
Stem					
N (%)	1.06 (0.21)	1.24 (0.27)	0.79 (0.04)	4.34	0.151 (NS)
P (%)	0.08 (0.01)	0.12 (0.01)	0.11 (0.02)	3.84	0.148 (NS)
K (%)	0.65 (0.04)	0.60 (0.03)	0.55 (0.02)	6.64	0.079 (NS)
Ca (%)	0.74 (0.05) a	0.28 (0.05) b	0.23 (0.01) b	91.90	0.002 (**)
Mg (%)	0.30 (0.01) a	0.25 (0.04) ab	0.16 (0.03) b	11.08	0.041 (*)
Na (%)	0.51 (0.01)	0.53 (0.28)	0.33 (0.01)	0.90	0.449 (NS)
Cl (%)	0.92 (0.08) a	0.39 (0.08) b	0.56 (0.01) b	36.12	0.008 (**)
Cu (ppm)	4.45 (0.49) a	4.95 (0.21) a	4.75 (0.35) b	2.05	0.327 (NS)
Zn (ppm)	81.8 (1.4) a	114.8 (33.0) a	80.0 (1.4) b	16.13	0.025 (NS)
Mn (ppm)	141.5 (14.8)	136.9 (89.5)	39.4 (2.5)	2.42	0.237 (NS)
Fe (ppm)	117.6 (6.2)	95.8 (19.4)	66.5 (1.84)	9.39	0.051 (NS)
NO3 (ppm)	40.0 (1.4)	33.5 (10.7)	36.5 (2.1)	0.53	0.633 (NS)
S (%)	0.09 (0.01) b	0.30 (0.08) a	0.08 (0.01) b	13.99	0.030 (*)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table A.9: Levels of nutrients in *E. grandis* seedlings

Nutrient	control	Ewington	F	*P*
Leaf				
N (%)	0.51 (0.05)	0.51 (0.01)	0.00	0.972 (NS)
P (%)	0.31 (0.11)	0.23 (0.02)	1.02	0.419 (NS)
K (%)	0.45 (0.21)	0.43 (0.04)	0.03	0.878 (NS)
Ca (%)	0.89 (0.12)	1.02 (0.08)	2.13	0.234 (NS)
Mg (%)	0.27 (0.10)	0.23 (0.03)	0.24	0.672 (NS)
Na (%)	0.72 (0.37)	0.60 (0.05)	0.21	0.689 (NS)
Cl (%)	1.44 (0.96)	1.54 (0.19)	0.02	0.904 (NS)
Cu (ppm)	5.00 (0.14)	5.35 (0.35)	1.69	0.323 (NS)
Zn (ppm)	170.6 (92.1)	21.0 (0.2)	5.28	0.148 (NS)
Mn (ppm)	451.4 (130.4)	198.2 (4.6)	7.58	0.110 (NS)
Fe (ppm)	30.25 (0.07)	52.65 (3.46)	83.56	0.012 (*)
NO3 (ppm)	41.5 (0.7)	40.5 (0.7)	2.00	0.293 (NS)
S (%)	0.116 (0.015)	0.082 (0.017)	4.05	0.182 (NS)
Stem				
N (%)	1.17 (0.07)	1.04 (0.04)	1.87	0.314 (NS)
P (%)	0.06 (0.01)	0.07 (0.01)	1.53	0.342 (NS)
K (%)	0.52 (0.04)	0.50 (0.14)	0.61	0.516 (NS)
Ca (%)	0.45 (0.04)	1.11 (0.01)	493.22	0.002 (**)
Mg (%)	0.29 (0.01)	0.19 (0.01)	150.19	0.007 (**)
Na (%)	0.28 (0.03)	0.22 (0.01)	10.63	0.083 (NS)
Cl (%)	0.34 (0.08)	0.54 (0.02)	12.07	0.074 (NS)
Cu (ppm)	1.90 (0.28)	3.05 (0.21)	21.16	0.044 (*)
Zn (ppm)	66.2 (2.4)	71.0 (3.2)	4.48	0.316 (NS)
Mn (ppm)	222.8 (12.8)	204.2 (8.2)	3.92	0.213 (NS)
Fe (ppm)	44.4 (7.1)	80.8 (2.6)	45.56	0.021 (*)
NO3 (ppm)	42.0	45.0 (2.8)	2.51	0.275 (NS)
S (%)	0.09 (0.01)	0.11 (0.01)	2.99	0.226 (NS)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table A.10: Levels of nutrients in *E. wandoo* seedlings

