School of Science
Department of Environmental and Aquatic Sciences

Seedling Growth and Physiological Responses of Perth's Eucalypts to Soil-Induced Stresses

Eiman Farifr

This thesis is presented for the Degree of
Master of Philosophy (Environmental Biology)
of
Curtin University of Technology

December 2010

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: ....

Date: ......15..10..2010

Abstract

Perth's Swan Coastal Sand Plain soils are typically nutrient impoverished, and the native trees of the region are therefore adapted to maximise nutrient uptake. Although the dune systems here are generally not known to be particularly saline or alkaline, there are areas that susceptible to salinity, flooded and elevated pH, especially those that have been modified by human activities. This study investigated the seedlings growth of three Eucalyptus species (*Corymbia calophylla, Eucalyptus gomphocephala and E. marginata*) to three environmental stress; salinity waterlogging and alkalinity in a greenhouse at Curtin University to assess their relative tolerance to these stressors, and hence understand more about their potential use in landscape restoration and rehabilitation. Knowing the seedling growth and physiological responses of three prominent Perth eucalypts to soil-induced stresses provides us with invaluable knowledge for rehabilitating and restoring Perth's urban bushland.

For the salt tolerance experiment, seedlings of the three species were subjected to 81 days growing in potting mix watered weekly with either 0, 50, 100, 150, 250 mM NaCl solutions. Measurements of relative plant growth, biomass allocation and leaf water loss and seedling survival suggested that *E. gomphocephala* was the most tolerant. Survival data suggests that *E. gomphocephala* seedlings have shown ability to cope with a weekly dosage of NaCl solution much greater than 0.25 M, and at least survived for more than 11 weeks under moderately saline conditions. *Corymbia calophylla*, and *E. marginata* were the least tolerance with more than half the seedlings succumbing to salt solutions > 250 mM NaCl.

A flooding experiment, caused by prolonged inundation of water, lasting for 70 days, all three species grew most vigorously in well watered condition but when waterlogged condition *E. gomphocephala* and *E. marginata* seedlings grew slowly and became more water stressed compared to *C. calophylla* seedlings. These finding suggest that although *E. gomphocephala* and *E. marginata* can occurs in wetter areas of Perth's Swan Coastal Plain they are not flood tolerant. *C. calophylla* is a common tree species in the moderately wet lower south-west of Western Australia; it is less common north of Perth where it is restricted to river valleys (Powell 2009). This may explain Marri's ability to physiologically tolerate seasonal flooding (i.e. no significant reduction in stomatal conductance or transpiration rate), despite a reduction in seedling growth.

A liming experiment, was conducted with 20% w/w crushed and sifted Tomala limestone add to potting mix to increase soil pH. The pot trial was conducted over 82 days. *E. gomphocephala* is restricted soils overlying limestone on Perth's Swan Coastal Plain, and according to total seedling dry weight data and calculated relative growth rates coped best in a limestone-enriched soil. However, when examining all the growth and physiological data collected *C. calophylla* appears to be the most tolerant, with no significant difference in leaf allocation or leaf water loss between the well-watered controls and the limestone-enriched treatments. *E. marginata* was the least tolerant with a 14% reduction in stomatal conductance.

As seedlings, *E. marginata* was the least tolerant to the three soil-induced stresses (i.e. flooding, salinity, alkalinity) imposed. The next most tolerant species, *E. gomphocephala* wasn't the most tolerant to an increase is soil alkalinity, although it displayed the least change in seedling dry weight and relative growth rate. *C. calophylla* was the most tolerant of the three eucalypts to the three stressors. However soil-induced stresses will last for longer than the 70-80 days when plants are growing in more natural environments than the seedlings were exposed to in these experiments. By itself, these results will assist Perth's urban land managers in understanding how these tree species respond at the seedling stage to three important soil-induced stressors, more work is required to understand how the observed responses after seedling physiology and how long the seedlings can tolerate these extreme changes in their growing environment.

Acknowledgments

I would like to thank my supervisor, Dr Philip Groom for his guidance and encouragement over the past 2 years. I have now completed my Master experience and achieving my academic aims. I am fortunate and proud to be one of team of Master Students had experiences under the supervision of Dr. Philip Groom, during my postgraduate study. I would also like to give big thank you to Professor: Jonathan Majer (Head of Department) and Associate Professor Jacob John (postgraduate coordinator). Many thank also to Peter Mioduszewski for assistance with the glasshouse experiment. I would also like to thank the staff of the Department of Environmental and Aquatic Sciences, who have provided access to facilities and equipment. Funding for this project was provided by the Centre for Ecosystem Diversity and Dynamics, Curtin University of Technology. This project was sponsored by The Libyan People's Bureau.

I would like to say a special thank you to my husband for his encouragement and assistance over the all time spent for this study. Big thank you also to my parents, my kids, my brothers and sisters and to my friends for their encouragement.

TABLE OF CONTENT

Chapter 1

Chapter 2

Seedling tolerance to short-term soil salinity

Chapter 3

Seedling tolerance to a short-term flooding event

Chapter 4

Seedling tolerance to changes in soil alkalinity due to the presence of limestone

Chapter 5

Chapter 1

General Introduction

Soil salinisation, waterlogged soils (flooding), and alkalinity (presence of limestone) are common land degradation problems. For example, soil salinity influences about 7% of the world's land region, or more than 900 million hectares (Szabolcs, 1994). greatly of this areas are agricultural territory. In Australia, around 5.7 million hectares of soil has potential to develop dry land salinity, which is expected to boost to 17 million ha by 2050 (Audit, 2001 *in National Land and Water Resources*). Salinisation, flooding and alkalinity may natural be a result of climatic and geological history or human activities such as irrigation practices, deforestation and replacement of native vegetation (Greenwood *et al.*, 1992). Waterlogging occurs in areas affected by salinity. The majority of the salinity problem in Western Australia is correlated to rain-fed dry ground agricultural areas, relatively than irrigated agriculture (Bari and Smettem, 2006). Western Australian soils tend to be rich by calcium carbonate with elevated pH (Moore 2004).

Perth, the capital of Western Australia, is situated on a coastal sand plain (the Swan Coastal Plain) that is approximately 30 km wide, bounded by the Gingin and Darling Scarps to the east, and the Indian Ocean to the west (Davidson 1995). The Swan Coastal Plain consists of three main dunal systems and alluvial plains roughly parallel to the coast (Seddon 2004). Fringing the coastline is the Quindalup dunes that consist of wind-blown lime and quartz sand. This flanks the Spearwood dunes of sand over limestone. Both these dune systems occur with 10 m of the true beach. The Bassendean dunes comprise the rest of Perth’s Swan Coastal Plain (except the clayey alluvial plains at the base of the Darling Scarp).

The three common eucalypt species of Perth’s Swan Coastal Plain occupy different habitats. *Eucalyptus gomphocephala* (common name ‘Tuart’) is restricted to the coastal Spearwood dunes, on brown or yellow sand over limestone (Powell 1990). *Eucalyptus marginata* (common name ‘Jarrah’) grows as a large tree on the lateritic soils of the Darling Range and a smaller tree (approximately 15 m) on more porous sands of the

Coastal Plain. It is a common species on the Plain, especially the better drained parts of the Bassendean dunes and the alluvial Pinjarra Plains. Jarrah invades the Tuart woodlands on the deeper sandy soils (Seddon 2004). *Corymbia calophylla*, formerly *Eucalyptus calophylla* (common name ‘Marri’), co-dominates the Darling Scarp vegetation, and has a similar distribution to that of Jarrah, but is more common on wetter, well drained soils (Seddon 2004). Southwestern Australian *Eucalyptus* species tend to have a natural moderate to high tolerance to salinity and flooding events (Benyon *et al.* 1999; Marcar *et al.* 1995, 2000).

Within south Western Australia the wettest regions on the more fertile soils are dominated by eucalypt woodland and forests (Beard 1990). On the drier, sandier soils this gives way to shrub-heath lands (Pate and Beard 1984). The eucalypts are the dominant tall trees on the Swan Coastal Plain, and hence are important part of the local ecosystems, supporting a range of invertebrate and bird life (Recher *et al.* 1996). Declining eucalypt tree health in some of the dominant eucalypts of south western Australia has been noticed since the 1970s, resulting mainly from fungal disease (*Phytophthora cinnamomi)* (e.g. *E. marginata, C. calophylla*) (Robinson 2008), and in combination with drought (e.g. *E. gomphocephala*) (Drake and Froend 2006). Little is known about seedling responses to soil-induced stresses of Perth’s eucalypts, expect for some work on flood and drought tolerance of *E. marginata* (Davison and Tay 1985, Stoneman *et al.* 1994). Seedling tolerance of *Eucalyptus* to waterlogging and salinity have been previously investigated by van der Moezel and co-workers (e.g. van der Moezel *et al.* 1988, 1989, 1991), but these have been confined to eucalypts of Western Australia’s dry and secondary salinisation prone agricultural regions.

Soil salinisation, waterlogging (=flooding) and alkalinity (=presence of limestone) are the three most common soil-related environmental factors effecting plant growth, survival and seedling establishment in Perth’s sandy soils. Species from the Swan Coastal Plain have not been previously assessed for their tolerance to these soil-induced stressors, and thus their suitability in rehabilitate disturbed environments is virtually unknown. This theses focuses on the growth and ecophysiological responses of seedlings from Perth’s three prominent eucalypts (Jarrah, Marri and Tuart) to short-term exposure to these three soil-induced stresses to assess their relative tolerances. Knowing the tolerances to these abiotic factors will assist in our understanding of optimal growth environments for these species on Perth’s Swan Coastal Plain.

Chapter 2

Seedling tolerance to short-term soil salinity

Introduction

Soil salinity may cause physiological dysfunction in plants, and hence influencing plant growth, due to changes in cell osmotic potentials, tissue ion toxicity, or tissue nutrient deficiencies (Munns and Terrmaat 1986, Yeo 1992). Under saline conditions the concentration of soluble salts increases in the rhizosphere, which influences root pressure and hence soil water availability, decreasing the uptake of water and certain soil nutrients (Kafkafi 1991). Sodium toxicity appears not to be widespread as chloride toxicity, because of low Ca^{2+} attentiveness in the substrates or poor soil aeration (Marschner 1995). Increasing salinity in the root medium interferes with uptake and translocation of Ca^{2+} and K^{+} and sometimes excludes K^{+} (Kalaji and Pietkiewiez 1993). Soil salt stress is a dominant factor influencing plant survival, growth and productivity within the genus *Eucalyptus* (Sun and Dickinson 1995, Benyon *et al.* 1999). Different species, and even provenances within species, differ in their ability to tolerate soil salinity (Morabito *et al.* 1994, Prat and Fathi-Ettai 1990).

Salinity is one the major environmental issues affecting the Australian vegetation (Bell 1999; Macaulay and Mullen 2006, Vadez *et al.* 2007, Song *et al.* 2008) with an estimated 5.6 million ha of salt affected land (Dale and Dieters 2005, Dunlop *et al.* 2007). Salinisation in Western Australia was in first observation reviewed by Wood (1924) who was the first person to document that the decline in native plant species' abundance was caused by increases in soil and stream salinity. About 77 percent of Western Australia land has affected by salinity as well as risk of increasing secondary dryland salinity (Marcar and Carwford 2004). Much of Perth's Swan Coastal Plain has groundwater salinity that rarely exceeds 1,000 mg/L total dissolved solids, with the greatest salinities occurring where soils impede rainfall infiltration and have high evaporation rates, or at salt water interfaces of rivers and deep aquifers (Davidson

1995). This low level of groundwater salinity is a type of ‘primary salinity’, resulting from the presence of naturally high dissolved salt concentrations in the soil solution, or marine incursions (Bennetts *et al.* 2005, Dunlop *et al.* 2007, van Dijk *et al.* 2007). Agricultural lands experience ‘secondary salinisation’, caused by the removal of deep-rooted, perennial native vegetation in favour of growing shallow-rooted, usually annual crops or pasture land, or other land uses that causes underlying water tables to rise (Kingsbury *et al.* 1984, Jain *et al.* 1985, Bell 1999; Bennetts *et al.* 2005, Kingwell and John 2007).

In a natural system that has the potential to be impacted by increased salinity due to anthropogenic interference, an understanding of how key components of the native flora might respond to salt stress is important. Various studies have been conducted to assess the suitablility of *Eucalyptus* tree species considered for potential planting in salt-affected landscaes (e.g. van der Moezel *et al.* 1988). *Eucalyptus camaldulensis* (River Red Gum), the most widely distributed eucalypt along Australian river systems, is a tree well know for its tolerant to salinity and waterlogging, having an >85% survival rate under a variety of saline soil condition (Sandhu and Qureshi 1986), although *E. camaldulensis* trees was second most tolerant to salt at 0.4% NaCl (out of 5 species), grown for one year in pot experiment (Panchaban and Srisataporn 1989). In southwestern Australia the decline in *C. calophylla* and *E. marginata* populations occurring in the 1960s and 1970s may have been a result of increased soil salinisation, in combination with a declining rainfall, and the prevalence of ‘dieback’ – a root fungal disease (Kimber 1981, Shearer 1992).

