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Comparative salt and waterlogging tolerance of an introduced grass (*Agropyron elongatum* [host] p. beauv.) and two herbaceous Australian species (*Podolepis gracilis* [lehm.] and *Danthonia caespitosa* gaud.)

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**COMPARATIVE SALT AND WATERLOGGING TOLERANCE OF
AN INTRODUCED GRASS (*AGROPYRON ELONGATUM* [HOST] P.
BEAUV.) AND TWO HERBACEOUS AUSTRALIAN SPECIES
(*PODOLEPIS GRACILIS* [LEHM.] AND *DANTHONIA CAESPITOSA*
GAUD.).**

BRYAN WILLIAM DUFF

**THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE AWARD OF
B.SC. (BIOLOGICAL SCIENCE) HONOURS**

**DEPARTMENT OF APPLIED SCIENCE
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USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

ABSTRACT

Increasing land degradation is recognised as an immediate worldwide threat. Human-induced soil salinity is probably the major cause of land abandonment, through its adverse effects on plant growth. Salinity is a major focus of environmental researchers because it is recognised that counter-strategies can potentially reclaim both artificially degraded lands and intrinsically saline areas. Currently, strategies to combat salinity require that land use is changed, since restoration to a past use is usually economically impractical or impossible.

Biological strategies show most promise. Revegetation of degraded soils with hardy plant species has met with considerable success, and shows promise for the future, given the vast, and as yet largely unknown, resources in plant genetic diversity. This diversity also gives rise to the need for testing of specific tolerances to soil salinity and associated soil conditions, such as waterlogging, to expedite revegetation programs.

Growth of the introduced grass *Agropyron elongatum*, known for its tolerance to stress, was examined in a glasshouse at a range of salinities, in waterlogged conditions and in high soil pH, conditions often found on some minesites in the southwest of Australia. An indigenous grass *Danthonia caespitosa* and an indigenous daisy *Podolepis gracilis* were similarly examined. These trials were undertaken in order to determine any potential usefulness in minesite revegetation, and to compare a known halophyte with the tolerances of previously untested Australian species.

A. elongatum was found to decrease in growth, but tolerate salt concentrations to 400 mM, and to be unaffected by waterlogging. Both Australian species were found to be at least as tolerant of salt as *A. elongatum*, but to be sensitive to hypoxia caused through waterlogging. Both grasses showed apparent growth stimulation at low salt concentrations, though this is not in accordance with past studies on monocotyledonous plants.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by any other person except where due reference is made in the text.

Bryan William Duff

17 December 1997

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CHAPTER 1. INTRODUCTION

1.1 Causes and Economic Importance of Soil Salinity

The effects of salinity on plant growth are well documented; according to Garfield (cited by Munns [1993]), this is the second-most frequently published plant physiology topic behind photosynthesis. Such a high level of scientific interest is indicative of the potential for significant economic loss caused through the adverse effects of salts on growth (hence yield) of crop species and also of the less obvious potential for harm to conservation efforts. As an extreme example, salinity in less-developed countries (LDCs) has been linked to the encroachment of desert on both crops and stands of natural vegetation (McWilliam, 1986). Desertification clearly is the ultimate result of unchecked salinisation (Mabbutt, 1978), yet such extreme conditions rarely cause concern in more-developed countries (MDCs) (Freer, 1978). This disparity is related to the amount of capital able to be directed at the problem of salinity (McWilliam, 1986), and also to the different capacities of cultures to firstly recognise and acknowledge the potential long-term effects, then to implement effective counter-strategies.

These two points also give some indication of the difficulty involved in assessing the importance and extent of salt-affected land (Flowers & Yeo, 1995); that is, the degree of estimated salt damage depends on the objective viewpoint of the assessors, and the assessors' understanding of the capacity of the population to

alter land-use practices. For example, rice is relatively salt-sensitive (Aslam, Qureshi & Ahmed, 1993), but is grown in sufficient quantity on salt-affected soils to support subsistence agriculture in parts of western Asia, India and Indonesia. However, in many of these same locations the rice yield is considered too low to support an increasingly important agricultural economy (Flowers & Yeo, 1995). It is, therefore, partially replaced by more salt-tolerant crop species (such as semi-dwarf rice), or the affected land is abandoned. More affluent nations, or those with less restricted options, might consider directing capital at research into biological or physical strategies to prolong the usefulness of the salt-affected land.

Of least importance in assessing the extent and impact of salinity at the international level is the amount of salt in the soils. Using salt concentration as the only parameter, some researchers have determined that as much as 10 million km² of the earth is affected; by confining these studies to land-use as secondarily affected by salt, the figure is given as around 1/3 of the approximately 1.2 million km² of irrigated land (Peck, 1993), and 1/7 of the approximately 15 million km² total cropland (Yeo & Flowers, 1989). It would seem therefore, more appropriate to confine generalisations to countries or regions with a single, definable economic base, enabling specific strategies to combat salinity which need only consider any region's economic practices and goals, and its peoples' capacities to change land-use wherever it is shown to be necessary.

A further complication in assessing the impact of salinity is that soil salt levels continually change through natural causes, and more importantly, increase through human land-use practices such as irrigation with salty water (Flowers & Yeo 1977; Ashraf 1994; Flowers & Yeo 1995). In addition, primary (natural, or intrinsic) salinity is usually associated with high or very low rainfall and undesirable soil characteristics. It is often economically impractical to change to new croplands (many countries simply have none available), so existing sites continue to be subjected to a salt input or to increased exposure of topsoil to natural underlying sources of salt.