Nutrient	control	Muja	Ewington	F	*P*
Leaf					
N (%)	1.30 (0.14)	1.11 (0.09)	1.05	3.54	0.163 (NS)
P (%)	0.10 (0.01)	0.08 (0.02)	0.14 (0.05)	1.80	0.307 (NS)
K (%)	0.86 (0.09) a	0.53 (0.09) b	0.70 (0.03) ab	9.74	0.049 (*)
Ca (%)	0.63 (0.06)	0.55 (0.03)	0.46 (0.06)	4.65	0.120 (NS)
Mg (%)	0.18 (0.01)	0.18 (0.04)	0.24	4.71	0.119 (NS)
Na (%)	0.97 (0.02)	0.84 (0.20)	1.11 (0.13)	2.53	0.311 (NS)
Cl (%)	0.96 (0.15)	0.73 (0.73)	1.17 (0.15)	2.93	0.415 (NS)
Cu (ppm)	2.10 (0.57)	2.85 (0.63)	3.55 (0.21)	4.10	0.139 (NS)
Zn (ppm)	22.6 (5.8)	26.4 (12.7)	30.4 (7.3)	2.18	0.412 (NS)
Mn (ppm)	325.3 (43.7)	252.8 (56.07)	359.0 (26.16)	3.08	0.187 (NS)
Fe (ppm)	65.2 (8.4) a	36.4 (4.5) b	27.9 (4.7) b	20.26	0.018 (*)
NO3 (ppm)	44.5 (2.1)	44.5 (4.9)	42.5 (0.7)	0.27	0.779 (NS)
S (%)	0.14 (0.02)	0.20 (0.02)	0.15 (0.01)	8.15	0.061 (NS)
Stem					
N (%)	1.16 (0.02)	1.11 (0.07)	1.12 (0.01)	1.10	0.437 (NS)
P (%)	0.12 (0.01)	0.07 (0.02)	0.09 (0.01)	5.46	0.100 (NS)
K (%)	0.48 (0.17)	0.60 (0.20)	0.74 (0.01)	1.46	0.361 (NS)
Ca (%)	0.82 (0.06)	1.10 (0.26)	0.68 (0.03)	4.00	0.142 (NS)
Mg (%)	0.30 (0.03)	0.27 (0.04)	0.33 (0.05)	2.57	0.224 (NS)
Na (%)	0.50 (0.03)	0.44 (0.05)	0.40 (0.01)	1.72	0.449 (NS)
Cl (%)	0.32 (0.02)	0.44 (0.02)	0.37 (0.05)	1.53	0.519 (NS)
Cu (ppm)	5.4 (0.6)	5.4 (0.1)	4.4 (0.4)	4.74	0.118 (NS)
Zn (ppm)	55.0 (5.6) a	17.7 (1.4) b	41.2 (1.6) ab	58.21	0.004 (**)
Mn (ppm)	226.1 (35.1)	289.5 (17.7)	260.6 (7.9)	3.77	0.152 (NS)
Fe (ppm)	119.0 (19.5)	102.1 (0.78)	110.9 (5.4)	1.05	0.451 (NS)
NO3 (ppm)	27.5 (6.4)	38.0 (4.2)	35.0 (4.2)	2.02	0.316 (*)
S (%)	0.28 (0.11) b	0.18 (0.07) b	0.63 (0.08) a	14.56	0.029 (*)