This chapter investigates the growth and physiological responses to increased levels of soil salinity caused by the addition of sodium chloride solutions (up to 0.25 M) of three common *Eucalyptus* species occurring within the Swan Coastal Plain, Western Australia.

Methods

Experimental Design

Seeds of the three eucalypt species were purchased from Nindethana Seed Service (Albany, Western Australia) sourced from local seed provenances, and germinated in shallow trays filled with white sand in a naturally lit glasshouse at Curtin University (Western Australia). Trays were initially partly submerged in a larger tray of water containing Previcur® fungicide (2 mL L^{-1}) to minimize seedling death resulting from fungal infection. Every 3-4 days the trays were rewatered. Seedlings remained in these trays until they had obtained a height of approximately 3 cm.

A total of 120 seedlings of each species were than transplanted into individual pots (7cm wide and 7cm long by 8cm deep) filled with soil at a ratio of four parts white sand to two parts peat. Transplanted seedlings were carefully watered twice weekly until the seedlings had 4-6 leaves or were approximately 6 cm tall. The day before applying the salinity treatments, five seedlings of each species were randomly selected for harvesting, with each seedling divided into stem, root and leaf components. Biomass of stem, root and leaves were recorded after drying the samples in a drying oven at 80ºC for 48 hours, or until constant mass was achieved. The remaining seedlings were randomly divided into five salinity treatments (0.00, 0.05, 0.10, 0.15 and 0.25 M NaCl) for each species. Each treatment was watered with the appropriate salt solution, twice a week for a total of 81 days. Solutions were poured onto the soil surface using a thin nozzle watering can to avoid wetting the leaves. The total number of leaves and height was recorded for each seedling prior to adding the salt solutions, and remeasured before the final harvest.

Physiological and Growth Measurements

In the days leading up to the final harvest, about ten seedlings per treatment and species were randomly chosen for chlorophyll and physiological measurements. Chlorophyll content (SPAD-502 meter, Konica Minolta, Japan), and stomata conductance (steady state porometer, LI-1600, Li-Cor, Nebraska, USA) were measured on the youngest fully expanded leaf. All measurements were recorded during the mid-morning in full sunlight. Stomata conductance was measured a second time 14 days afterwards to assess for physiological recovery after watering with tap water. Percentage relative water content was measured on a different subset of seedlings as [(saturation weight – dry weight)/(fresh weight – dry weight)] x 100 of the youngest fully expanded leaves. Saturation weight was obtained by floating leaf discs in deionised water overnight in a darkened container.

At the end of the experiment, seedling were harvested into stem, leaf and root components. For each seedling all leaves were digitally scanned fresh and total leaf area measured using the image J software (http://rsb.info.hih.gov/ij). All plant material was oven dried at 80^{o}C for 48 hours, and the dry weights of each component recorded. Various growth, biomass and leaf area allocation parameters were then calculated for each treatment and species. These included relative growth rate (RGR), leaf area ration (LAR), leaf weight ratio (LWR) and shoot to root ratio as defined by McGraw and Garbutt (1990).

Statistical Analysis

Interaction between species and treatment was analysed by factorial ANOVA and statistical difference between treatments within species was analysed by one-way ANOVA using SPSS version 16.0 (SPSS inc. Chicago, USA). Homogeneity of variances was assessed using Levene's test and log transformed as required , with data presented as untransformed means. Scheffe's test was used for multiple comparisons of means where significance level of 0.05 was found.

Results

Survival

Both *C. calophylla* and *E. marginata* exhibited similar survival patterns with 34-42 of the original 120 seedlings surviving after 11 weeks subjected to weekly watering of 0.15 M NaCl solution, and 11-15 seedlings surviving at 0.25 M solution (Table 2.1). For the same solutions, *E. gomphocephala* had 70-82 seedlings survive compared to 99% survival for the control treatment.

Salinity treatment had effect in leaf number, which increased at lower salt concentration in all species, but decreased at higher salt concentration as compared with the control (Table 2.2) where symptoms of leaf salt damage such as burned leaves of the plant was noticed at concentrations > 0.1 M for both *C. calophylla* and *E. marginata*, but not as obvious in *E. gomphocephala*.

Table 2.1 Percentage of surviving seedlings after 11 weeks growing in soils watered with NaCl solutions. 0 M solution is deionised water.

	0 M	0.05 M	0.10 M	0.15 M	0.25 M
C. calophylla	100	85	73	35	12
E. gomphocephala	99	90	84	68	58
E. marginata	99	80	68	28	9

Table 2.2 Mean (±SE) number of leaves per seedlings after 81 days growing in soils watered with NaCl solutions. 0 M solution is deionised water. Similar letters indicate means that are not significantly different as determined by Scheffe post-hoc tests. Means derived from data of 5 seedlings.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	15.66 ±1.45^a	13.33 ± 2.40	10.33 ±0.33^a
0.05 M	11.66 ±1.45^a	14.66 ± 0.66	9.33 ±0.66^a
0.10 M	10.66 ± 1.33^a	12.00 ± 1.15	8.33 ±0.88^a
0.15 M	9.66 ± 1.45^a	12.66 ± 0.33	7.33 ± 1.33.^{a}
0.25 M	8.00 ± 1.15^b	12.00 ± 1.15	5.33 ± 0.66^b
P value	*	NS	*

P values: * <0.05, ** <0.005, *** <0.0005, NS= not significant

Plant Growth

All three species produced significantly shorter seedlings at NaCl concentrations >0.15 M (Table 2.3). There was no significant difference in height between seedlings growing in the control treatment (0 M) compared to the 0.05 and 0.10 M treatments, even though the control *C. calophylla* seedlings were on average 12 cm taller (Table 2.3). Photographs of the seedlings showed that an increase in salt concentration caused a reduction in seedling height for all three species (Fig. 2.1) and supports the data provided in Table 2.3.

All species had a lower relative growth rate (RGR) compared with the 0 M treatment (Table 2.4), with *E. gomphocephala* having the highest RGR for every treatment. *E. gomphocephala* RGR varied the least (between 41-47.3 mg g^{-1} day^{-1}). Both *C. calophylla* and *E. marginata* had a 32 and 44% respective in RGR at 0.25 M compared to the control, compared with 87% for *E. gomphocephala* (Table 2.4). Overall there was a decrease in RGR with increasing salinity concentration, with the greatest decrease occurring between the 0.15 and 0.25 M treatments. None of the three eucalypts displayed any significant difference in shoot-to-root (StR) biomass ratios between salinity treatments (Table 2.5).

Table 2.3 Mean (± SE) seedling height to increasing salinity treatments after 81 days growing in soils watered with NaCl solutions. Similar letters indicate means that are not significantly different as determined by Scheffe post-hoc tests.. Means derived from data of 5 seedlings.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	28.70 ±1.53^{a}	15.00 ± 0.88^{a}	10.50 ±1.10^{a}
0.05 M	16.70 ±1.00^{a}	10.40 ± 0.88^{a}	10.30 ±0.76^{a}
0.10 M	16.0 ± 0.71^{a}	16.00 ± 1.14^{a}	10.60 ±0.90^{a}
0.15 M	11.2 ± 0.95^{b}	10.80 ± 0.72^{b}	4.30 ± 0.42^{b}
0.25 M	9.70 ± 0.47^{b}	5.80 ± 0.59^{b}	3.30 ± 0.36^{b}
P value	**	***	***

P values: * <0.05, ** <0.005, *** <0.0005, NS= not significant

Table 2.4 Relative seedling growth rate (mg g^{-1} day^{-1}) to increasing salinity treatments after 81 days growing in soils watered with NaCl solutions. Relative growth rate was calculated based on mean pre-experiment and final harvest data and therefore no statistical analysis was undertaken.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	30.9	47.3	39
0. 05 M	28.4	46.0	36.9
0.10 M	20.9	42.5	35.2
0.15 M	18.5	43	20.11
0.25 M	9.96	41.3	17.3

Table 2.5 Mean (±SE) biomass partitioning (shoot-to-root ratio) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.0 M	5.84 ± 0.64	7.70 ± 1.46	13.74 ± 1.73
0.05 M	3.53 ± 0.07	4.41 ± 0.52	4.96 ± 1.38
0.10 M	3.20 ± 0.39	5.75 ± 0.94	11.50 ± 6.13
0.15 M	8.41 ±2.90	7.36 ± 1.74	6.30 ±1.95
0.25 M	7.15 ± 2.93	6.33 ± 1.36	3.02 ± 0.63
P value	NS	NS	NS

P values: NS= not significant

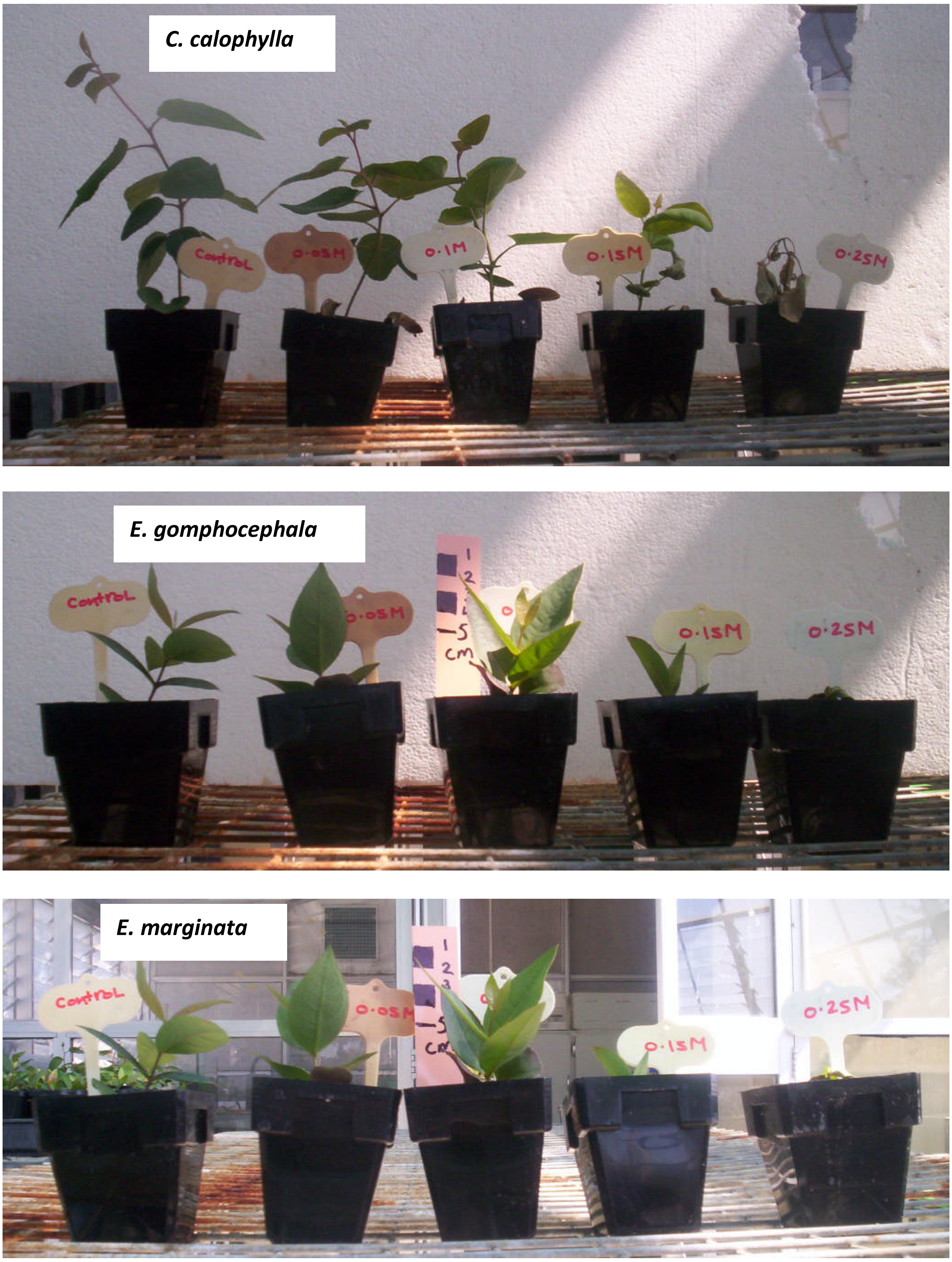

Fig. 2.1 Photographs showing relative heights of the three eucalypts species subjected to different salinity treatments.

Leaf Investment

Increased soil salinity had no significant effect on the relative biomass allocation in leaf mass compared with the total plant mass (Leaf Weight Ratio) for any of the three eucalypts species (Table 2.6), but did influence the Specific Leaf Area and Leaf Area Ratio, at least for *E. gomphocephala* and *E. marginata* (Tables 2.7 and 2.8).