Soil salinity is not a new problem. Records show that ancient peoples frequently moved to new farmlands when existing land became contaminated; the Punjab in India for example has created a severe salinity problem over many centuries of traditional farming practices. However, the world now has something like 6 billion people, and arable land is increasingly being seen as a very finite resource (Yeo & Flowers, 1989).

The focus nowadays, in both LDCs and MDCs, is on production or selection of vegetation which will tolerate certain levels of salt (hence acknowledging the relative permanence of the problem), or on reclamation of contaminated land through phytological and/or physical remediation (Yeo & Flowers 1989; Barrett-Lennard & Qureshi 1994; Flowers & Yeo 1995). The former strategy has the advantage of being potentially able to claim previously unusable land; intrinsically

saline soils which have been ignored in the past may be entirely suitable to some halophytic varieties (McWilliam, 1986).

Naturally-saline soils may be caused by weathering of parent rock, wet or dry precipitation, surface or subterranean water flow, and are often the sites of ancient sea-beds. Agriculturally useful halophytes may be used to tap the resources of these normally unproductive areas. Both of the above strategies depend on the production or selection of plants with some degree of salt tolerance, whether for immediate and prolonged use, such as crops, or for a long-term program of reclamation.

1.2 Soil Salinity in Australia

A further cause of salinity, and one which is well known in Australia, is the exposure of topsoil to rising water-tables (Mulcahy 1973; Belford, Dracup & Tennant 1990). Unconfined aquifers are leachate sumps, and hence are slightly to moderately saline (even exceeding that of sea-water where dissolving parent rock contributes sufficient metallic chlorides NaCl, CaCl₂ or MgCl₂). Salts brought to the rootzone by rising water are concentrated by evaporation (Mulcahy, 1978). In the Murray River catchment, irrigation with this water or surface water in conjunction with past clear-felling of deep-rooted trees which previously kept the water table low through transpiration, has created a serious salinity problem; of particular importance since the region represents around 80% of Australia's irrigated lands (Mabbutt, 1978).

In Western Australia, tree-clearing has probably contributed to the salinisation of over half of the dryland farming areas (Mabbutt, 1978). Salinity and waterlogging is widespread in those areas; some water tables have risen from depths of 20m to within 2m of the soil surface. Over the past few decades, crop production has decreased, some land has been abandoned, concern has arisen over the salinity of the domestic water supplies, into which saline water drains, and ominously, much of the world's driest continent is considered to be increasingly susceptible to desertification (Mabbutt, 1978). Unlike LDCs, Australia has the capital and resources to keep the problem in check, and recent tree-planting programs (Schofield, 1991), and improved irrigation techniques along with extensive drainage, have helped begin a long program of reclamation. Research has shown that smaller plant types may also have a role in lowering water tables (Barrett-Lennard, 1996). Groundwater recharge can also be reduced through evapotranspiration from near the soil surface.

Elsewhere in Australia, salinity is considered a less immediate economic problem (McWilliam, 1986). Intrinsically saline areas are often located where periodic flooding, unpredictable rainfall or infertile topsoil preclude farming, and xeromorphic and/or halophytic indigenous vegetation is suitable for supporting relatively few stock animals per km² on large commercially viable properties in arid inland areas (Mabbutt, 1978). Primary salinity is estimated to occupy around 5.3% of the continent (Peck, 1993).

1.3 Soil Salinity in Southwestern Australia

The state of Western Australia has an economy based primarily on mining, followed by agriculture/timber, and tourism. Extensive clear-felling of eucalypts (and in some places, extravagant irrigation methods) have caused salinity/waterlogging problems through elevation of water tables in parts of the southwest of the state (George, 1990). Tree planting programs, with government-provided incentives for farmers, have begun in recent years. Some farmers have turned partly or wholly to tree farming, with the two-fold benefits of improving their own soil while profiting in the near future from the timber industry (Bartle, 1991).

The tourism industry is based partly on mining operations, mainly in the north of the state, but more importantly on natural areas of interest such as old-growth forests in the southwest. These have been extensively cleared since European settlement in the 19th century, to both exploit the hardwoods and make room for crops (especially wheat) and pastures. Probably less than 10% of forest in that part of the state has been unaffected by European settlement, and no more than 35% of original wooded land remains. Nevertheless, salinity/waterlogging problems have been regarded as so far confined to isolated sites, and most attention has been directed at educating farmers about containment. The Western Australian Department of Agriculture regularly issues journals and newsletters which report on the costs and benefits of various salt and waterlogging

containment methods (Bathgate & Evans 1990; Barrett-Lennard, Frost, Vlahos & Richards 1991; George 1991).

Inland from the state's capital city Perth, wheat is the major crop, and the area is a major contributor to the economy. Erosion of the topsoil and salinity have been of major concern in the past. Studies pertinent to the worldwide concern over desertification have been undertaken in this and other parts of the state (Mabbutt, 1978).

Western Australia is a major contributor of mineral ores to the world market, including diamonds and iron ore in the north, gold, nickel and silver in the inland southern half, and bauxite (and coal for local power production) in the southwest. Mining operations are a tourist attraction in themselves, but can exacerbate pre-existing hydrogeological problems, and drastically alter environments over wide areas (Freer 1978; Barrett & Bennett 1994). State and federal legislation is in place which requires that mining companies contribute to conservation efforts, including remediation of spoiled or denuded land (Scheltema, 1991). In the past, this has resulted in the planting of stands of contaminant-tolerant, especially halophytic, trees and smaller woody species, which were selected for the similarities in soil conditions found in their natural habitats. There has been little regard for their ecological 'relevance' to the new situation. Over recent years, however, the focus has been on conservation using indigenous species (Barrett & Bennett, 1994).