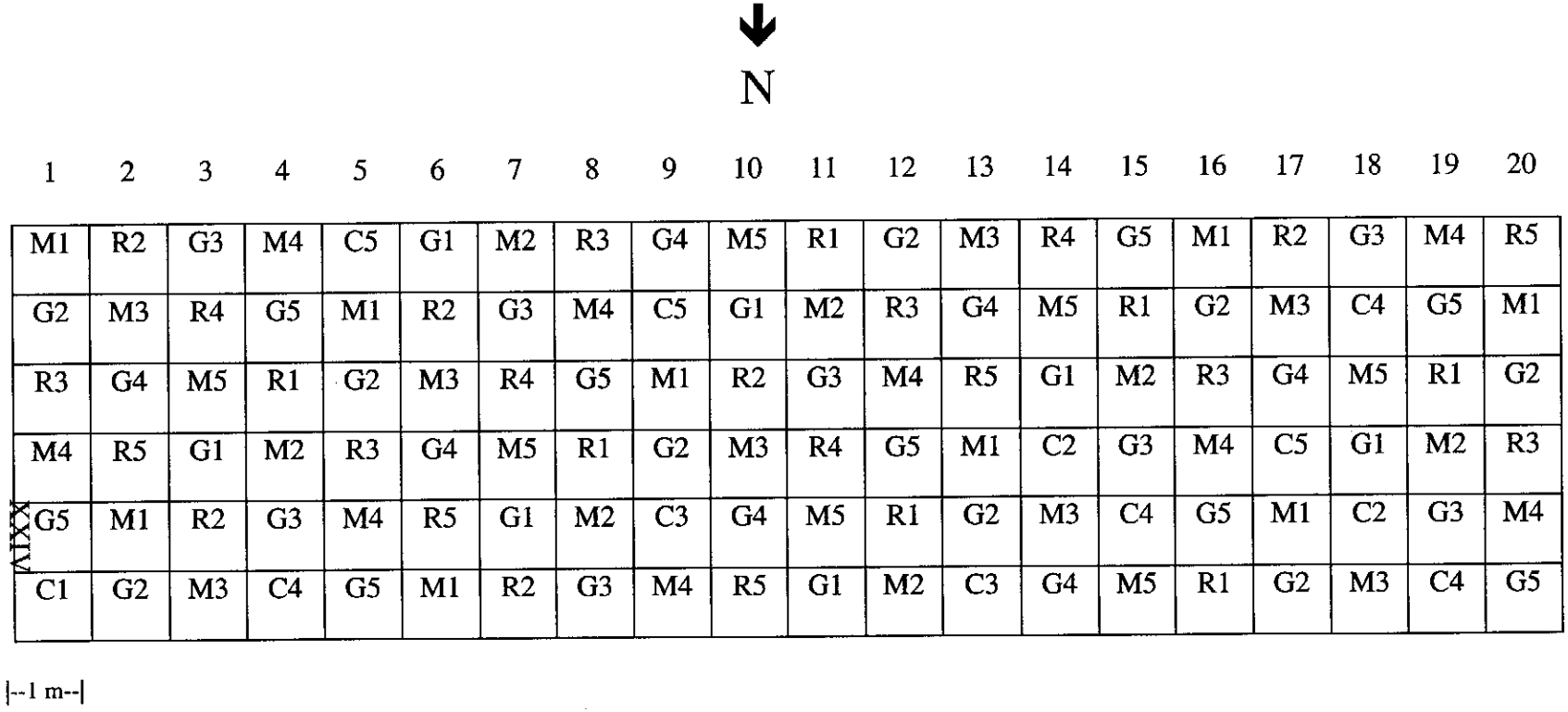
***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table A.11: Levels of nutrients in *C. rupestris* seedlings