Table 2.6 Mean (± SE) leaf weight ratio (g g^{-1}) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	0.61 ± 0.01 ^{a}	0.66 ± 0.02 ^{a}	0.73 ± 0.01 ^{a}
0.05 M	0.51 ± 0.07 ^{a}	0.67 ± 0.04 ^{a}	0.66 ± 0.06 ^{a}
0.10 M	0.61 ± 0.02 ^{a}	0.65 ± 0.04 ^{a}	0.70 ±0.02 ^{a}
0.15 M	0.64 ± 0.03 ^{a}	0.76 ± 0.04 ^{a}	0.71 ± 0.03 ^{a}
0.25 M	0.49 ± 0.16 ^{a}	0.63 ± 0.18 ^{a}	0.69 ± 0.04 ^{a}
P value	NS	NS	NS

P values: NS= not significant

Table 2.7 Specific leaf area (m^2 g^{-1}) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings..

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	193.0 ± 17.1 ^{a}	185.9 ± 9.0^{a}	158.1 ± 6.9^{a}
0.05 M	120.7 ± 6.8 ^{a}	88.9 ± 8.6^{ab}	98.2 ± 8.7^{b}
0.10 M	127.8 ± 4.0 ^{a}	98.2 ± 30.8^{b}	93.8 ± 1.8^{b}
0.15 M	123.3 ± 6.8 ^{a}	111.5 ± 9.9^{b}	104.8 ± 4.4^{b}
0.25 M	159.2 ±15.1 ^{a}	117.2 ± 2.5^{b}	103.6 ± 3.9^{b}
P value	NS	**	**

P values: * <0.05, ** <0.005, *** <0.0005, NS= not significant

Table 2.8 Mean (±SE) leaf area ratio (m^2 g^{-1}) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	119.1 ± 13.1 ^{a}	122.9 ± 6.5 ^{a}	115.7 ± 6.1 ^{a}
0.05 M	62.8 ± 10.3 ^{a}	60.1 ± 7.0 ^{b}	63.4 ± 2.8 ^{b}
0.10 M	78.8 ± 1.0 ^{a}	63.3 ± 19.6 ^{b}	66.2 ± 2.7 ^{b}
0.15 M	79.2 ± 4.8 ^{a}	84.7 ± 6.1 ^{a}	74.2 ± 1.8 ^{b}
0.25 M	109.0± 25.2 ^{a}	75.1 ± 12.1 ^{b}	72.1 ± 2.9 ^{b}
P value	NS	**	**

P values: * <0.05, ** <0.005, *** <0.0005, NS= not significant

Relative Chlorophyll Content

Increasing salt concentration had significant difference of relative chlorophyll content in all *Eucalypts* species (*E. calophylla*, *gomphocephala* and *marginata*, all $P< 0.001$), although increasing salinity treatment reduced relative chlorophyll content. *E. gomphocephala* had the greater chlorophyll content in all treatment compared with other species, and there is slightly different of chlorophyll content between control and other treatment in this specie. *E. calophylla* chlorophyll content with 0.25 M having approximately 40% (17.1 SPAD units) as the control (44.3 SPAD units). *E. marginata* there is no great different of chlorophyll content between treatments up to 0.15 M, but with 0.25 M the chlorophyll content having roughly 60% (28.8 SPAD units) as the control (46.5 SPAD units) (Table 2.9).

Table 2.9 Relative chlorophyll content (SPAD units) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings..

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	44.3 ± 1.8 ^{a}	52.9 ± 1.6 ^{a}	46.5 ± 1.6 ^{a}
0.05 M	41.8 ± 2.1 ^{a}	56.8 ± 2.0 ^{a}	47.2 ± 3.1 ^{a}
0.10 M	38.1 ± 1.2 ^{a}	50.6 ± 1.9 ^{a}	48.1 ± 2.7 ^{a}
0.15 M	34.5 ± 2.6 ^{a}	52.4 ± 1.2 ^{a}	47.4 ± 2.7 ^{a}
0.25 M	17.1 ± 1.8 ^{b}	46.3 ± 1.4 ^{a}	28.8 ± 2.5 ^{b}
P value	***	**	**

P values: * <0.05, ** <0.005, *** <0.0005, NS= not significant

Relative Water Content

No data was obtained for *C. calophylla* and *E. marginata* with 0.15 and 0.25 M NaCl as the plants died before measurements could be taken. Leaf relative water content decreased with increasing salt concentration for all species, except *C. calophylla* (Table 2.10).

Table 2.10 Relative water content (%)after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings. of three eucalypts species subjected to varying salinity regimes. Data were unavailable for *C. calophylla* and *E. marginata* in the 0.15 and 0.25 M NaCl treatments.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	88.18 ± 3.88 [a]	81.60 ±6.48 [a]	82.54 ± 1.67 [a]
0.05 M	73.35 ± 15.15 [a]	82.27 ± 7.36 [a]	82.33 ± 8.87 [a]
0.10 M	61.86 ±17.2 [a]	78.40 ± 18.82 [b]	74.43 ± 6.73 [ab]
0.15 M	NA	76.20 ± 6.40 [a]	NA
0.25 M	NA	74.76 ± 16.44 [a]	NA
P value	NS	*	*

NA= not available,
P values:*<0.05, ** <0.005 , *** <0.0005, NS= not significant

Stomatal Conductance and Transpiration

No data is presented for *C. calophylla* and *E. marginata* for 0.15 and 0.25 M treatments as plants died before measurements were taken. Increasing salinity concentration caused substantial decline of stomatal conductance in the three species (Table 2.11). This decline was great from 0.05 M and above; however the difference between treatments were significantly for *E. gomphocephala* and *E. marginata*; for *C. calophylla* the difference between treatments was not significant.

The transpiration rates of *E. gomphocephala* and *E. marginata* were found to have been significantly affected with increasing salinity treatment levels (Table 2.12), with the maximum reduction occurring within *E. gomphocephala* seedlings with a 95% decrease at 0.25 M with control treatment. In comparison seedling transpiration rates were less impacted in *E .marginata* (85% decrease) and *C. calophylla* (65% decrease).

Table 2.11 Stomatal conductance (mol m^{-2} s^{-1}) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings. No data were presented for *C. calophylla* and *E. marginata* with 0.15 and 0.25 M NaCl treatments.

Treatments	*C. calophylla*	*E. gomphocephala*	*E .marginata*
0.00 M	0.051 ± 0.01^{a}	0.23 ± 0.08^{a}	0.33 ± 0.11^{a}
0.05 M	0.023 ± 0.01^{a}	0.05 ± 0.02^{a}	0.04 ± 0.00^{a}
0.10 M	0.020 ± 0.00^{a}	0.07 ± 0.00^{a}	0.05 ± 0.02^{ab}
0.15 M	NA	0.02 ± 0.01^{ab}	NA
0.25 M	NA	0.02 ± 0.02^{ab}	NA
P value	NS	*	*

NA= not available,
P values:*<0.05, ** <0.005 , *** <0.0005, NS= not significant

Table 2.12 Transpiration (mmol m^{-2} s^{-1}) after 81 days growing in soils watered with NaCl solutions.. Similar letters indicate means that are not significantly different. Means derived from data of 5 seedlings. No data were presented for *C. calophylla* and *E. marginata* with 0.15 and 0.25 M treatments NaCl treatments.

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*
0.00 M	1.57 ± 0.35^{a}	7.68 ± 2.68^{a}	9.95 ± 3.19^{a}
0.05 M	0.59 ± 0.32^{a}	1.56 ± 0.80^{a}	1.24 ± 0.19^{a}
0.10 M	0.83 ± 0.18^{a}	1.47 ± 0.46^{a}	1.14 ± 0.53^{a}
0.15 M	NA	0.67 ± 0.43^{a}	NA
0.25 M	NA	0.34 ± 0.60^{b}	NA
P value	NS	*	*

NA= not available,
P values:*<0.05, ** <0.005 , *** <0.0005, NS= not significant

Discussion

Kozlowski (1997) mentions that "salt tolerance of plants is difficult to quantify because it varies appreciably with many environmental factors and plant factors". Comparisons between the salt tolerance of our studied eucalypt species demonstrated that *C. calophylla* was the least tolerant to soil salinity. Declining seedling growth began when 0.10 M NaCl solutions, or greater were provided. The next tolerant was *E. marginata* and most tolerant *E. gomphocephala*. This was supported by a 68% (*C. calophylla*), 56% (*E. marginata*) and 13% (*E. gomphocephala*) reductions in relative growth rate between the highest NaCl concentration (0.25 M) and the control (0 M). Although *E. gomphocephala* relative growth rate declined with an increased salinity, it was always greater than the other eucalypts. Survival data suggests that *E. gomphocephala* seedlings have shown ability to cope with a weekly dosage of NaCl solution much greater than 0.25 M, and at least survived for more than 11 weeks under moderately saline conditions.

As has been documented for other eucalypts, and indeed other plant species, growing plants in every increasing saline soil will ultimately have a negative effect on seedling growth, leaf biomass and leaf area allocation, with the amount of salinity tolerated depending on species, provenance or genotype (van der Moezel and Bell 1990). In the current experiment, it could be concluded that *E. gomphocephala* and *E. marginata* are more likely to invest less leaf area (on both a total leaf dry mass and total dry plant mass basis) at the same time as salinity levels increase, the long-term (i.e. greater than 11 weeks). Although, implications of growing these eucalypts under these stressful conditions is unknown, particularly as a continue watering of NaCl solutions may eventually cause soil salt levels to rise to toxic levels. Growth data appeared to have no significant influence on seedling height in the 0.05 and 0.10 M treatments compared to control treatment (0 M). This suggests continue cells elongation in plants under low salinity concentrations and is similar to results obtained on other eucalypts (Qureshi *et al.* 2000, Miyamoto *et al.* 2004). However, a decrease growth in high level of salinity is mostly a result of decreasing cell turgor potential which has implications for cell elongation and cell division (Ashraf and Khan 1994, Qureshi *et al.* 2000). A reduction in leaf production in response to increased salinity is due to a shortening of the length of the leaf elongating zone and decreasing the growth intensity in its central and distal portions (Lazof and Bernstein 1998;, Bernstein *et al.* 1993).

Little is known about the salt tolerance of *E. gomphocephala*, a species restricted to coastal limestone soils. Salt tolerance of *E. gomphocephala* may be attributed to one, or more, of the following: (1) salt exclusion; (2) osmotic effects (Bell 1999); (3) storage of accumulated salts in mature leaves. Salt exclusion from the shoot has been described as a vital mechanism of salt tolerance (van der Moezel *et al.* 1988; Kozlowski 1997; Bell 1999). Due to the energy demanding production of osmoregulators, an overall reduction in growth is expected (van der Moezel *et al.* 1988). It has been described that salt excluding species will generally have a lower overall growth rate than salt stressed non-excluding species (van der Moezel *et al.* 1988, Bell 1999). The three species may have the capacity for osmotic adjustment in saline environments. This involves the production of various organic solutes in the cytoplasm, which help mitigate high salt concentrations accumulating in the vacuole, yet have no adverse effect on enzyme and cell membrane function (Kozlowski 1997). Generally however, salinity reduces vegetative growth of all non-halophyte species (Kozlowski 1997) including those involved in the trial, irrespective of potential mechanisms of avoiding or tolerating saline conditions.

Relative chlorophyll content significantly decreased in the three eucalypt investigated. *C. calophylla* had the lowest relative chlorophyll content compared with the control treatment, while *E. gomphocephala* had least reduction of chlorophyll content. Studies have shown increasing salinity levels results in lower chlorophyll concentration (Chavan and Karadge 1986) which has been discussed as being attributed to the disintegration of chloroplasts due to increased chlorophylls activities (Kozlowski 1997, Rawat and Banerjee 1998). A reduction in chlorophyll content reduces an individual's ability to photosynthesize and effects overall plant growth (Muhammad *et al.* 2007).

Relative water content also decreased with increasing salt concentration especially in *E. marginata* and *E. gomphocephala*, but not *C. calophylla,* despite *C. calophylla* being overall the least tolerant. The results suggest that *C. calophylla* had the ability to avoid the water stress induced by salinity than *E. marginata* and *E. gomphocephala*. With an increase in NaCl solution concentration, water uptaken by plants is slowed down thereby causing a change in leaf RWC. Plant performance under stressful conditions is a result of the sum of the species adaptive mechanisms, including changes in leaf physiology. Stomatal conductance and transpiration in this study was found to decline significantly with increasing salt concentration, except for *C. calophylla*, with *E. gomphocephala* having the greater decrease. This may be due to an increase in abscisic acid signalling from the roots in response to the resulting decline in soil water potentials (Zhang and Davies 1991). Another reason for decreased stomatal conductance and transpiration may be due to an increase in intercellular CO_2 concentrations caused by an increase in mesophyll salt concentration, resulting in a decreasing stomatal aperture (Josefa *et al*. 2003).