There is ample evidence that increased salinity in this region is a result of settlement by Europeans. Concern was expressed as long ago as 1897 that the Northam/Toodyay rivers were becoming increasingly saline (Mulcahy, 1978). Significantly, at that time only a comparatively small part of the catchment area had been cleared for (wheat) farming, suggesting that tree-clearing in relatively small sections of bushland affected soil salinity both locally and remotely, via surface or shallow sub-surface drainage. Research was underway by the 1930s into the causes and effects of local salinity. A generalisation was made, and remains valid today, which proposed that the usual result of an unaffected hydrogeological cycle was a very slight net increase of salinity nearest the coast, increasing with decreasing rainfall and increasing evaporation further inland, and that European-style farming practices affected the cycle's equilibrium. Replacement of deep-rooted trees with short-lived (annual) crops and pastures caused a reduction in evapotranspiration, hence an increase in groundwater recharge (Mulcahy, 1978). Salt concentration near the soil surface increased through evaporation in the hot dry summers of a Mediterranean-type climate.

Although salt input through wet and dry precipitation decreases further inland, the topography is not conducive to effective discharge to the ocean; indeed this region is characterised by chains of salt lakes which likely represent the remnants of ancient rivers (Mulcahy, 1973). The lack of a gradient necessary for adequate drainage, combined with the artificial increase in groundwater recharge, is obviously a major contributor to salinity/waterlogging problems in the wheatbelt, but also of importance is the predominance of lateritic layers underlain by

relatively impermeable kaolinitic clays (Mulcahy 1978; Belford *et al.* 1990). The latter (pallid) zones are associated with significant amounts of soluble salts, and usually occur over the granitic bedrock which prevails east of the Darling escarpment (Mulcahy, 1973). Relatively permeable sedimentary calcareous rock underlies most of the Swan Coastal Plain, west of the scarp. The granitic bedrock of the Western Australian Shield contains little chloride, hence the salts in the pallid zone are probably derived secondarily from precipitation followed by evaporative concentration (the pallid zones are themselves formed by leaching) (Mulcahy, 1973).

The westernmost section of the shield is termed the zone of bauxitic laterites. Bauxitic soils overlay a laterite-associated pallid zone in this area (Mulcahy, 1973). Winter rainfall is relatively high, more so toward the coast, as is the amount of evaporation occurring during the summer months. Natural drainage is ineffective in the eastern part of this zone, though less so toward the west, where permanent rivers follow a steep gradient to the coastal plain. Most of the western and southern section of the zone is eucalypt (especially jarrah, *E. marginata*) forest; clearing for agriculture occurs mostly in the eastern parts of the forest, where rainfall is lower. Cleared sections of the jarrah forest have contributed to salinity levels in catchment areas; of most concern is the deteriorating quality of domestic water supplies (Peck, 1993), and as mentioned, the impact on individual farms (George, 1990).

Of most relevance to the current study are the mining operations in the zone of bauxitic laterites, in particular gold mining towards the west of the zone, southeast of Perth. Soil at these sites is adversely affected by waste products from chemical treatment, topographic alterations which affect water drainage, and disruption to soil strata caused during the ore-extraction process. Salts and heavy metals are deposited in tailings at the soil surface; the normal curative effects of leaching are inhibited partly by the natural characteristics of the geological zone, and by an artificially elevated water table. Hence the soils acquire a heterogeneous distribution of salt concentrations, waterlogging near the surface, and a predominantly alkaline chemistry due to intrinsic parent materials and introduced contaminants. Such conditions strongly inhibit the settlement and growth of local plant species, impeding efforts at restoration.

1.4 Strategies for Continued Use or Recovery of Saline and Waterlogged Land

Assessment of the economic impacts of salinity must take into account not only the available remediative or palliative strategies, but the capacity of a people to put these into effect. In addition, its capacity must be carefully defined to include its own assessment of the costs/benefits of land-care strategies as they pertain to economic goals, and naturally, the return expected of the land itself. This point is reiterated here because it is important to recognise that no single strategy can be applied to all cases, and that even where a set of containment measures have a high probability of success, the cost of their implementation may exceed the

predicted benefits in the short and/or long term (Yeo & Flowers 1989; Flowers & Yeo 1995).

Hence reviews of the salinity problem vary considerably not only in quantitative assessment of its impact and scope, but also in prognoses of the efficacies of different containment strategies. McWilliam (1986) for example, states that effective drainage of saline water is the only effective long-term solution, and that development of salt-tolerant crops may be merely a method of postponing technological programs. Flowers and Yeo (1995) on the other hand, recommend that containment should involve a co-ordinated blend of physical and biological measures; that is, that neither approach will succeed in the absence of the other. Both reviews stress the importance of minimising artificial inputs of salt, particularly through improved water management by reviewing irrigation practices. Biological programs are in place, but results so far have been mixed. The next section briefly describes biological containment programs. For the sake of brevity they are considered as discrete disciplines, though the approach should be unified in practice, and reckoned in combination with physical/topographical containment programs.

1.5 Salt in Crops and Pastures

Agriculture has been the major focus of attempts to develop salt-tolerant plant varieties (Flowers & Yeo, 1995). The human population, and that of LDCs in particular, has reached a level where farmland is considered an irreplaceable

necessity rather than a source of economic gain (Tal, 1985). Inadequate drainage is usually given as the major cause of salt accumulation in croplands, but it is the input of water and comparative lack of evapotranspiration on annually harvested land which first creates the need for artificial drainage in many situations. Subsistence agriculture has shown that reliance on a single, staple crop is possible only through management of soil water, including effective drainage (which is often practical on the smaller plots found in subsistence farming), and periodic transfer of attention to other plots which have had time to recover their fertility. The rice paddies of Asia are one obvious example, especially pertinent since rice requires a semi-aquatic environment but is moderately sensitive to salt.