Nutrient	control	Ewington	F	*P*
Leaf				
N (%)	1.00 (0.07)	0.96 (0.01)	0.61	0.515 (NS)
P (%)	0.10 (0.02)	0.06 (0.01)	10.81	0.081 (NS)
K (%)	1.05 (0.03)	0.61 (0.01)	522.35	0.002 (**)
Ca (%)	0.60 (0.01)	0.10 (0.01)	1826.31	0.001 (**)
Mg (%)	0.32 (0.01)	0.21 (0.01)	37.73	0.025 (*)
Na (%)	0.80 (0.01)	0.21 (0.01)	5965.20	<0.001 (***)
Cl (%)	2.69 (0.08)	0.28 (0.01)	2021.42	<0.001 (***)
Cu (ppm)	2.40 (0.14)	3.85 (0.07)	168.20	0.006 (**)
Zn (ppm)	19.2 (0.28)	8.8 (0.4)	832.00	0.001 (**)
Mn (ppm)	109.2 (5.9)	117.0 (4.6)	7.82	0.134 (NS)
Fe (ppm)	26.55 (9.55)	59.65 (0.64)	23.94	0.039 (*)
NO3 (ppm)	16.00 (2.83)	18.5 (0.71)	1.47	0.349 (NS)
S (%)	0.12 (0.01)	0.10 (0.01)	15.38	0.059 (NS)
Stem				
N (%)	0.65 (0.06)	0.81 (0.19)	13.58	0.079 (NS)
P (%)	0.17 (0.04)	0.14 (0.03)	1.32	0.646 (NS)
K (%)	0.50 (0.01)	0.63 (0.10)	3.72	0.193 (NS)
Ca (%)	0.42 (0.01)	0.55 (0.16)	1.35	0.365 (NS)
Mg (%)	0.19 (0.02)	0.29 (0.05)	7.90	0.107 (NS)
Na (%)	0.28 (0.01)	0.43 (0.08)	6.77	0.121 (NS)
Cl (%)	0.47 (0.18)	0.54 (0.09)	0.23	0.681 (NS)
Cu (ppm)	1.75 (0.50)	7.85 (1.91)	19.13	0.048 (*)
Zn (ppm)	25.4 (1.3)	21.9 (2.8)	8.87	0.217 (NS)
Mn (ppm)	68.9 (2.8)	72.2 (9.6)	1.58	0.335 (NS)
Fe (ppm)	38.10 (11.88)	87.55 (21.28)	8.23	0.103 (NS)
NO3 (ppm)	42.00 (1.41)	43.50 (2.12)	0.69	0.493 (NS)
S (%)	0.08 (0.01)	0.18 (0.02)	72.48	0.014 (NS)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Appendix 7 – Map of substrate trial (Chapter 4)



Species

C = *E. camaldulensis*
G = *E. grandis*
M = *E. maculata*
R = *E. rudis*

Substrate

1 = potting mix
2 = potting mix + lime
3 = cow manure
4 = cow manure + lime
5 = lime

Appendix 8 – Map of Nitrogen and Phosphorus Trial (Chapter 5)

(see Appendix 5, Ewington map for original species planted)

10		Gomp N	Clad N+P		Rob P			Cam	Wand P		Gran P	Res
9	Res P		Gomp P	Clad N		Rob N	Mac P		Cam	Wand N		Gran N
8	Gran P	Res N		Gomp N	Clad P		Rob N+P	Mac N		Rud P	Wand N+P	
7		Gran N	Res N+P		Gomp N+P	Clad		Rob	Mac P		Cam	Wand
6			Gran N+P	Res N		Gomp	Clad N+P		Rob P	Mac		Rud
5	Cam P	Wand P		Gran	Res P		Gomp P	Clad N		Rob N	Mac N+P	
4		Rud P	Wand N		Gran P	Res		Gomp	Clad P		Rob P	Mac N
3	Mac N+P		Rud	Wand P		Gran N	Res N+P		Gomp N+P	Clad		Rob
2	Rob N+P	Mac		Cam P	Wand N+P		Gran N+P	Res		Gomp N	Clad N+P	
1		Rob N	Mac N+P		Rud P	Wand N+P		Gran	Res N+P		Gomp P	Clad
	12	11	10	9	8	7	6	5	4	3	2	1

|--- 1 m---|

Treatment

C	Control
N	Nitrogen
P	Phosphorus
N+P	Nitrogen and Phosphorus

Species

Cam	*E. camaldulensis*
Clad	*E. cladocalyx*
Gomp	*E. gomphocephala*
Gran	*E. grandis*
Mac	*E. maculata*
Res	*E. resinifera*
Rob	*E. robusta*
Rud	*E. rudis*
Wand	*E. wandoo*