Chapter 3

Seedling tolerance to a short-term flooding event

Introduction

Waterlogging causes the top soil to become saturated with water which the air space between the soil particles generally replaced by water. This reduces the amount of oxygen availably for the growing roots and adds subsequently affects the physiological activates of the species (Marschner 1995). Anoxic conditions affects basic metabolic and physiological processes eventually lead to a decline in plant growth (Kozlowski 1984), as well as waterlogging has been shown to impact some main physiological functions (Smith and Moss 1998). A common physiological response to soil anoxia is stomata closure and root-zone saturation (Heinrich 1990, McEvoy 1992, Marcar 1993) because waterlogged plants are unable to uptake soil water and nutrients , resulting in a type of water stress to occur. This then negatively impacts other physiological processes that rely on leaf gas exchange, such as photosynthesis, resulting in a decline in carbohydrate production and hence growth rates (Musgrave and Ding 1998). The inability to uptake water, and hence soluble soil nutrients, will also reduce plant growth, leaf chlorophyll content, abscission and cause early leaf senescence and a reduction in leaf area (Bradford 1982, Huang *et al.* 1994).

In addition long-term waterlogging forces plants to switch from an aerobic to an anaerobic-respiration pathway, leading to a decrease in photosynthesis and growth rates (Kozlowski 1984, Bray *et al.* 2000). Shoot to root ratio is usually increased as a result of flooding because the shoot tends to grows more than the root (Pezeshki 2001, Kozlowski 1984). Flood tolerance and adventitious rooting are often correlated (Kozlowski 1997). Adventitious roots usually grow at the base of the shoot, the hypocotyl and the upper part of the tap root which allows them to reside closer to the surface, usually near well aerated soil (Blom and Voesenek 1996). These new roots have increased water absorption, can oxidize the rhizosphere and increase the flow of gibberellins and cytokinins to leaves (Kozlowski 1997).

Recent studies on the short term tree and seedling response to waterlogging and flooding (Heinrich 1990, McEvoy 1992, Jolly and Walker 1996) of *E. largiflorens*, indicated that stomatal closure does not occur until after 32 days of flooding. Low levels of oxygen in the root zone trig root tips resulted in root death within a few days of waterlogging and change in plant water availability because to the flood result. Thus no increase in tree transpiration after flooding was observed (Jolly and Walker 1996). Flooding tolerance differs between *Eucalypts* species (Clemens *et al.* 1978, van der Moezel *et al.* 1988), and it has been documented that there is a negative effect on the growth of *E. marginata* seedlings after three months of continual flooding stress (Davison and Tay 1985).

The three most common eucalypt species, (*Corymbia calophylla, E. gomphocephala, E. marginata*) on Perth's Swan Coastal Plain and adjacent Darling Scarp on a range of soil types, some of which are prone to prolonged waterlogging (Powell 2009). This study investigates the impact of 70 days of continuous waterlogging (flooding) on seedling growth and physiology to determine their relative flood tolerance.

Methods

Experimental Design

Seedlings were grown from seed using the same procedures and soil mixture outlined in the previous '*Seedling tolerance to soil salinity*' chapter. In August 2009, about 85 germinated seeds per species were planted directly into individual square pots (7c m wide and 7 cm long by 8 cm deep) filled with soil at a ratio of four parts white sand to every two parts peat, each pot containing one seedling. Transplanted seedlings were watered twice weekly using tap water until the seedlings had 4-6 leaves or were approximately 6 cm tall. The day before applying the flooding treatment, five seedlings of each species were randomly selected for harvesting, with each seedling divided into stem, root and leaf components. Biomass of stem, root and leaves were recorded after drying the samples in a drying oven at 80ºC for 48 hours, or until constant mass was achieved.

The flooding trial started on 8 August 2009 and finished 70 days later. Forty seedlings per species (20 underwent flooding; 20 were controls) which placed in 60 L clear plastic tubs. Flooding was achieved by filling the tubs with tap water so the water level was approximately 1 cm above the soil surface. Another three tubs had the same method except with drainage holes, were watered three times a week for the duration of the flooding treatment, and used as control (C). After three weeks all the seedlings were removed and all the tubs drained and cleaned with tap water to remove the algae which constructed on the tub walls. Seedlings were than returned in to the tubs and refilled with fresh tap water. Plants were allowed to establish in a glasshouse for 11 weeks prior to the application treatments. Pots were randomly ordered within tubs.

Physiological and Growth Measurements

At the end of the experiment, approximately ten plants per species and treatment were chosen randomly for chlorophyll and physiological measurements. Stomata conductance (steady state porometer, LI-1600, Li-Cor, Nebraska, USA), measurements were made on a youngest fully expanded leaf of each seedling. Since the tubs were located under shading, all plants to be measured were moved into full sunlight for 15 minutes prior to taking measurements Chlorophyll content (SPAD-502 meter, Konica Minolta, Japan), and photochemical yield (modulated chlorophyll fuorometer), were measured on the youngest fully expanded leaf. All measurements were recorded during the mid-morning in full sunlight. Midday measurements of maximum photochemical efficiency of photosystem II (F_v/F_m) were recorded after 10 min as leaf dark by leaf clips which are attached to fluorometer manufacturer. The Fv/Fm ratio can be used as a comparable measure of relative photosynthetic performance. Percentage relative water content was measured on a different subset of seedlings as [(saturation weight – dry weight)/ (fresh weight – dry weight)] x 100 of the youngest fully expanded leaves. Seedling height and leaf number were measured in the end of the experiment.

An additional 10 seedling per species and treatment were harvest at the end of the experiment, each seedling was separated into stem, leaf and root components. For each seedling all leaves were digitally scanned fresh and total leaf area measured using the image J software (http://rsb.info.hih.gov/ij). All plant material was oven dried at 80ºC for 48 hours, and the dry weights of each component recorded. Various growth, biomass and leaf area allocation parameters were then calculated for each treatment and species. These included total dry weight, shoot to root ratio, leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), and relative growth rates (RGR), the latter calculated as per McGraw and Garbutt (1990).

Statistical Analysis

The data collected from frequent measurements were summed and mean for each individual seedling previous to any data analysis. If required, data transformations (normalised) were applied to stabilise error variance. These data were then analysed using independent t-tests with the statistical program SPSS with significances determined at P values < 0.05

Results

Corymbia calophylla

Total seedling dry weight (P=0.0001) and plant height (P=0.002) was negatively influenced by 70 days of flooding in *C. calophylla* (Table 3.1), with flood-affected seedlings noticeably smaller than their well-watered counterparts (Fig. 3.1). At the end of the experiment only 20% of the flooded seedlings had died. Despite no significant difference between flood and control seedlings in all other growth parameters measured (Table 3.1), *C. calophylla* seedlings subjected to flooding had a lower relative growth rate than the well-watered controls. Flooded seedlings had significantly higher leaf chlorophyll content (*P*=0.0001) and photochemical efficiency (*P*=0.05) (Table 3.2) but similar stomatal conductance and transpiration rates.

Table 3.1. Growth parameters of *C. calophylla* seedlings under well-watered (control) and 70 days of inundation (flooded) conditions. Values are mean ± SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth rate.

	Control	Flooded	P (*t*-test)
Total dry weight (g)	2.35 ± 0.22	0.86 ± 0.08	**
Seedling height (cm)	30.2 ± 2.1	18.0 ± 1.1	***
LAR mm ^{2}g^{-1})	119.1 ± 13.1	63.0 ± 10.3	NS
LWR(g g^{-1})	0.61 ± 0.01	0.60 ± 0.10	NS
SLA (mm ^{2}g^{-1})	193.1 ±17.1	153.3 ±38.2	NS
Leaf number	15.7± 1.4	11.66 ± 0.9	NS
Root: shoot ratio (g g^{-1})	5.8 ± 1.4	4.5 ± 1.0	NS
RGR (mg g^{-1} day^{-1})	35.7	21.5	NA-

NA= not applicable,
P values:*<0.05, ** <0.005 , *** <0.0005, NS= not significant

Table 3.2. Physiological data for *C. calophylla* seedlings under well-watered (control) and 70 days of inundation (flooded) conditions. Values are mean ± SE for 10 seedlings.

	Control	Flooded	P (*t-test)*
Chlorophyll content (relative units)	45.3 ± 1.8	25.3 ± 2.2	***
Stomatal conductance (mol m^{-2} s^{-1})	0.07 ± 0.01	0.05 ± 0.01	NS
Transpiration (mmol m^{-2} s^{-1})	1.57 ± 0.35	1.43 ± 0.31	NS
F_v/F_m (relative units)	0.78 ± 0.01	0.70 ± 0.01	*
RWC (%)	88.8 ± 3.9	87.0 ±3.5	NS

P values: *<0.05, ** <0.005 , *** <0.0005, NS= not significant

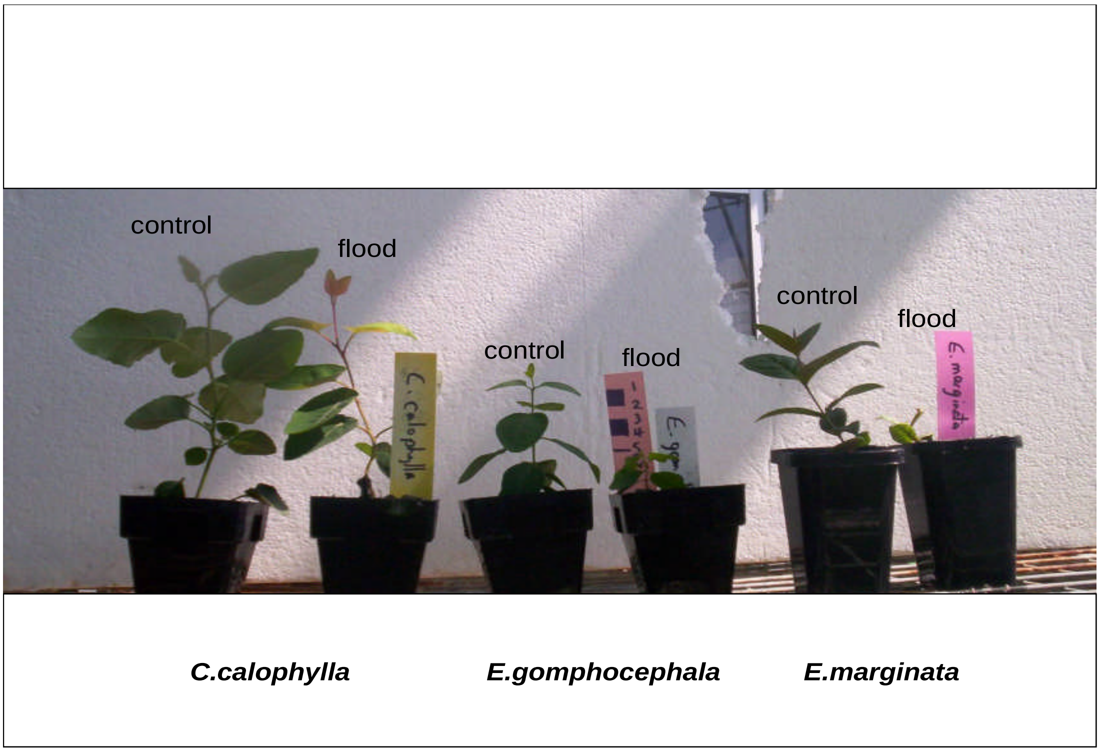

Fig 3.1. Seedling height comparison between control and flooded in the three *Eucalyptus* species.

Eucalyptus gomphocephala

After 70 days of flooding, the total dry weight and height of the seedlings were significantly lower ($P<0.005$), than the well-watered seedlings 60 (Table 3.3, Fig. 3.1). At the end of the experiment 65% of the flooded seedlings had died. Despite no significant difference between flood and control seedlings in all other growth parameters measured (Table 3.3), *E. gomphocephala* seedlings subjected to flooding had a 32% decrease in relative growth rate than the well-watered controls. Flooded seedlings had significantly higher leaf chlorophyll content ($P=0.01$), and lower stomatal conductance and transpiration rates (Table 3.4).

Table 3.3. Growth parameters of *E. gomphocephala* seedlings under control and flooding condition. Values are mean ±SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth ratio.

	Control	Flooded	P (*t*-test)
Total dry weight (g)	0.39 ± 0.06	0.18 ± 0.02	**
Seedling height (cm)	16.6 ± 1.1	9.8 ± 0.7	***
LAR(($mm^2 g^{-1}$)	122.9 ± 6.5	131.2 ± 33.7	NS
LWR($g\ g^{-1}$)	0.66 ± 0.02	0.65 ± 0.02	NS
SLA ($mm^2 g^{-1}$)	185.9 ±9.0	200.4 ± 48.49	NS
Leaf number	13.3± 2.4	18.7 ± 1.8	NS
Root: shoot ratio ($g\ g^{-1}$)	7.7 ± 1.5	5.3 ± 0.7	NS
RGR ($mg\ g^{-1}\ day^{-1}$)	43.1	29.4	

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Table 3.4. Physiological data for *E. gomphocephala* seedlings under well-watered (control) and 70 days of inundation (flooded) conditions. Values are mean ±SE for 10 seedlings.