Physical approaches to water management are often less feasible in larger-scale agriculture, where the aim is for maximum yield at minimum cost (Bathgate & Evans 1990; George & Frantom 1991). Financial strategies are used in more affluent countries to offset the economic impact of drought or flood. Such strategies include fund borrowing, calculation of the most conservative cropping rates, long-term grain storage and emergency government funds. McWilliam (1986) suggests that salinity has not yet been considered a problem worthy of similar approaches in most of these same countries. Certainly in Australia, salinity has had local, rather than regional economic effects, and as mentioned, is only considered a problem wherever other environmental restraints on agriculture such as drought or flooding are not. The irrigated farmlands of northern Victoria however, have recently undergone widespread changes to irrigation techniques, in conjunction with improved drainage methods. Yet it has

been estimated that soil salinity/waterlogging in that area could take 100-200 years to decrease to pre-European settlement levels if the new cropping methods were effective immediately. A similar figure was given for Western Australia's Blackwood River to regain its status as a source of potable water, if saline input were to cease completely and immediately. Hence there seems a clear need for salt/waterlogging-tolerant crop species, especially in countries where salt input is an unavoidable aspect of providing water to otherwise barren land.

Biological approaches have been most extensively studied with agriculture as the focus. Research endeavours can be conveniently classified as selection of appropriate crop types, production of novel hybrids or genetic improvement of existing crop types.

1.5.1 Selection of salt-tolerant crop varieties

This approach has arguably achieved the greatest success. Halophytic and xerophytic species have been widely transplanted around the world, and incorporated into economies as sources of fuelwood, fodder or oilseed (Flowers & Yeo, 1995). For example, *Salicornia* has been the focus of extensive field-trialling in the Middle East (Charnock, 1988). Varieties of this euhalophyte (salt-tolerant physiology) have been shown to succeed in severe environments, and even thrive on irrigation with seawater. Its nutritional value as fodder for goats has been judged superior to the crops it has replaced. Locally, *Atriplex* (saltbush) species have been widely transplanted as forage for sheep in southwestern

Australia. Recently, however, its nutritional value has been found to be lower than expected (Warren, 1995).

Yet these are not true crop species. The world depends on only a few hundred plant species for its nutrition, and probably only 12 species could account for most of this dependence. Of the 1 560 halophytic plants so far known, none have shown potential as a human food source on a useful scale. Flowers and Yeo (1995) suggest that a similar approach, that of domesticating (breeding usefulness into) a halophyte may be the most practical option. This is supported by past successes in increasing the yields from *Triticale* and *Hordeum* (though neither are true halophytes).

1.5.2 Hybrids (crop species + halophyte)

Triticale, a wheat/ryegrass hybrid, has been mentioned above. However, successful hybridisation programs are uncommon. Salt tolerance seems to be a complex genetic trait, further complicated by the environmental/physiological relationships of halophytes (eg; aridity/tissue succulence) (Tal 1985; Flowers & Yeo 1995).

Hybridisation has been successful where a single gene can confer immunity to disease or insect pests, followed by simple back-crosses to dilute the undesirable phenotypic features of the resistant parent in following generations. Salt tolerance at the whole plant level, however, entails complex genetic control of

growth strategies, physiologies (eg; slow growth, succulence, glands) in the halophytic parent (Warne, 1995) which creates difficulties when attempting to dilute this parent's phenotype.

Flowers and Yeo (1995) found very few substantiated reports, or trade patents, for salt-tolerant hybrids. Therefore it might be concluded that the crossing of entire genomes is not yet a practical option.

1.5.3 Genetic improvement of salt tolerance in existing crop genotypes

All major crops are relatively salt-sensitive or only moderately tolerant, by coincidence or through usage over centuries. All approaches so far mentioned have ultimately required empirical testing of results, and most have shown that crop yield is not finally dependant on an absolute value of a species' tolerance of osmotic stress or of salt effects (Ralph 1989; Flowers & Yeo 1995). Screening in controlled conditions has not identified any clonal lines which regularly and predictably give a higher yield than another conspecific in field conditions. These observations give some clue as to the difficulties which might arise in genetic manipulation of crop species, supported by the previously-mentioned lack of patents relating to salt-tolerant plant varieties.

A genetically 'improved' plant variety appears no more likely to show a higher yield compared to an unimproved variety because of the heterogeneous distribution of salts in cropland (Ralph 1989; Flowers & Yeo 1995). In other

words, a plot with an average soilwater conductivity of 100mS/cm may provide higher yields than an equivalent plot with an average conductivity of 70mS/cm, if there are sufficient patches of low salinity, or other growth-maintaining qualities within that plot.

This limitation may apply to any method of crop improvement, but genetic manipulation has additional constraints which give it particular relevance. One such constraint is that there has been limited success in identifying genes which specifically confer tolerance to salts (Tal, 1985). Most candidate genes probably contribute to salt tolerance only as a secondary result of other physiological control mechanisms (Warne, 1995). Tolerance is likely to be conferred by complex synergistic genetic mechanisms (Tal 1985; Rausch, Kirsch, Low, Lehr, Viereck & Zhigang, 1996). A search of the literature revealed no concerted attempts to identify transcription factors which might open entire gene 'boxes' conferring resistance to salt-induced stress. It has been proposed that a more realistic aim would be to produce high-yield transgenic crop plants able to exploit the patchiness of saline soils, rather than transgenic halophytes with a lower yield (Flowers & Yeo, 1995).