⇑
N

Appendix 9 – Statistical analysis of plants in inoculation trial (Chapter 7)

Table A.12: Mean heights (cm) of inoculated seedlings at WO5H 0-12 mths

Species	n	Control	n	Inoculated	*P*
0 months					
Eucalyptus calophylla	9	21.4 (4.1)	9	20.4 (2.1)	0.527 (NS)
E. camaldulensis	5	17.8 (2.4)	9	15.0 (2.8)	0.083 (NS)
E. diversicolor	9	17.3 (2.1)	9	16.0 (1.8)	0.170 (NS)
E. patens	4	9.5 (1.3)	5	9.6 (1.8)	0.929 (NS)
E. robusta	7	20.0 (2.0)	9	20.1 (1.8)	0.908 (NS)
E. rudis	7	15.9 (1.6)	9	16.2 (1.6)	0.651 (NS)
Allocasuarina fraseriana	4	17.7 (2.3)	6	14.5 (1.9)	0.061 (NS)
Kunzea ericifolia	5	30.0	6	30.0	1.000 (NS)
Calistachys bilobum	5	31.6 (1.1)	4	30.8 (1.7)	0.838 (NS)
2 months					
Eucalyptus calophylla	9	22.1 (5.2)	9	21.1 (3.0)	0.453 (NS)
E. camaldulensis	5	18.8 (4.7)	8	15.6 (3.2)	0.378 (NS)
E. diversicolor	9	17.8 (3.2)	8	16.4 (3.6)	0.057 (NS)
E. patens	3	10.0 (2.0)	5	10.2 (3.3)	0.929 (NS)
E. robusta	6	20.5 (4.5)	9	20.2 (3.5)	0.894 (NS)
E. rudis	7	17.9 (4.3)	7	16.9 (2.0)	0.588 (NS)
Allocasuarina fraseriana	2	11.5 (6.4)	4	8.5 (1.3)	0.362 (NS)
Kunzea ericifolia	5	30.2 (4.0)	6	31.7 (4.9)	0.915 (NS)
Calistachys bilobum	5	39.4 (7.7)	4	31.8 (1.3)	0.095 (NS)
4 months					
Eucalyptus calophylla	9	27.1 (9.9)	9	24.7 (4.6)	0.511 (NS)
E. camaldulensis	5	21.8 (6.4)	7	17.9 (5.0)	0.258 (NS)
E. diversicolor	8	20.6 (4.1)	7	19.0 (6.9)	0.581 (NS)
E. patens	3	18.3 (8.5)	4	17.8 (5.1)	0.913 (NS)
E. robusta	7	26.9 (8.6)	9	28.9 (5.0)	0.456 (NS)
E. rudis	7	21.0 (6.8)	7	23.4 (4.1)	0.434 (NS)
Allocasuarina fraseriana	4	14.0 (7.1)	4	10.8 (5.1)	0.544 (NS)
Kunzea ericifolia	4	41.2 (10.1)	6	36.0 (4.0)	0.276 (NS)
Calistachys bilobum	5	50.0 (15.8)	4	42.8 (8.1)	0.436 (NS)
6 months					
Eucalyptus calophylla	9	28.8 (6.9)	9	28.2 (3.6)	0.933 (NS)
E. camaldulensis	5	23.2 (6.6)	7	20.1 (3.1)	0.305 (NS)
E. diversicolor	7	24.1 (5.7)	7	22.0 (6.6)	0.527 (NS)
E. patens	3	32.0 (17.4)	4	21.0 (3.65)	0.262 (NS)
E. robusta	7	31.1 (8.0)	9	30.0 (9.7)	0.805 (NS)
E. rudis	7	25.1 (7.2)	7	29.1 (3.5)	0.209 (NS)
Allocasuarina fraseriana	4	9.5 (0.6)	4	18.5 (10.5)	0.137 (NS)
Kunzea ericifolia	4	48.5 (15.2)	6	41.3 (4.3)	0.295 (NS)
Calistachys bilobum	5	56.2 (22.9)	4	47.8 (9.7)	0.517 (NS)
12 months					
Eucalyptus calophylla	9	35.4 (15.2)	9	34.2 (6.4)	0.827 (NS)
E. camaldulensis	5	30.4 (9.6)	7	27.9 (4.8)	0.556 (NS)
E. diversicolor	7	29.1 (4.1)	7	27.2 (6.0)	0.478 (NS)
E. patens	3	38.5 (27.2)	4	27.2 (10.7)	0.463 (NS)
E. robusta	7	43.7 (20.0)	9	37.0 (8.6)	0.377 (NS)
E. rudis	7	29.4 (5.6)	7	31.9 (5.6)	0.434 (NS)
Allocasuarina fraseriana	3	20.0 (3.6)	4	22.7 (4.3)	0.406 (NS)
Kunzea ericifolia	3	47.3 (15.4)	4	44.2 (14.2)	0.588 (NS)
Calistachys bilobum	5	72.6 (35.4)	3	67.0 (11.3)	0.843 (NS)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table A.13: Mean crown diameters (cm) of inoculated seedlings at WO5H 0-12 mths