	Control	Flooded	P (*t*-test)
Chlorophyll content (relative units)	39.8 ± 1.3	19.2 ± 1.4	*
Stomatal conductance (mol $m^{-2}\ s^{-1}$)	0.23 ± 0.08	0.08 ± 0.03	NS
Transpiration (mmol $m^{-2}\ s^{-1}$)	7.68 ± 2.68	2.64 ± 1.23	NS
F_v/F_m (relative units)	0.76 ± 0.01	0.72 ± 0.02	NS
RWC (%)	81.6 ± 6.5	68.9 ± 7.0	NS

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Eucalyptus marginata

For *E. marginata* seedlings, the total dry weight (*P*=0.005), plant height (*P*=0.001), leaf number (*P*=0.012) were negatively influenced by 70 days of flooding (Table 3.5), with flood-affected seedlings noticeably smaller than their well-watered counterparts (Figure 3.1). At the end of the experiment 90% of the flooded seedlings had died. Flooded seedlings had a lower leaf area ratio (LAR) than the control seedlings, but did not differ in their leaf weight ratio or specific leaf area (Table 3.5), with approximately half the relative growth rate. Flooded seedlings had significantly higher leaf chlorophyll content (*P*=0.0001) and photochemical efficiency (*P*=0.03) (Table 3.6) and substantial decreases in stomatal conductance (97% decrease) and transpiration rate (95%) and relative water content (20%).

Table 3.5. Growth parameters of *E. marginata* seedlings under control and flooding condition. Values are mean ±SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth ratio.

	Control	Flooded	P (*t*-test)
Total dry weight (g)	0.49 ± 0.06	0.18 ± 0.02	**
Seedling height (cm)	13.2 ± 1.4	4.8 ± 0.5	**
LAR(mm ^{2}g^{-1})	119.1 ± 13.2	83.3 ± 10.0	**
LWR(g g^{-1})	0.73 ± 0.02	0.60 ± 0.07	NS
SLA (mm ^{2}g^{-1})	161.8 ±15.0	142.1 ± 34.4	NS
Leaf number	10.33± 0.33	4.33 ± 0.88	*
Root: shoot ratio (g g^{-1})	14.9 ± 3.2	3.5 ± 1.4	**
RGR (mg g^{-1}g day^{-1})	39.4	18.8	

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Table 3.6 Physiological data for *E. marginata* seedlings under well-watered (control) and 70 days of inundation (flooded) conditions. Values are mean ±SE for 10 seedlings.

	Control	Flooded	P (*t*-test)
Chlorophyll content (relative units)	43.6 ± 3.2	25.4 ± 2.1	***
Stomatal conductance (mol m^{-2} s^{-1})	0.33 ± 0.11	0.01 ± 0.00	*
Transpiration (mmol m^{-2} s^{-1})	9.95 ±3.19	0.47 ± 0.31	**
F_v/F_m (relative units)	0.78 ± 0.01	0.72 ± 0.01	*
RWC (%)	82.5 ± 1.7	65.3 ± 5.8	*

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Discussion

According to survival, growth and physiology data of the three species examined, *E. marginata* (Jarrah) seedlings were the least flood tolerant. This is not surprising as *E. marginata* is known to be sensitive to waterlogging (Davison and Tay 1985) and normally inhabits well-drained sites on Western Australia's Darling Range and localised pockets of Perth's Swan Coastal Plain, with waterlogging initiating similar symptoms as Jarrah trees infected with the soil-borne water mould, *Phytophthora cinnamomi* (commonly known as 'dieback') (Davison 1988).

After 70 days of continuous flooding *E. marginata* seedlings exhibited significant stomatal closure (3% of the control seedlings), much more than the other two species (*E. gomphocephala* = 35%; *C. calophylla* = 71%). For waterlogged *E. marginata* seedlings a declining stomatal conductance was associated with a decline in photosynthesis process (lower F_v/F_m ratio), leaf chlorophyll content, decreased growth rate and an increase in seedling mortality. The reduction in shoot relative water content is indicative of the interruption of water uptake as a result of prolonged flooding. Waterlogging *E. marginata* seedlings continuously for 14 days causes a greater resistance to water movement into or through seedlings (Davison and Tay 1985) but did not result in a decrease in stomatal conductance. This suggests that flooding has an immediate effect on water transport through the root and stem xylem without compromising leaf water loss. The longer *E. marginata* seedlings are exposed to waterlogging or flooding conditions, the more xylem vessels become blocked (Davison and Tay 1985) resulting in a sharp decline in stomatal conductance and root hydraulic conductivity, as has been reported for other plant species (Davies and Flore 1986a, b, Syvertsen *et al.* 1983)**.** The high mortality of *E. marginata* seedlings under flooded condition may also be related to the roots inability to survive and function in anaerobic environments.

Eucalyptus gomphocephala (Tuart) is restricted to a narrow coastal band in the Perth region, occurring on brown or yellow sand over limestone occasionally near rivers (Powell 2009). *E. gomphocephala* was the next most flood tolerant eucalypt studied. Although all species displayed significant changes in total seedling dry biomass and seedling height (see Fig. 3.1) only those species which had greater than 50% mortality (Marri and Tuart) showed a reduction in stomatal conductance and leaf water loss. For these non-flood tolerant species, the anaerobic conditions created by periods of prolonged waterlogging are preventing water uptake from the soil by ‘sufficating’ the root system (De Simone *et al.* 2002). This has long term implications for seedling growth and survival, as has been documented for other *Eucalyptus* species. It is unlikely that *E. gomphocephala* seedlings are capable of surviving extended periods of flooding, preferring to inhabit well-drained soils in the Perth region.

Corymbia calophylla (Marri) was the most tolerant to 70 days of continuous flooding with 80% of the seedlings surviving. Like *E. marginata*, *C. calophylla* is a common tree species in the moderately wet lower south-west of Western Australia; it is less common north of Perth where it is restricted to river valleys (Powell 2009). This may explain Marri’s ability to physiologically tolerate seasonal flooding (i.e. no significant reduction in stomatal conductance or transpiration rate), despite a reduction in seedling growth. Flooding in south-western Australian ecosystems tends to occur in the winter-early spring months, drying out in the long, hot and dry summers. It is unlikely that *C. calophylla* seedlings will experience periods of flooding lasting more than 100 days, as Perth experiences a bimodal climate of dry, hot summers and cool, wet winters. Waterlogged soils, caused by the June-August winter rains, tend to dry out during the spring-summer-autumn months (Sept-April). In recent years this has become more apparent as Perth winter rainfalls declines. Marri's ability to tolerate habitats prone to flooding may no longer be as important in the future as Perth's climate becomes drier, as it's ability to tolerate summer droughts.

Chapter 4

Seedling tolerance to changes in soil alkalinity due to the presence of limestone

Introduction

Western Australian soils of calcareous origin are high in pH (Moore 2004). The alkalinity-acidity salt of Western Australia soils have been categorised according to their plant toxicity (Szabolcs 1989). The common field crops have a preference to a neutral or slightly acidic soil (pH 7) whereas some plants, however, prefer more acidic or alkaline conditions. The pH of soil solution is dependent relative on mineral weathering conditions, and mineral weathering raising pH by releasing base cations (Ca, Mg and K), and therefore a soil which is rich in simply weatherable minerals tend mostly have a higher pH and higher soil solution concentration of Ca, Mg and K (Jarvan 2004).

Several nutrient cations such as aluminium (Al^{3+}), zinc (Zn^{2+}), copper (Cu^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}) and cobalt (Co^{2+}) are soluble and available for absorbing by plants when pH value below 5.0, even though their availability may result in ion toxicity as soil pH decreases (Jakobsen 1993b). These cations are less available in soil in more alkaline environment and usually the soil shows indications of nutrient deficiency, comprise thin plant stems, yellowing and mottling of leaves, and slow and/or short growth. Some elements require a specific pH range. Phosphorus (P) uptake requires a soil pH between 6.0 and 7.5, otherwise it becomes chemically immobile, forming insoluble compounds with iron (Fe) and aluminium (Al) in acid soils and with calcium (Ca) in calcareous soils. (Jakobsen 1993b and references therein).

Soils that contain calcium sulphate (gypsum), sodium bicarbonate and calcium carbonate can impact of seedling growth because of the effect of increasing soil pH, on micro and macro nutrient availability, particularly phosphorus, nitrogen, copper, zinc, manganese and iron (Alloway and Jackson 1991). The pH (in soil solution) of these soils have been documented as being either slightly acid (calcium sulphate), low alkaline (carbon carbonate) and alkaline (sodium bicarbonate) (Szabolcs 1989). An excessive addition of calcium carbonate in the soil can cause calcium phosphates precipitation phenomenon. It plays an important role in controlling phosphorus activity and its availability in soil solution. The general reaction that explains the interaction of a liming material such as $CaCO_3$ with water to form OH^- ions is as follows (Thomas and Hargrove 1984).

$$CaCO_3 + H_2O \text{ (in soil)} \rightarrow Ca^{2+} + HCO_3^- + OH^-$$

The overall reaction of lime with an acid soil can be expressed by the following equation with OH^- reacts with indigenous H^+ or H^+ that has formed from the hydrolysis of Al^{3+}.

$$2Al + 3\,CaCO_3 + 3\,H_2O \rightarrow 3\,Ca + 2Al\,(OH)_3 + 3CO_2$$

Species from the genus *Eucalyptus* naturally occur in a range of different environments and soil characteristics from alkaline, calcareous or acidic soils acid (Stace *et al.* 1968, Hall *et al.* 1975, James *et al.* 2002). Characteristically, differing tolerance to calcareous circumstances in *Eucalyptus* has been investigated in relationship between transpacific populations of plants raised from seed collected from trees growing on acidic or alkalinised soils (Parsons and Specht 1967, Anderson and Ladiges 1978, Florence 1981). For example, *Eucalyptus obliqua* has received particular attention to assess the response of potentially useful species to the pH of the growing medium (e.g. Anderson and Ladiges 1978, Anderson and Ladiges 1982, Anderson 1982).

About 35 seedlings of *Eucalyptus* species were grown under amended medium with limestone and dolomite (1:1) and pH range of 5.1 to 8.9 to study a response of ornamental eucalypts from acidic and alkaline habitats (Symonds *et al.* 2001). Results indicted that the seedlings growth was generally greater under acidic (pH 5.1-5.6) than under more alkaline conditions. Species demonstrated a range of responses to changes in soil pH, including species that were unaffected even at pH 8.9 such as *E. erythrocorys* and *E. extensa.* The tolerance to high pH is associated with a capability to maintain relatively low Ca and Mg and P and Fe concentration ratios <5. The tolerance of six clones from five provenances of *E. camaldulensis* was examined under controlled conditions of waterlogged, highly saline and highly alkaline soils in a greenhouse where leaves produced under salinity and alkalinity impacts were similar in ion content to those produced prior to the test condition (Richard *et al.* 1996).

Some *Eucalyptus* species grow in soils of relatively high pH, high bicarbonate (HCO_3^-), and low iron (Fe) concentration is concluded their potential to live in calcareous or alkaline soils (Kinzel 1983, James *et al.* 2002). The tolerance of 5 semi-arid Western Australia species (*E. gracilis*, *E. halophila*, *E. kondininensis*, *E. loxophleba* and *E. platypus* var. *heterophylla*) to alkaline conditions, bicarbonate, and low iron availability was examined by James *et al.* (2002). The study showed that seedlings growing in medium of pH 9, caused a reduction in plant height and leaf production for most of the study species, compared with a species growing in media of pH 6. Iron concentrations in the youngest fully expanded leaves were reduced for seedlings in the pH 9 solution. *Eucalyptus halophila* was an exception to this. *Eucalyptus* species may be important in the amelioration of salt-affected (van der Moezel *et al.* 1991, Bell 1999) and alkaline lands (Gupta *et al.* 1988), the treatment of limestone quarries (Ruthrof 1997) and mine waste process that often very alkaline, acidic or saline (Singh 1989, Bell *et al.* 1993).

The research presented in this chapter focuses on the tolerance of three Perth eucalypts to soils enriched by natural limestone, as measured by changes in growth and physiology over 80 days.

Methods

Experimental Design

Seedlings were grown from seed using the same procedures outlined in the previous '*Seedling tolerance to soil salinity*' chapter. One hundred and twenty seedlings of each species were than transplanted into square individual pots (7 cm wide and 7 cm long by 8 cm deep) filled with soil at a ratio of four parts white sand to two parts peat, each pot containing one seedling. Transplanted seedlings were watered twice weekly until the seedlings had 4-6 leaves or were approximately 6 cm tall. The day before applying the alkalinity treatment, 10 seedlings of each species were randomly selected for harvesting, with each seedling divided into stem, root and leaf components. Biomass of stem, root and leaves were recorded after drying the samples in a drying oven at 80ºC for 48 hours, or until constant mass was achieved.