Tissue culture has highlighted this difficulty; tolerant cell lines do not reliably develop into tolerant whole plants (Daines & Gould 1985; Flowers & Yeo 1995). Tolerance is a property of the whole plant, including control of membrane function, water/solute relations in the apoplast, photosynthetic capacity etc. Field

testing, with yield as the important criterion, seems to be the only true indicator of the value of a particular crop genotype.

1.6 Woody Species in Conservation and Land Rehabilitation

Trees and shrubs can be an economically profitable crop (eg; timber, paper, fuelwood) (Barrett-Lennard *et al.* 1991; Bartle 1991), but are considered separately because they are not harvested annually. They are relatively long-lived, hence can both remediate salt/waterlogged areas (George, 1991), and return affected sites to more socially-accepted conditions (ie: in conservation efforts) (Freer, 1978).

Strategies for production and selection of suitable varieties are similar to those used in agriculture, but differ in that there is considerably greater variation in stress tolerances of undomesticated species. In addition, the yield of woody species is usually an unimportant consideration in conservation.

Australian species have been widely used locally and internationally in control of salinity and waterlogging (van der Moezel, Walton, Pearce-Pinto & Bell 1989a; Barrett-Lennard & Qureshi 1994), as a source of fuelwood and for aesthetic value. Screening of eucalypts (van der Moezel, Watson & Bell, 1989b), casuarinas (El-Lakany & Luard 1982; van der Moezel, *et al.* 1989a), acacias and *Atriplex*, has resulted in a useful database which details relative and absolute

values for salt and waterlogging tolerances in a number of species, including comparison between provenances within these species and some clonal lines.

Complicating factors which are mentioned in the reports of these screening experiments include predicted (and observed) variation in results according to different growth stages, different periods and levels of prior acclimation (Marcar, 1993), intraspecific variation (Sands, 1981) and seasonal growth variations.

Trees have been widely used in Australia over recent years in replanting programs, which aim to repair degradation caused through past clearfelling practices of both the timber and agriculture industries (George, 1991). The target in this 'Decade of Landcare' (Schofield 1991; Scheltema 1991), is the replanting of a billion trees in ecologically appropriate areas. Farmers have traditionally resisted planting trees in valuable crop space, but the federal government is currently providing incentives, and education as to the importance of conservation in sustainable agriculture.

1.7 Herbaceous Varieties in Conservation

Conservation does not necessarily mean reclamation of affected land with trees and shrubs, especially since there is little likelihood of returning the original vegetation to newly saline soil. Necessarily, salt tolerant species of various morphologies must be identified for use in revegetation. This should include herbaceous varieties, particularly since grass and heathlands have also been

subjected to salt/waterlogging. Ecological considerations could be included in selecting the most appropriate plant type for revegetation from a database of the tolerance abilities of candidate species.

Herbaceous plants have some advantages over woody species in rapid revegetation: rapid growth to reproductive stage, shallow roots (hence may avoid all but the most severe waterlogging) and dense root mats which can rapidly bind topsoil and encourage microbiotic colonisation. These root mats may also quickly alter soil structure to enhance further settlement of plants, including subsequent generations of offspring. Initial settlement may be followed by a rapid linking-up of 'islands' of colonisers. Rapid colonisation may occur through asexual reproduction or seasonal reseeding if planting is initially successful (many grasses for instance, are *r* strategists whose populations increase at an intrinsic rate), hence planting programs could be 'one-off' and inexpensive. Such strategists may make use of the patchiness found in saline soils to aggressively colonise initially small areas.

A program of reclamation with herbaceous species should include; identification of candidate species from severe environments, identification of more-tolerant genotypes through screening, field testing of success to adult and subsequent reproduction, and identification of any markers (physiological, chemical) which could predict field success.

The current study focusses in part on testing Australian herbaceous species, identified as growing naturally near contaminated soils, for their tolerances to salinity and waterlogging, with the aim of determining their usefulness in revegetation programs.

1.8 Physiologies of Salt Tolerance, Waterlogging Tolerance and Alkalinity Tolerance

1.8.1 Salt tolerance

It is possible to separate species by broad delineation of their tolerances; marine algae (some Rhodophyta can survive 4X seawater salt concentration), euhalophytes (physiologically specialised, benefitting from some level of salt, eg; *Salicornia* spp.), miohalophytes (relatively high tolerances, usually through foliar salt exclusion, eg; barley) and glycophytes (low tolerances, eg; rice).

1.8.1.1 Halophytes

Flowers, Troke & Yeo (1977) group halophytes for convenience of discussion as having an optimal external salt requirement of between 20-500 mM concentration (commonly between 100-200 mM), and a lethal salt concentration of usually much greater than 300 mM (with growth inhibition occurring at greater than 100 mM [Ayala & O'Leary, 1995]), and glycophytes as a separate group by exclusion from these considerations. Salt tolerance is however, a

continuous rather than discrete variable, affected by factors such as; light intensity, light period, temperature, humidity (ie; factors affecting photosynthesis, transpiration [Munns, 1993]), growth stage (Ashraf, 1994), prior acclimation, and physiological adjustments to very small changes in ion concentrations (Flowers *et al.*, 1977).

Halophytes generally respond well to external salts (Ayala & O'Leary, 1995), though it has not been shown that these plants are innately dependant on ion concentrations above those found in normal soil conditions. Experiments under culture conditions show that growth responses are highest when NaCl is the salt used, and lowest when CaCl₂ is used. There are interspecifically variable responses to monovalent and divalent cations, and also to different anions; Cl⁻ has been found to increase relative growth rates across a wide range of species tested (Flowers *et al.*, 1977).