Species	n	Control	n	Inoculated	*P*
0 months					
Eucalyptus calophylla	9	13.6 (2.3)	9	14.0 (1.6)	0.267 (NS)
E. camaldulensis	5	10.8 (2.1)	9	10.1 (1.2)	0.274 (NS)
E. diversicolor	9	10.9 (1.7)	9	10.3 (1.6)	0.577 (NS)
E. patens	4	11.0 (2.9)	5	9.9 (1.5)	0.482 (NS)
E. robusta	7	10.4 (1.9)	9	10.7 (2.0)	0.716 (NS)
E. rudis	7	9.6 (1.4)	9	9.5 (1.4)	0.841 (NS)
Allocasuarina fraseriana	4	11.0 (2.8)	6	13.9 (4.5)	0.348 (NS)
Kunzea ericifolia	5	5.3 (1.6)	6	4.4 (1.1)	0.316 (NS)
Calistachys bilobum	5	23.7 (1.2)	4	19.0 (9.3)	0.121 (NS)
2 months					
Eucalyptus calophylla	9	14.3 (1.5)	9	14.3 (1.7)	1.000 (NS)
E. camaldulensis	5	11.1 (3.4)	8	10.4 (1.6)	0.172 (NS)
E. diversicolor	9	12.2 (2.6)	8	10.2 (1.8)	0.090 (NS)
E. patens	3	12.3 (2.4)	5	10.4 (2.4)	0.314 (NS)
E. robusta	6	12.4 (3.5)	9	11.1 (1.9)	0.345 (NS)
E. rudis	7	9.4 (1.8)	7	9.5 (0.6)	0.850 (NS)
Allocasuarina fraseriana	2	16.8 (4.6)	4	12.4 (4.9)	0.353 (NS)
Kunzea ericifolia	5	13.6 (2.9)	6	10.6 (4.4)	0.225 (NS)
Calistachys bilobum	5	32.0 (2.4)	4	25.6 (6.9)	0.091 (NS)
4 months					
Eucalyptus calophylla	9	14.3 (3.4)	9	16.7 (2.5)	0.109 (NS)
E. camaldulensis	5	11.4 (2.9)	7	10.8 (4.7)	0.802 (NS)
E. diversicolor	8	11.9 (2.0)	7	10.5 (4.3)	0.435 (NS)
E. patens	3	15.2 (4.3)	4	12.8 (2.9)	0.412 (NS)
E. robusta	7	14.8 (6.2)	9	14.9 (5.5)	0.972 (NS)
E. rudis	7	9.6 (2.7)	7	9.9 (3.0)	0.150 (NS)
Allocasuarina fraseriana	4	10.8 (3.9)	4	10.0 (5.9)	0.882 (NS)
Kunzea ericifolia	4	18.5 (9.0)	6	15.6 (4.9)	0.522 (NS)
Calistachys bilobum	5	48.6 (4.4)	4	38.5 (12.1)	0.123 (NS)
6 months					
Eucalyptus calophylla	9	16.2 (4.5)	9	18.0 (4.7)	0.425 (NS)
E. camaldulensis	5	11.6 (3.3)	7	11.1 (4.0)	0.832 (NS)
E. diversicolor	7	11.6 (3.8)	7	11.1 (2.8)	0.783 (NS)
E. patens	3	17.5 (7.3)	4	11.3 (4.9)	0.291 (NS)
E. robusta	7	19.0 (9.7)	9	15.8 (6.5)	0.446 (NS)
E. rudis	7	9.2 (2.6)	7	12.0 (2.8)	0.075 (NS)
Allocasuarina fraseriana	4	5.5 (1.8)	4	6.4 (4.6)	0.698 (NS)
Kunzea ericifolia	4	19.6 (9.8)	6	17.4 (4.1)	0.665 (NS)
Calistachys bilobum	5	54.2 (10.0)	4	38.9 (11.5)	0.069 (NS)
12 months					
Eucalyptus calophylla	9	25.6 (16.1)	9	30.8 (32.9)	0.678 (NS)
E. camaldulensis	5	15.6 (4.4)	7	15.0 (5.1)	0.827 (NS)
E. diversicolor	7	14.9 (3.6)	7	14.5 (3.3)	0.845 (NS)
E. patens	3	30.0 (22.5)	4	19.1 (4.3)	0.379 (NS)
E. robusta	7	29.7 (27.4)	9	21.4 (6.6)	0.390 (NS)
E. rudis	7	13.8 (2.9)	7	14.5 (2.6)	0.636 (NS)
Allocasuarina fraseriana	3	11.0 (1.7)	4	12.9 (3.8)	0.708 (NS)
Kunzea ericifolia	3	27.5 (8.5)	4	23.5 (2.0)	0.678 (NS)
Calistachys bilobum	5	69.3 (25.2)	3	54.7 (18.7)	0.502 (NS)