For each species, the remaining seedlings were randomly divided into two treatments. The first treatment had no additional limestone added, and was the control. For the second treatment (the ‘limestone’ treatment) pots were filled with crushed local Tamala limestone, which had been passed through a 3 mm sieve, and mixed at a rate of 20% by soil weight. Overall 55 seedlings per treatment were used per species. Seedlings in both treatments were watered twice a week with fresh tap water for 12 weeks experiment period.

Physiological and Growth Measurements

At the end of experiment, about ten seedlings per treatment and species were randomly chosen for chlorophyll and physiological measurements. Chlorophyll content (SPAD-502 meter, Konica Minolta, Japan), stomatal conductance and transpiration (steady state porometer, LI-1600, Li-Cor, Nebraska, USA) were measured on the youngest fully expanded leaf. All measurements were recorded during the mid-morning in full sunlight The number of leaves and seedling height were measured for ten plants which chosen randomly at the end of the experimental.

An additional ten seedling per species and treatment were harvest at the end of experiment, each seedling was divided into stem, leaf and root components. For each seedling all leaves were digitally scanned fresh and total leaf area measured using the image J software (http://rsb.info.hih.gov/ij). All plant material was oven dried at 80ºC for 48 hours, and the dry weights of each component recorded. Various growth, biomass and leaf area allocation parameters were then calculated for each treatment and species. These included total dry weight, shoot to root ratio, leaf area ration (LAR), leaf weight ratio (LWR), Specific leaf area (SLA) and growth rate (RGR), as defined by

McGraw and Garbutt (1990). Dried leaf material were then ground separately through a 40 µm mesh and 2 g subsamples were analysed by the Western Australia's Chemistry Centre for macro and micro element concentrations N was analysed by Kjeldahl digestion and titration the remaining elements were digested by a concentrated $HNO_3:HClO_3:H_2SO_4$ solution.. Other elements were analysed by Inductively coupled plasma atomic emission spectroscopy (ICP-AES) (e.g. Cu, Fe, Mg, Mn, Zn) and ion-exchange chromatography (Cl, B, S, P, K)

Statistical Analysis

Data was analysed using independent *t*-tests with the statistical program SPSS. Means were determined to be significantly different between treatments (control and limestone) at P values <0.05.

Results

Corymbia calophylla

After 82 days of the limestone the total seedling dry weight (*P*=0.008), plant height (*P*=0.001) and specific leaf area (*P*=0.004) were negatively impacted by the presence of limestone in *C. calophylla* seedlings (Table 4.1). *C. calophylla* seedlings growing in the limestone enhanced soils had a lower relative growth rate than the well-watered controls by approximately 20% (Table 4.1). Limestone seedlings had no significant difference in all Physiology parameters compared with control (Table 4. 1).

Table 4.1 Growth and physiological parameters of *C. calophylla* seedlings after 82 days under well-watered (control) and limestone-enhanced conditions. Values are mean ±SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth ratio.

	Control	limestone	P value (*t*-test)
Total dry weight (g)	2.35 ± 0.22	1.37 ± 0.16	**
Seedling height (cm)	30.2 ± 2.1	15.83 ± 1.30	**
LAR (mm ^{2}g^{-1})	119.1 ± 13.1	67.90 ± 2.41	NS
LWR (g g^{-1})	0.61 ± 0.01	0.69 ± 0.02	NS
SLA (mm ^{2}g^{-1})	193.1 ± 17.1	97.80 ± 4.76	**
Leaf number	15.7± 1.4	10.00 ± 1.18	NS
Root: shoot ratio (g g^{-1})	5.8 ± 1.4	5.89 ± 0.75	NS
RGR (mg g^{-1}g day^{-1})	30.5	24.3	-
Chlorophyll content (relative units)	44.28 ± 1.76	40.67 ± 2.22	NS
Stomatal conductance (mol m^{-2}s^{-1})	0.06 ± 0.01	0.03 ± 0.01	NS
Transpiration (mmol m^{-2} s^{-1})	2.02 ± 0.38	1.11 ± 0.76	NS

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Eucalyptus gomphocephala

E. gomphocephala seedlings exhibited a negative effect in most of the growth parameters measured in relation to the occurrence of limestone in the soil, except for an increase in leaf number (Table 4.2). This is supported by a decrease in the relative growth rate. Limestone-effected seedlings had significantly less leaf chlorophyll content (P=0.02) and a decreased stomatal conductance (*P*=0.05), but not transpiration (*P*=0. 19) than well watered seedlings.

Table 4.2 Growth and physiological parameters of *E. gomphocephala* seedlings after 82 days under well-watered (control) and limestone-enhanced conditions. Values are mean ±SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth ratio.

	Control	limestone	*P* value (*t*-test)
Total dry weight (g)	0.39 ± 0.06	0.35 ± 0.01	*
Seedling height (cm)	16.6 ± 1.1	8.33 ± 0.9	**
LAR (mm ^{2}g^{-1})	122.9 ± 6.5	87.21 ± 7.3	**
LWR (g g^{-1})	0.66 ± 0.02	0.72 ± 0.01	NS
SLA (mm ^{2}g^{-1})	185.9 ± 9.0	121.0 ± 11.5	**
Leaf number	13.3± 2.4	17.0 ± 1.34	**
Root: shoot ratio (g g^{-1})	7.7 ± 1.5	7.29 ± 0.25	NS
RGR (mg g^{-1} day^{-1})	36.8	32.9	-
Chlorophyll content (relative units)	42.92 ± 1.63	37.62 ± 1.43	*
Stomatal conductance (mol m^{-2} s^{-1})	0.33 ± 0.09	0.09 ± 0.02	*
Transpiration (mmol m s^{-1})	6.95 ±3.13	1.97 ± 0.59	NS

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Eucalyptus marginata

Seedling total dry weight (*P*=0.001), leaf area ratio (*P*=0.001), leaf weight ratio (P=0.004) and specific leaf area (P=0.01), and root: shoot ratio (*P*=0.001) were negatively affected after growing in limestone enriched soils for 82 days (Table 4.3) with an approximately a 30% decreased in relative growth rate. Limestone-affected seedlings had a significant decrease in leaf chlorophyll content (*P*=0.01) and stomatal conductance (*P*=0.05). There was also a decrease in transpiration rate (approximately 70%).

Table 4.3 Growth parameters of *E. marginata* seedlings after 82 days under well-watered (control) and limestone-enhanced conditions. Values are mean ±SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth ratio.

	Control	limestone	*P* value (*t*-test)
Total dry weight (g)	0.70 ± 0.05	0.32 ± 0.02	**
Seedling height (cm)	10.8 ± 1.6	7.50 ± 0.9	NS
LAR (mm ^{2}g^{-1})	118.8 ± 5.1	72.0 ± 1.8	**
LWR (g g^{-1})	0.73 ± 0.01	0.58 ± 0.03	**
SLA (mm ^{2}g^{-1})	161.5 ± 5.8	142.1 ± 34.4	*
Leaf number	8.0 ± 0.6	8.2 ± 0.6	NS
RGR (mg g^{-1}g day^{-1})	33.6	23.5	
Chlorophyll content (relative units)	46.47 ± 1.62	30.05 ± 2.85	*
Stomatal conductance (mol m^{-2} s^{-1})	0.49 ± 0.10	0.07 ± 0.02	*
Transpiration (mmol m^{-2} s^{-1})	14.29 ± 2.71	4.79 ± 2.58	NS

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Leaf Chemistry

Table 4.4 shows that boron, iron and manganese were the only mineral nutrients that showed an overall significant decrease in leaf content resulting from liming compared with the control treatment. Boron's decrease was greatest for *C. calophylla* (18% that of the control) whereas Iron's decrease varied from 56-67% and Manganese's decrease varied from 31-43%. There was no significant increase in leaf Calcium concentration in response to liming in either *C. calophylla* and *E. gomphocephala* (Table 4.4). Leaf Magnesium concentrations decreased with increasing alkalinity in all species. There was a significant effect ($P<0.05$) of pH on leaf Fe content in three *Eucalyptus* examined (Table 4.4). There was no effect of pH on leaf K concentration in all *Eucalyptus* species.

Table 4.4 Leaf chemistry of three Eucalyptus seedlings after 82 days growing under well-watered (control) and limestone-enhanced conditions. Data represents chemical analysis of ground dried material from leaves of 10 seedlings.

	B mg/kg	Ca %	Cu mg/kg	Fe mg/kg	K %	Mg %	Mn mg/kg	Na %	P %
C. calophylla									
Control	27	0.86	4.8	84	1.36	0.23	120	1.06	0.11
Limestone	5	0.87	1.8	50	1.8	0.12	42	0.57	0.08
E. gomphocephala									
Control	33	0.92	22	89	1.48	0.2	220	0.83	0.19
Limestone	11	1.4	30	50	1.52	0.18	95	0.79	0.18
E. marginate									
Control	21	1.38	17	61	1.56	0.27	140	1.04	0.14
Limestone	13	1.18	37	41	1.59	0.23	43	1.46	0.08
P-value	*	NS	NS	*	NS	NS	*	NS	NS

P values: *< 0.05, ** <0.005 , *** <0.0005 , NS= not significant

Discussion

The differences in species high pH tolerance in experiments has been reported in *Eucalyptus* (Anderson and Ladiges 1978). In this study limestone was added to the soil to determine how it might influence the growth and physiology of three local Perth eucalypt species. *E. gomphocephala* is restricted soils overlying limestone on Perth's Swan Coastal Plain, and according to total seedling dry weight data and calculated relative growth rates was the most tolerant to the limestone-enriched soils. However, when examining all the growth and physiological data collected *C. calophylla* appears to be the most tolerant, with no significant difference in leaf allocation or leaf water loss between the well-watered controls and the limestone-enriched treatments. *E. marginata* was the least tolerant with a 14% reduction in stomatal conductance. Liming (i.e. the presence of bicarbonate) increases soil pH and is well known to decrease the growth of Eucalypts (Ladiges 1977, Ladiges and Ashton 1977, Anderson 1982, Gupta *et al.* 1988) and was represented by decreased relative growth rate, seedling height and reduced leaf production in the three target species of this study. The leaf chemistry analyses for the high pH tolerant *C. calophylla* and the intolerant *E. marginata* gives an insight into the basis of discrepancy reaction to changing pH.

Liming causes an increase in soil pH, and affects the ability for the seedlings to uptake some mineral nutrients. According to the leaf chemistry results an increase in Ca and decrease in Mg concentrations in the liming trial in the intolerant species , it is probable have been negative effect on some physiological functions and growth ratio on seedlings studied. It has previously been show that a significant increase in soil pH relates to a decrease total Fe content in plant tissues (White and Robson 1990; Tang *et al.* 1991, Farrell *et al.* 1996). James *et al.* (2002) found that lower Fe concentrations of newly produced leaves of *Eucalyptus* species growing in a growth media of pH 9 demonstrates that the availability and translocation of Fe within seedlings is reduced as a result of both the presence of bicarbonate and the reduced concentration of soil Fe, ultimately limiting seedling growth rate. Similar results were obtained in the present study. Manganese and Boron are both made unavailable to plants with an increase in soil pH, and *Eucalyptus* species are known to tolerate a range of soil acidity-alkalinity (van der Moezel *et al.* 1991).

Chapter 5

General Discussion

The overstorey health and population size of Perth’s eucalypts have reduced since the late 1990s, in both relatively undisturbed and urban landscapes (Grigg *et al.* 2009). A study by Crosti *et al.* (2007) within Perth’s Kings Park Bushlands demonstrated a significant increase in abundance for three dominant eucalypt species between 1939 and 1999, although Grigg *et al.* (2009) noted a decline in the bushland adjacent to large expanses of irrigated turf. In this case, a decline in *Eucalyptus marginata* (Jarrah) and *Corymbia calophylla* (Marri) may be due to a mangesium deficiency, caused by alkaline run-off (pH = 8.4) into the bush reserve from surrounding irrigated turf (Grigg *et al.* 2009). This alkalinity is due to the high levels of calcium carbonate in the underground water, as the bush reserve occurs on a limestone escarpment. In contrast *Eucalyptus gomphocephala* (Tuart) shows no symptoms of decline in the Kings Park bushland (Grigg *et al.* 2009), mainly because this species in indigenous to calcareous, high pH soils. The main factors responsible for vegetation change in this urban bushland are weed invasion, modified fire regimes, disease and seed predation (Crosti *et al.* 2007).