Halophytes have been useful in studies of physiological responses to salt, showing clear variation in growth and morphology according to types and concentrations of salts applied, and have assisted in elucidating cellular and whole-plant responses to external salts. Studies of these plants have provided evidence that growth reduction is due to reduced cell division rather than reduced cell growth, and have also provided support for a model of plant growth which is controlled by cell wall extensibility more directly than turgor effects (Casas, Bressan & Hasegawa, 1991). In broad terms, halophytes may be considered separate from glycophytes in having a higher optimal external salt

concentration, and a higher tolerance of high salt concentrations. These may represent two distinct and different mechanisms (Ayala & O'Leary, 1995). An optimum lower salt concentration may be equated with a glycophytic requirement of specific ions as micronutrients in varying concentrations, while a high upper lethal limit is certainly a specialised stress response (Flowers *et al.*, 1977).

Given that halophytic ability is usually determined empirically, according to responses to environmental salt concentrations, rather than by physiological adaptation, Jennings' definition (cited by Greenway & Munns, [1980]) may be appropriate; '(halophytes are)...the native flora of saline soils'.

1.8.1.2 Glycophytes

Glycophytes may be defined as those plants which are less able to tolerate a salt concentration as high as that tolerated by halophytes. This represents the vast majority of plants. In research, these plants have proven useful in elucidating the physiological effects of salinity (Greenway & Munns, 1980), and provide comparison with halophytes, especially where a glycophyte and halophyte are closely related. Crop species show a range between glycophytic and miohalophytic abilities (some crop species have halophytic ancestors). Plants can show an intraspecific range of salt tolerances; native inhabitants of saline soils can often be found growing in non-saline areas, while the reverse is true of some species normally considered to be non-halophytic, further demonstrating the

blurred distinction between halophytes and glycophytes. *E. camaldulensis* for example, usually not considered a halophyte in the strictest sense, can be found growing naturally throughout mainland Australia in a wide range of environmental conditions (Farrell, Bell, Akilan & Marshall, 1996a).

1.8.1.3 Mechanisms of salt tolerance

Having mentioned that halophytic ability is a continuous variable, it must be pointed out that it is also evident that true (eu-) halophytes may be characterised physiologically, primarily by adaptations which facilitate ion (particularly Na^+) uptake and compartmentalisation to decrease water potential in tissue (Flowers *et al.* 1977; Flowers & Yeo 1986; Glenn, Olsen, Frye, Moore & Miyamoto 1994). Such a mechanism ensures a gradient in water potential between the plant and the soil. This is associated in dicotyledonous halophytes with increased tissue water (ie; succulence) (Flowers & Yeo, 1986). Monocotyledonous halophytes may take up Na^+ , and utilise organic solutes for intracellular osmotic adjustment, usually accompanied by a decrease in water content (Glenn, 1987). Ion uptake has been shown to be an effective osmotic adjustment. The level of adjustment varies between species, but NaCl alone can account for 75-93% of osmotic potential in plant tissues (Flowers *et al.*, 1977). Vacuoles are the likely site of ion concentration. It is generally accepted that cytoplasmic ion concentration is 1/3 to 1/2 that of the vacuole, and that tissue damage occurs only when supply of ions exceeds demand, causing apoplastic saturation. Na^+ is toxic at higher concentrations, as is K^+ , both inhibiting protoplasmic enzymes. Shoot ion

concentration in comparison to root mass has been used to define halophytic ability, since ion supply is a function of root surface area (Flowers & Yeo, 1986).

Glycophytic tolerance is more correctly an avoidance strategy, provided primarily by ion exclusion, mostly from the leaves, with concomitant accumulation of Na^+ in roots and stems (Greenway & Munns, 1980). The roots often have a bi-layered endodermis, affording increased control of ion uptake, and while Na^+ enters the root via the symplast, it is actively retained in the root xylem parenchyma and 'leaked' Na^+ is actively resorbed from the xylem.

The halophyte/glycophyte distinction can blur interspecifically, but even within a species, such as *Spartina patens*, the tolerance ability can vary between ecotypes across the halophytic/glycophytic boundary (Hester, Mendelsohn & McKee, 1996). It is possible that tolerance to salt is an evolutionary adjustment of the level of ion uptake relative to ion exclusion, occurring separately in approximately one-third of angiosperm orders. This view is supported by the apparent lack of metabolic adaptations seen in halophytes (Flowers *et al.*, 1977), although anatomical adaptations, such as the salt-extruding glands of *Atriplex*, occur in some species. In addition, some plants (particularly monocots) tolerate high external salt by exclusion of ions at the roots, combined with temporary or long-term suspension or minimisation of growth. Many halophytes are, in fact, slow growers (Flowers *et al.*, 1977).

It is possible that the high ion content of halophytes is not induced by high external salt, but is a constitutive aspect of halophytic growth (Flowers *et al.*, 1977). For example, many halophytes are C4s and require Na⁺ as an essential component of pyruvate transport (Luttge, 1993), whereas Na⁺ is not an essential micronutrient of C3 plants. In addition, some plants may change from C3 to C4 pathways when exposed to water stress. *Mesembryanthemum crystallinum* for example, can be induced to change from C3 photosynthesis to CAM in high external salt (Luttge, 1993). This causes malic acid synthesis to increase, which is an organic osmoticum (anionic counter to Na⁺ in the vacuoles of photosynthetic leaf cells). The role of organic osmotica in countering osmotic stress is not yet resolved. This is discussed further in section 1.9.3. Cellular organelles are implicated in this mechanism (Robinson & Jones, 1986), and some tentative support for organic osmotica as a response to stress is offered by evidence that temporary sequestration of inorganic ions may occur in organelles prior to compartmentalisation in vacuoles.