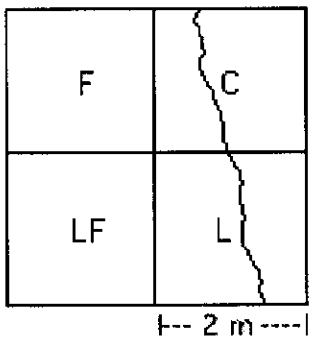
***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

Table A.14: Mean basal diameters (mm) of inoculated seedlings at WO5H 0-12 mths

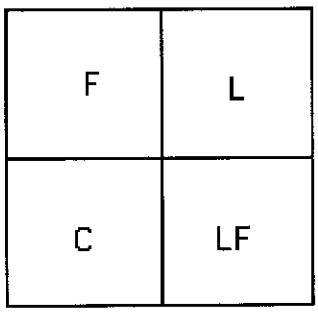
Species	n	Control	n	Inoculated	*P*
0 months					
Eucalyptus calophylla	9	1.88 (0.33)	9	1.85 (0.28)	0.802 (NS)
E. camaldulensis	5	1.70 (0.28)	9	1.48 (0.18)	0.093 (NS)
E. diversicolor	9	1.37 (0.26)	9	1.47 (0.20)	0.366 (NS)
E. patens	4	1.58 (0.14)	5	1.50 (0.16)	0.440 (NS)
E. robusta	7	1.64 (0.21)	9	1.58 (0.23)	0.581 (NS)
E. rudis	7	1.67 (0.19)	9	1.76 (0.22)	0.437 (NS)
Allocasuarina fraseriana		NA			
Kunzea ericifolia	5	2.41 (0.53)	6	2.40 (0.37)	0.977 (NS)
Calistachys bilobum	5	10.08 (1.21)	4	10.02 (1.84)	0.958 (NS)
2 months					
Eucalyptus calophylla	9	2.22 (0.68)	9	2.04 (0.38)	0.513 (NS)
E. camaldulensis	5	2.12 (0.24)	8	1.72 (0.56)	0.158 (NS)
E. diversicolor	9	2.00 (0.44)	8	1.65 (0.50)	0.148 (NS)
E. patens	3	1.85 (0.435)	5	2.26 (0.49)	0.435 (NS)
E. robusta	6	2.04 (0.55)	9	2.09 (0.54)	0.862 (NS)
E. rudis	7	1.92 (0.53)	7	2.03 (0.32)	0.648 (NS)
Allocasuarina fraseriana		NA			
Kunzea ericifolia	5	3.22 (1.09)	6	2.50 (0.97)	0.279 (NS)
Calistachys bilobum	5	9.13 (2.44)	4	8.88 (1.88)	0.876 (NS)
4 months					
Eucalyptus calophylla	9	2.71 (0.61)	9	2.95 (0.53)	0.379 (NS)
E. camaldulensis	5	2.33 (0.42)	7	1.94 (0.51)	0.180 (NS)
E. diversicolor	8	2.72 (0.82)	7	2.21 (0.98)	0.291 (NS)
E. patens	3	2.10 (0.76)	4	3.00 (0.70)	0.164 (NS)
E. robusta	7	2.79 (1.03)	9	2.85 (0.58)	0.456 (NS)