As seedlings, *E. marginata* was the least tolerant to the three soil-induced stresses (i.e. flooding, salinity, alkalinity) imposed. This is not surprising as *E. marginata* does not occur in habitats that are excessively wet, calcareous or saline. *E. marginata* is near its northern limits in Perth (Seddon 2004). A few isolated populations further north of Perth (i.e. Mt Lesueur, 300 km north) implies that the species historically had a wider distribution in southwestern Australia when conditions were much wetter (Powell 2009). Currently *E. marginata* dominates the upland Darling Range, extending to the southcoast of Western Australia near Albany, on well-drained lateritic soils. *E. marginata* mortality is highest in habitats with impeded drainage, usually following periods of exceptionally heavy rainfall (Davison and Tay 1985). The association of tree death with poorly drained sites and exceptional rainfall, indicates that short-term waterlogging may also be important contributory factors. However one can not discount the effect of the introduced water mould pathogen, *Phytophthora cinnamomi* has on adult jarrah deaths. This pathogen, also known as ‘jarrah dieback’ is a major threat to the ecology of native plant communities in southwestern Australia (Shearer 1994) and is widely distributed on the Swan Coastal Plain and adjacent Darling Range (Shearer and Dillon 1995, 1996).

The next most tolerant species, *E. gomphocephala* has demonstrated significant decline in adult crown health and numbers since the mid-1990s (Robinson 2008) and is the most restricted in habitat preference (i.e. calcareous soils). However *E. gomphocephala* wasn’t the most tolerant to an increase is soil alkalinity, although it displayed the least change in seedling dry weight and relative growth rate. This may be because the limestone mixture used for the experiments wasn’t reflective of the natural subsurface calcium carbonate concentration experienced by tuart plants in the field. It may also be because alkalinity tolerance is developed over longer periods than the experiments were conducted. Nevertheless the coastal distribution of *E. gomphocephala* suggests an ability of this species to tolerate soil alkalinity.

C. calophylla was the most tolerant of the three eucalypts to the three stressors, occurring on more wetter soils, replaced with *E. rudis* trees on the poorer drained soils (Seddon 2004). *E. rudis* (Flooded Gum) usually grows in habitats that experience periodic flooding (i.e. floodplains, fringing wetlands and water courses). Species that occupy such habitats tend to be salt tolerant (at least to NaCl induced salinity) than species naturally occurring in ‘drier’ locations (Groom and Lardner 2009) Both *E. marginata* and *C. calophylla* co-dominate the overstorey on the lateritic Darling Ranges and co-occur in patches on Perth’s Swan Coastal Plain yet display very different tolerances to soil-induced stresses. This may relate to habitat preferences as species that can tolerate flooding tend to occur (or can be found) in waterlog-prone areas. These may also be prone to the effects of primary and secondary salinisation (van der Moezel et al 1988, 1991). Further investigation into why *C. calophylla* seedlings are more tolerant than it’s congeners, particularly in relation to hydraulic architecture and water use, is required to further substantiate the claims documented in this thesis.

Knowing the seedling growth and physiological responses of three prominent Perth eucalypts to soil-induced stresses provides us with invaluable knowledge for rehabilitating and restoring Perth’s urban bushland. However soil-induced stresses will last for longer than the 70-80 days that the seedlings were exposed to in these experiments. Seedling survival and growth depends on a range of factors including soil water holding properties, groundwater depth, occurrence of pathogens etc. Adult plant tolerances may be vastly different to those experienced by seedlings.

References

Alloway, B.J., and Jackson, A. P. (1991) The behaviour of heavy metals in sewage sludge-amended soils. *The Science of the Total Environment* **100**, 151-176.

Anderson, C. A., Ladiges, P. Y. (1978) A comparison of three populations of *Eucalyptus obliqua* L'Herit. Growing on acid and calcareous soils in southern Victoria. *Australia Journal of Botany* **26**, 93-109.

Anderson, C. A. (1982) The effect of high pH and P on the development of lime-chlorosis in two seedling populations of *Eucalyptus obliqua* L'Herit. *Plant and Soil* **69**, 199-212.

Anderson, C.A., and Ladiges, P.Y. (1982) Lime-chlorosis and the effect of ®re on the growth of three seedling populations *of Eucalyptus obliqua* L'Herit *Australia Journal of Botany* **30**, 47- 66.

Arshi, A., Abdin, M. Z., and Iqbal, M. (2002) Growth and metabolism of Senna as affecter by salt stress. *Biology Plant* **45**, 295-298.

Ashsrf, M. Y., and Khan, A. H. (1994) Solute accumulation and growth of sorghum grown under NaCl and $Na_2 SO_4$ Salinity stress. *Sci, Int*. 6(**4**), 377-379.

Audit (2001) "Australia Dry land Salinity Assessment 2000". National Land and Water Resources Audit, Canberra, Australia.

Baker, J. T., and Allen, L. H. (1993) Contrasting crop species responses to CO_2 and temperature: rice, soybean and citrus. *Vegetation* **104**, 239-260.

Bari, M. A., and Smettem, K. R. J. (2006) A daily salt balance model for stream salinity generation processes following partial clearing from forest to pasture. *Hydrology and Earth System Sciences* **10**, 519-534

Beard, J. S. (1990) Plant life of Western Australia. Kangaroo Press, Kenthurst, Australia.

Bell, D. T., Wilkins, C. F., van der Moezel, P. G., and Ward, S. C. (1993) Alkalinity tolerance of woody species used in bauxite waste rehabilitation, Western Australia. *Restoration Ecology* **1**, 51-58.

Bell, D. T. (1999) Australia trees for the rehabilitation of waterlogged and salinity-damaged landscapes. *Australia Journal of Botany* **49**, 697-716.

Bennetts, D. A., Webb, J. A., Stone, D. J. M., and Hill D. M. (2005) Understanding the salinisation process for ground water in an area of south-eastern Australia, using hydrochemical and isotopic evidence. *Journal of Hydrology* **323**, 178-192.

Benyon, R. G., Marcar, N. E., Crawford, D. F., and Nicholson, A. A. (1999) Growth and water use of *Eucalyptus camaldulensis* and *E. occidentalis* on a saline discharge site near Wellington, NSW, Australia. *Agricultural Water Management* **39**, 229-244.

Blom, C. W. P., and Voesenek, L. A. C. J. (1996) Flooding: the survival strategies of plants. *Trends in Ecology & Evolution* **11**, 290-295.

Bradford, K. J. (1982) Regulation of shoot responses to root stress by ethylene, abscisic acid and cytokinin, pp. 599-608. *In* P.F. *Wareing, (ed.). Plant Growth Substances*. Academic Press, New York

Bray, E. A., Bailey, J., and Weretilnyk, E. (2000) Responses to abiotic stresses, pp. 1158- 1249. *In* Buchanan, [B.B., W. Gruissem and R.L. Jones (eds)]. *Biochemistry and Molecular Biology of Plants*. Courier Companies, Inc. Waldorf, USA

Chavan, P. D., and Karadge, B. A. (1986) Growth, mineral nutrition, organic constituents and rat of photosynthesis in *Sesbania grandflora* grown under saline conditions. *Plant and Soil* **93**, 395-404.

Clemens, J., Kirk, A. M., and Mills, P. D. (1978) The resistance to waterlogging of three *Eucalyptus* species- the effect of waterlogging and an ethylene releasing substance on *E. robusta, E. grandis* and *E. saligna*. *Oecologia* **34**, 125-131

Crosti, R., Dixon, K.W., Ladd, P.G. and Yates, C.J. (2007) Changes in the structure and species dominance in vegetation over 60 years in an urban bushland remnant. *Pacific Conservation Biology* **13,** 158-170.

Dale, G., and Dieters, M. (2005) Economic returns from environmental problems: Breeding salt- and drought tolerant Eucalyptus for salinity abatement and commercial forestry. *Ecological Engineering* **31**, 175-182.

Davies, F. S., and Flore, J. A. (1986a) Flooding, gas exchange and hydraulic conductivity of highbush blueberry. *Physiologia Plantarum* **67**, 545-551.

Davies, F. S., and Flore, J. A. (1986b) Short-term flooding effects on gas exchange and quantum yield of rabbiteye blueberry. *Plant Physiology* **81**, 289–292.

Davies, P. J. (1995) Plant Hormones. *Dordrecht: Kluwer Academic*. Pp.13-38.

Davies, W. J., Wilkinson, S., and Loveys, B. (2002) Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. *New Physiology* **153**, 779-760.

Davison, E. M and Tay, F. C. S. (1985) The effect of waterlogging on seedlings of *Eucalyptus marginata. New Phytologist* **101**, 743-753.

Davison, E.M. (1988) The role of waterlogging and *Phytophtora cinnamomi* in the decline and death of *Eucalyptus marginata* in Western Australia. *Geo Journal* **17,** 239-244.

Davidson**,** W.A. (1995). Hydrogeology and Groundwater Resources of the Perth Region, Western Australia. *Geological Survey of Western Australia*. Bulletin **142**.

De Simone, O., Haase, K., Mueller, E., Junk, W., Gonsior, G., and Schmidt, W. (2002) Impact of root morphology on metabolism and oxygen distribution in roots and rhizosphere from two Central Amazon floodplain tree species. *Plant Biology* **29,** 1025-1035.

Drake, P. and Froend, R. (2006) The role of water relations in the health of tuarts? Tuart Decline Research Findings Bulletin No.3. Tuart Health Research Group, Murdoch University, Perth, Australia.

Dunlop, J. E., Horrigan, N., McGregor, G., Kefford, B. J., Choy, S., and Prasad, R. (2007) Effect of spatial variation on salinity tolerance of macroinvertebrates in Eastern Australia and implications for ecosystem protection trigger values. *Environmental Pollution* **151**, 621-630.

Farrell, R. C. C., Bell, D. T., Akilan, K., and Marahall, J. K. (1996) Morphological and physiological comparisons of clonal lines of Eucalyptus camaldulensis. II. Responses to waterlogging/ salinity and alkalinity. *Australian Journal of Plant Physiology* **23**, 509-518.

Florence, R. G. (1981) The biology of the eucalyptus forest. In [The biology of Australia Plant.] (Eds Pate, J. S., and McComb, A. J.) pp. 147-80. University of Western Australia Press: Nedlands.

Gomes, A.R.S., and Kozlowski, T.T. (1980a) Effects of flooding on *Eucalyptus camaldulensis* and *Eucalyptus globulus* seedlings. *Oecologia* **46**, 139–142.

Greenwood, E. A. N., Milligan, A., Biddiscombe, E. F., Rogers, A. L., Beresford, J. D., Watson, G. D., and Wright, K. D.(1992) Hydrologic and salinity changes associated with tree plantation in a saline agricultural catchment in south-western Australia. *Agricultural Water Management* **22**, 307- 323.

Grigg, A., Glose, Lambers, H., Ruthrof, K.X., and Dixon, K.W. (2009) Ecophysiology of *Eucalyptus marginata* and *Corymbia calophylla* to decline in an urban parkland. *Austral Ecology* **34,** 499-507.

Groom, P.K. and Lardner, T.D. (2009) Seedling growth responses of *Banksia littoralis* and *Melaleuca preissiana* to soil salinity. *Journal of the Royal Society of Western Australia* **92,** 1-4.

Gupta, G. N., Prasad, K. G., Subramaniam, V., and Manivachakam, P. (1988) Effect of alkalinity on survival and growth of tree seedlings. *Journal of the Indian Society of Soil Science* **36**, 537- 542.

Hall, N., Johnston, R.D., and Chippendale, G.M. (1975) Forest Trees of Australia. Australian Government Publishing Service. Canberra.

Heinrich, P. A. (1990) The ecophysiology of riparian river red gum *(Eucalyptus camaldulensis)* Final Report, *Forestry section,* University of Melbourne, Australia.

Huang, B. R., Johnson, J. W., Nesmith, D. S. and. Bridges, D.C. (1994) Growth, physiological and anatomical responses of two wheat genotypes to waterlogging and nutrient supply. *Journal of Experimental Botany* **45**, 193-202.

Jacobsen, S. T. (1993a) Interaction between Plant Nutrients. III. Antagonism between Potassium, Magnesium and Calcium. *Acta Agriculturae Scandinavica Section B* **43**, 1-5.

Jacobsen, S. T. (1993b) Interaction between Plant Nutrients. IV. Interaction between Calcium and Phosphate. . *Acta Agriculturae Scandinavica Section B* **43**, 6-10.

Jain, B.L., Muthana, K.D., and Goyal, R.S. (1985) Performance of tree species in salt-affected soils in arid regions. *Journal of Indian Society of Soil Science* **33**, 221-224.

James, S. A., Bell, D. T., and Robson, A. D. (2002) Growth response of highly tolerant Eucalyptus species to alkaline pH, bicarbonate and low iron supply. *Australian Journal of Experimental Agriculture* **42**, 65-70.

Jarvan, M. (2004) Available plant nutrients in growth substrata depending on various lime materials used for neutralizing of bog peat. *Agronomy Research,* **2**, 29-37.

Jolly, I.D., Walker, G.R. (1996) Water use response of *Eucalyptus largiflorens* (F. Muell.) to flooding. *Australia Journal of Ecology* **21**, 173-183.

Josefa, M. N., Gariddo, C., Martinez. V., and Carvajal, M. (2003) Water relations and xylem transport of nutrients in pepper plants grown under two different salt stress regimes. *Plant Growth Regular* **41**, 237-245.