Specific uptake of K⁺ or Na⁺ varies between plants (Glenn, Pfister, Brown, Thompson & O'Leary, 1996), and between ratios of K⁺/Na⁺, so it is difficult to generalise on ion specificity as a factor in salt tolerance (Flowers *et al.*, 1977). At high concentrations of different salt types, Na⁺ is taken up in selective transfer of K⁺ in the majority of angiosperms. In addition, ion/tissue specificity varies between species. In halophytes, approximately 90% of Na⁺ is found in the shoots and 80% in leaves. This is in contrast to glycophytes, in which Na⁺ is retained in

the roots. Although rapidly transported to leaves, neither cations nor anions are readily exported, even from senescing leaves (Flowers *et al.*, 1977).

There is evidence that halophyte enzymes are no less inhibited in their function by high salt concentrations in the vacuoles than those of glycophytes (Daines & Gould, 1985). High Na^+/K^+ ratios, absolute levels of each, and high Cl^- concentrations are known to inhibit many enzyme activities. Enzyme activity may be improved by acclimation or by high substrate concentration. There is also evidence that more than one form of some enzymes (eg; malate dehydrogenase) exists in both glycophytes and halophytes, but that halophytic cells contain proportionately more of the less-inhibited forms (Daines & Gould, 1985).

The role of active transport in vacuolar sequestration and in flux across root cell membrane is not yet fully elucidated, though active transport of Na^+ in glycophyte roots has been shown, and Na^+/H^+ antiporters would fulfill theoretical requirements (Rausch, *et al.*, 1996). Certainly, changes in tonoplast permeability (and that of organelle membranes) is important, as it has been shown to be affected by changes in concentrations of ions applied experimentally. The difference between tonoplast permeability in root cells and leaf cells could account for selective transfer (antagonistic exchange) of K^+ (less readily across membrane) and Na^+ (more readily) (Rausch *et al.*, 1996). In *Salicornia*, Na^+/K^+ pumps are implicated in ion exchange across the tonoplast; thus Na^+ would be sequestered temporarily in organelles (to avoid excess cytoplasmic concentration). In other halophytes, Na^+ may move into the apoplast prior to

removal in the transpiration stream to leaf surfaces. Na^+ supply would, therefore, rarely exceed demand in these species.

Controlled experiments in salt-stress effects and tolerance mechanisms are of necessity relatively simple. Soil salinity is however a complex combination of many factors. Besides the physical effects on soil structure, and the effects on chemistry and root-zone biota which certainly contribute to overall plant response, the salts themselves vary in proportionate ionic concentrations, and this both temporally and spatially. $\text{Na}^+/\text{Ca}^{2+}$ ratios for example, are important to the permeability of root cell membranes, affecting control of ion uptake. Na^+ displaces Ca^{2+} (important in membrane structure) in cation exchange, which occurs not only in the soil solution but also at root cell surfaces. When the $\text{Na}^+/\text{Ca}^{2+}$ ratio is high, tolerance is often low. Further complicating this relationship is the exchangeability of other cations in the soil solution, particularly Mg^{2+} .

Clearly, salt tolerance in plants is a complex trait. The following sections deal briefly with two further soil conditions often associated with salinity that plants may encounter.

1.8.2 Waterlogging

1.8.2.1 Effects of waterlogging on plant growth

Saturated soil contains insufficient oxygen to support normal root cell function (Barrett-Lennard, Davidson & Galloway 1990; Marcar 1993). Active transport at the root cell membranes is inhibited by up to 95% (the difference between oxygenic respiration and fermentation pathways) (Barrett-Lennard *et al.*, 1990). The immediate effect is root-tip injury (Farrell *et al.*, 1996a), a lack of control of ion uptake (Marcar, 1993), and an inhibition of osmotic control (van der Moezel *et al.* 1989b; Galloway & Davidson 1993). Ion toxicity, especially that of sodium, may be manifest through tissue damage, while osmotic imbalance varies as to its observable effects according to plant type. Grasses for example, seldom wilt when exposed to waterlogging until tissue damage occurs.

Many plants can survive for long periods in waterlogged conditions by temporary cessation or minimisation of growth. Oxygen content, for instance, usually takes at least 8 days to decline to zero, depending on temperature. However, there is evidence that root-tip death occurs in hypoxic conditions relatively quickly in both halophytes and glycophytes, though the former may be more injury-tolerant (Galloway & Davidson, 1993). In addition, waterlogging is associated with numerous secondary soil effects, many of which are deleterious to plant survival. Nitrifying bacteria cannot survive anoxic conditions, hence nitrate levels in the

root zone decrease to zero in approximately 15 days. Exudates of roots, decomposing microbiota and a new population of anaerobic microbiota contribute to further changes to soil chemistry. These include increased levels of carbon dioxide, ethylene, hydrogen sulfide, and methane, though these may take days (over 100 days in the case of methane) depending on temperature and soil constituents, to reach toxic levels (Setter & Belford, 1990).

1.8.2.2 Waterlogging tolerance mechanisms

Parenchymatous air-spaces (aerenchyma) are found in the roots of some plants subjected to waterlogging (Setter & Belford, 1990). These provide a continuous air-filled channel to the atmosphere, hence an uninterrupted supply of oxygen to the roots. Aerenchyma development may be increased by the onset of waterlogging, and for several species the extent of this development has been shown to be related to the plant's age and prior exposure to waterlogging. Older plants with existing aerenchyma often have a higher survival rate than younger plants which have yet to develop aerenchyma through exposure to waterlogging (Setter & Belford, 1990).