E. rudis	7	2.57 (0.66)	7	2.60 (0.33)	0.912 (NS)
Allocasuarina fraseriana		NA			
Kunzea ericifolia	4	3.78 (0.90)	6	3.13 (0.77)	0.254 (NS)
Calistachys bilobum	5	10.01 (2.17)	4	9.77 (1.48)	0.852 (NS)
6 months					
Eucalyptus calophylla	9	3.25 (0.81)	9	3.51 (0.34)	0.391 (NS)
E. camaldulensis	5	2.73 (0.64)	7	2.47 (0.56)	0.460 (NS)
E. diversicolor	7	3.18 (1.00)	7	3.05 (0.74)	0.527 (NS)
E. patens	3	3.81 (2.10)	4	2.95 (0.76)	0.473 (NS)
E. robusta	7	3.13 (1.03)	9	3.04 (0.83)	0.865 (NS)
E. rudis	7	2.42 (0.44)	7	2.68 (0.16)	0.164 (NS)
Allocasuarina fraseriana		NA			
Kunzea ericifolia	4	4.51 (2.12)	6	3.90 (1.07)	0.564 (NS)
Calistachys bilobum	5	9.85 (3.48)	4	9.92 (1.74)	0.975 (NS)
12 months					
Eucalyptus calophylla	9	5.61 (4.28)	9	5.10 (1.05)	0.732 (NS)
E. camaldulensis	5	2.96 (0.84)	7	2.88 (0.26)	0.822 (NS)
E. diversicolor	7	3.76 (1.36)	7	3.74 (0.72)	0.973 (NS)
E. patens	3	5.98 (1.77)	4	4.93 (0.69)	0.549 (NS)
E. robusta	7	6.62 (6.98)	9	4.33 (1.46)	0.351 (NS)
E. rudis	7	2.75 (0.57)	7	2.93 (0.39)	0.510 (NS)
Allocasuarina fraseriana		NA			
Kunzea ericifolia	3	6.60 (1.88)	4	5.34 (0.38)	0.132 (NS)
Calistachys bilobum	5	12.67 (5.70)	3	11.07 (2.23)	0.727 (NS)

***P =< 0.001; **P =< 0.01; *P =< 0.05; NS = ≥ 0.05

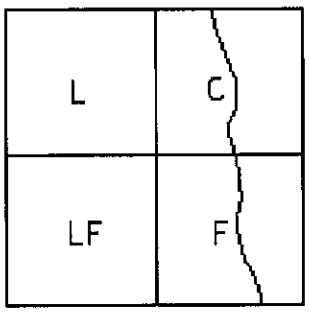
Appendix 10 – Layout of fascining trial (Chapter 8)



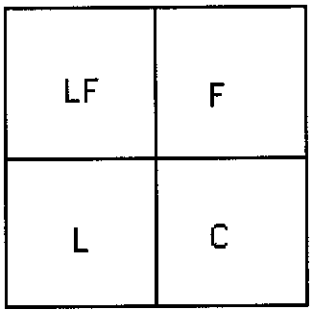
plot 1



plot 2



plot 3



plot 4

⇓
N

C = control
F = fertiliser
L = lime
L+F = lime + fertiliser
= buried by erosion