Kafkafi, U. (**19**91) Root growth under stress In: Salinity in the Plant Roots. The Hidden Half [E. Waidel, A. Eshel and U. Kafkafi, (eds.)] Marcel Dekker, New York.

Kalaji, M. H., and Pietkiewiez, S. (1993) Salinity effect on plant growth and other physiological process ACTA. *Physiologiae Planarum* **15**, 89-124.

Kingsbury, R. W., Epstein, E., and Pearcy, R. W. (1984) Physiological responses to salinity in selected lines of wheat. *Journal Plant Physiology* 74, 417-23.

Kingwell, R., and John, M. (2007) The influences of farm landscape shape on the impact and management of dry land salinity. *Agricultural Water Management* **89**, 29-38.

Kinzel, H. (1983) Influence of limestone, silicates and soil pH on vegetation. In [*Physiological plant ecology. III. Responses to the chemical and biological environment*]. (Eds. Lange, O. L., Nobel, P. S., Osmond, C. B., and Ziegler, H.) pp. 201-244.Verlag, Berlin.

Kozlowski, T. T. (1984) Plant responses to flooding of soil. *Bioscience* **34**, 162-167.

Kozlowski, T. T. (1997) Responses of woody plants to flooding and salinity. *Tree Physiology* Monograph No.1.

Ladiges, P. Y. (1977) Differential susceptibility of two populations of *Eucalyptus viminalis* Labill. to iron chlorosis. *Plant and Soil* **48**, 581-597.

Ladiges, P. Y. and Ashton, D. H. (1977) A comparison of some populations of Eucalypts *viminalis* Labill. Growing on calcareous and acid soil in Victoria, Australia. *Journal of Ecology* **2**, 161-178.

Lazof, D., Bernstein, N. (1998) The NaCl-induced inhibition of shoot growth: the case for disturbed nutrition with special consideration of calcium nutrition. *Adv Botany Research* **29**, 113–189.

Macaulay, S., and Mullen, I. (2006) Predicting salinity impact of land-use change: Groundwater modelling with airborne electromagnetic and field data, SE Queensland, Australia. *International Journal of Applied Earth Observation and Geoinformation* **9**, 124-129.

Marcar, N. E. (1993) Waterlogging modifies growth, water use and ion concentrations in seedlings of salt-treated *Eucalyptus camaldulensis*, *E. tereticornis*, *E. robusta* and *E. globulus*. *Australia Journal of Plant Physiology* 20, 1-13.

Marcar, N. E., Crawford, D., Leppert, P., Jovanovic, T., Floyd, R., and Farrow, R. (1995) “Tree for Saltland: a guide to selecting native species for Australia,” CSIRO, Division of Forestry, Canberra, Australia.

Marcar, N. E., Hossain, A. K. M. A., Crawford, D. F., and Nicholson, A. T. (2000) Evaluation of tree establishment treatments on saline seeps near Wellington and Young in New South Wales. *Australia Journal of Experimental Agriculture* **40**, 99-106.

Marcar, N. E., Crawford, D. F., Saunders, A., Matheson, A. C., and Arnold, R. A. (2002) Genetic variation among and within provenances and families of *Eucalyptus grandis* W. Hill and *E. globulus* Labill. subsp. *globulus* seedlings in response to salinity and waterlogging. *Forest Ecology and Management* **146,** 109-146.

Marcar, N. E., and Crawford, D. F. (2004) “Tree for saline Landscapes” RIRDC Publication Number 03/108, Canberra. Australia.

Marschner, H. (1995) Mineral Nutrition of Higher Plants. Academic Press, London. p 889**.**

McEvoy, P. K. (1992) Ecophysiological comparisons between *Eucalyptus camaldulensis*, *E. largiflorens* and *E. micropcarpa* on the Murray River floodplain. M.Sc., *Faculty of Agriculture and Forestry*. The University of Melbourne.

McGraw, J. B., and Garbutt, K. (1990) Demographic growth analysis, *Ecology*. 7**1**, 1199-1204.

Miyamoto, S., Riley, T. Gobran, G., and Petticrew, J. (2004) Effects of Saline Water Irrigation on soil Salinity, Pecan Tree growth and Nut production. *Irrigation Science* **7**, 83-89.

Moore, G. (Ed.) (1998) Soil guide: a handbook for understanding and managing agricultural soils. Agriculture Western Australia, Perth.

Muhammad, J., Rehman, S. U., Lee, K. J., Kim, H., and Rha, E. S. (2007) Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Scientia Agricola* **64,** 111-118.

Munns, R., and Termaat, A**. (**1986) Whole-plant responses to salinity. *Australian Journal of Plant Physiology* **13,** 143–160.

Musgrave, M. E. and Ding, N. (1998) Evaluating wheat cultivars for waterlogging tolerance. *Crop Science* **38**, 90-97.

Panchaban, R. K. and Srisataporn, S. (1989) Effect of salinity on growth of fast growing tree. *Agriculture Journal* **17**, 91-99.

Parsons, R. F., and Specht, R. L. (1967) Lime chlorosis and other factors affecting the distribution of *Eucalyptus* on coastal sand in southern Australia. *Australia Journal of Botany* **15**, 95-105.

Pate, J.S and Beard, J. S. (eds) (1984) *Kwongan: plant life of the sandplain*. University of Western Australia Press.

Pezeshki, S.R. (2001) Wetland plant responses to soil flooding. *Environment and Experiment Botany* **46**, 299–312.

Pospisilova, J. (2003) Participation of phytohormones in the stomatal regulation of gas exchange during water stress. *Biology Plant* **46**, 491-506.

Powell, R. (1990) *Leaf and branch: trees and tall shrubs of Perth*. Department of Conservation and Land Management, Perth, Western Australia.

Powell, R. (2009) *Leaf and Branch. Trees and Tall Shrubs of Perth.* Department of Environment and Conservation, Perth, Western Australia.

Prat, D. and Fathi -Ettai, R. A. (1990) Variation in organic and mineral components in young Eucalyptus seedlings under saline stress. *Physiology Plant* **79**, 479-486.

Qureshi, T. M., Aslam, M., Ashraf, M.Y., and Hussain, F. (2000) Performance of Eucalyptus camaldulensis under different types of salt- affected soil. *Journal of Agricultural Biology* **2**, 45-95.

Rawat, J. S., and Banerjee, S. P. (1998) The influence of salinity on growth biomass production and photosynthesis of *Eucalyptus camaldulensis* Dehnh. and *Dalbergia sisoo* Roxb. seedlings. *Plant and Soil* 20**5**, 163-169.

Recher, H.F., Majer, J.D. and Ganesh, S. (1996) Eucalypts, arthropods and birds: on the relation between foliar nutrients and species richness. *Forest Ecology and Management* **85,** 177-195.

Richare, C. C., Farrell, R. C., David, T., and Bell, D. T. (1996) Morphological and physiological comparisons of conal lines of *Eucalyptus camaldulensis.* II. Responses to waterlogging, salinity and alkalinity *.Australia Journal Plant Physiology* **23**, 509-518.

Robinson, R. (2008). Forest health surveillance in Western Australia: a summary of major activities from 1997 to 2006. *Australian Forestry* **71**, 202–211.

Rodriguez, P., Torrecillas, A., Morales, M. A., Ortuno, M. F., and Sanchez-Blanco, M. J. (2005) Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. *Environmental and Experimental Botany* **53**,113-123.

Ruthrof, K. X. (1997) Improving the success of limestone quarry revegetation. *Cave and Karst Science* **24**, 117-125.

Sandhu, G.R., and Qureshi, R.H. (1986) Salt affected soils of Pakistan and their utilization. *Reclamation and Revegetation Research* **5,** 105–113.

Seddon, G. (2004) *Sense of Place. A Response to an Environment. The Swan Coastal Plain, Western Australia.* Facsimile edition, Blooming Books, Melbourne, Australia

Shearer, B.L. (1994) The major plant pathogens occurring in native ecosystems of south-western Australia. *Journal of the Royal Society of Western Australia* **77,** 113-122.

Shearer, B.L., and Dillon, M. (1995) Susceptibility of plant species in *Eucalyptus marginata* forest to infection by *Phytophthora cinnamoni*. *Australian Journal of Botany* **43,** 113-134.

Shearer, B. L., and Dillon, M. (1996) Impact and disease centre characteristics of *Phytophthora cinnamomi* on the Swan Coastal Plain, Western Australia. *Australian Journal of Botany* **44,** 79-90.

Singh, B. (1989) Rehabilitation of alkaline wasteland on the gangetic alluvial plains of Uttar Pradesh, India, through afforestation. *Land Degradation and Rehabilitation* **1**, 305-310.

Smith, M., and Moss, J. S. (1998) An experimental Investigation using stomatal conductance and Fluorescence of the flood sensitivity of *Boltonia decurrens* and its competitors. *Journal of Applied Ecology* **35**, 553-561.

Song, J., Yuan Yuan, Z., Jia, Y., Du, X., and Wang, B. (2008) Effect of salinity on germination, seedlings emergence, seedling growth and ion accumulation of a euhalophyte Suaeda salsa in an intertidal zone and on saline land. *Aquatic Biology* **88**, 331-337.

Stace, H.C.T., Hubble, G. D., Brewer, R., Northcote, K. H., Sleeman, J. R., Mulcahy, M. J., and Hallsworth, E. G. (1968) A Handbook of Australian Soils. Rellim Technical Publishers, Glenside, SA.

Stoneman, G.L., Turner, N.C., and Dell, B. (1994) Leaf growth, photosynthesis and tissue water relations of greenhouse-grown *Eucalyptus marginata* seedlings in response to water deficits. *Tree Physiology* **14**, 633-646.

Sun, D., and Dickinson, G. R**.** (1995) Salinity effects on tree growth, root distribution and transpiration of *Casuarina cunninghamiana* and *Eucalyptus camaldulensis* planted on a saline site in tropical north Australia. *Forest Ecology and Management* **77**, 127-138.

Symonds, W. L., Campbell, L. C., and Clemens, J. (2001) Response of ornamental *Eucalyptus* from acidic and alkaline habitats to potting medium pH. *Scientia Horticulturae* **88,** 121-131.

Syvertsen, J. P., Zablotowicz, R. M., and Smith, M. L. (1983) Soil temperature and flooding effects on two species of citrus. I. Plant growth and hydraulic conductivity. *Plant and Soil* **72**, 3–12.

Szabolcs, I. (1984) Soil and salinisation. *In* " Handbook of plant and Crop Stress" pp.311. Marcel Dekker, New York, USA.

Szabolcs, I. (1989) *Salt affected soils,* Boca Raton, CRC Press.

Tang, C., Robson, A.D., and Dilworth, J. (1991) Inadequate iron supply and high bicarbonate impairs the symbiosis of peanuts (*Arachis hypogaea* L.) with different *Bradyrhizobium* strains. *Plant and Soil* **138**, 159–168.

Thomas, G. W., and Hargrove, W .L. (1984) The chemistry of soil acidity. In "Soil Acidity and Liming" (F.Adams, ed.), 2nd ed., *Agronomy Monograph* pp 12.

Vadez, V., Krishnamurthy, L., Serraj, R., Gaur, P. M., Upadhyaya, H. D., Hoisington, D. A., Varshney, R. K., Turner, N. C., and Siddique, K. H. M. (2007) Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. *Field Crops Research* 1**04**, 123-129.

van der Moezel, P. G., Watson, L.E., Pearce-Pinto, G. V. N., and Bell, D. T. (1988) The response of six Eucalyptus species and Casuarina obesa to the combined effect of salinity and waterlogging. *Australian Journal of Plant Physiology* **15**, 645-754.

van der Moezel, P. G., Watson, L.E., and Bell, D. T. (1989) Gas exchange responses of two Eucalyptus species to salinity and waterlogging. *Tree Physiology* **5**, 251-257.

van der Moezel, P. G., and Bell, D. T. (1990) Saltland reclamation: selection of superior Australian tree genotypes for discharge sites. *Proceedings of the Ecological Society of Australia* **16**, 545-549.

van der Moezel, P. G., Pearce-Pinto, G.V.N., and .Bell, D.T. (1991) Screening for salt and waterlogging tolerance in Eucalyptus and Melaleuca species. *Forest Ecology and Management* **40**, 27-37.

van Dijk, A.I. J. M., Hairsine, P. B., Pena Arancibia, J., and Dowling, T. I. (2007) Reforestation, water availability and stream salinity: A multi-scale analysis in the Murray-Darling Basin, Australia. *Forest Ecology and Management* **251**, 94-109.

White, P. F., and Robson, A. D. (1990) Responses of lupins (*Lupinus angustifolius* L.) and peas (*Pisum sativum* L.) to Fe deficiency induced by low concentrations of Fe in solution or by addition of HCO_3^-. *Plant and Soil* **125**, 39-47.

Yeo, A. R. (1992) Variation and inheritance of sodium transport in rice. *Plant and Soil* **146,** 109-116.

Zhang, J., and Davies, W.J. (1991) Antitranspirant activity in xylem sap of maize plants. *Journal Experiment Botany* **42**, 317-321.