Adventitious roots above the zone of waterlogging occur in some tolerant species, though these have also been observed in plants with no tolerance of waterlogging (van der Moezel *et al.* 1989b; Setter & Belford 1990).

Saline soils are often waterlogged. Ionic and osmotic imbalance caused by the lack of oxygen is exacerbated by the abundance of salt ions (Marcar, 1993). Many halophytes are, however, also tolerant of waterlogging due to their halophytic physiology. Compartmentation of ions in the tissues moderates the need to regulate ion uptake, so an excess of both water and ions is perhaps of lesser significance to their growth strategies. More-tolerant species may therefore be simply those which are able to more efficiently compartmentalise salts. Inhabitants of saline/waterlogged environments are often found to be slow growers, indicating an ability to suspend or minimise growth (Setter & Belford, 1993).

Exclusion of Na^+ and Cl^- , together with adventitious aerenchymatous roots, has been suggested as contributing to the relative tolerance of *Casuarina obesa* to salt/waterlogging (van der Moezel, Watson, Pearce-Pinto & Bell, 1988). It must be noted, however, that the physiological effects of combined salinity and waterlogging are not well understood.

1.8.3 Alkalinity

A further effect of salinity which is often noted in Australian drylands is high soil pH, caused by high levels of intrinsic sodium. White encrusted sodic soils are often used to show the effects of unchecked salinity in agricultural journals. However, alkalinity need not be derived entirely from primary salt sources. Agricultural soil additives and industrial contaminants can increase pH through

direct or indirect input of excess hydroxides. Soil pH is a large and complex topic, hence will be dealt with here only in terms of its direct effects on plant growth.

The immediate effects of alkalinity are the changes to the ion-exchange processes in soil solutions. Iron, manganese, copper, zinc and other micronutrients can precipitate from the solution in high pH, making them unavailable for uptake by plant roots. Calcium can be replaced by sodium where high sodium concentrations occur, leading to a loss of root cell membrane integrity and further inhibition of micronutrient uptake. This will be exacerbated in soils which are further affected by salinity and waterlogging, in which osmotic stresses also contribute (Bell & van der Moezel, 1991). High pH can also have a caustic effect on plant tissue (Brady, 1990).

Plants can counter moderately high pH through their normal physiological activity (Brady, 1990). Uptake of cations and exudation of acidic substances into the rootzone can control alkalinity. However, if the pH is excessively high, normal plant activity is inhibited and the alkalinity can be compounded by further effects on a changing soil chemistry and the loss of rootzone microbiota which would normally moderate pH levels.

1.9 Conservation in Saline and Waterlogged Environments

1.9.1 Conservation and sustainable agriculture

It seems clear that soil salinity is a multi-faceted problem with no simple all-encompassing solution. Physical measures vary in their long-term effectiveness (McFarlane & Cox 1990; McFarlane, Negus & Ryder 1990), and can incur both direct costs in their implementation and indirect costs in their often unpredictable effects on the hydrological cycle (McFarlane, Engel, Ryder & Eales 1990; Barrett-Lennard & Qureshi 1994). Biological strategies show promise but are hindered from the outset by a poor correlation between halophytic abilities of plants and economic usefulness, and further by a lack of success to date in combining these qualities. Nationally, deep-rooted perennials have been widely planted, lowering elevated water tables and reducing surface salt. This practice has benefitted agriculture directly, and can be viewed as truly remediative since it returns land to a more natural condition.

Internationally, the most successful strategy has probably been the simplest one; to select halophytes from saline habitats and transplant them to otherwise economically valueless land elsewhere. This approach requires that the transplanted species can be incorporated into a region's trading economy, either directly or by reducing a dependence on imports, or has some value in conservation. An advantage in the short term is that salinity in that area becomes

a mere soil condition (which may even be most profitably maintained; through irrigation with seawater for example), but the long-term disadvantages are the new dependence on that particular species remaining economically appropriate, and the unchecked increase in salinity (Barrett-Lennard & Qureshi, 1994).

In Australia, dryland farming has been the major economic base, and salinity is largely secondarily derived from less than two centuries of European-style land-use. Thus though a problem has been created which can match those of less affluent countries, the practices are not culturally entrenched. For this reason, Australia is in the enviable position of being able to contain the spread of degraded land simply by educating landowners as to the causes. While individual farms and properties may be beyond immediate financial recovery, causing some alarm to the agriculture industry, the industry itself is not in apparent immediate danger. Attention can be partly directed therefore to conservation, an essential component of a nation's economic success (Freer, 1978). It is perhaps from these efforts in combination with more directly agriculture-based research that effective long-term solutions will be found.

1.9.2 Screening for salt and waterlogging tolerant woody species

There has been some relatively comprehensive testing of indigenous Australian woody species over the past decade (Marcar & Termaat 1990; Marcar 1993; Farrell *et al.* 1996a; 1996b). This has been essential to the agriculture industry due to the proven benefits of planting species with a high rate of water use in

strategic positions on salt-affected farmlands. A useful database of trees and shrubs able to succeed in severe environments has developed from these studies, and this database is of international interest given that Australian species have been successful transplants in the Middle East and elsewhere (Marcar & Termaat 1990; Marcar 1993; Barrett-Lennard & Qureshi 1994).

The usual protocol has been to identify those species which naturally inhabit severe environments, with special emphasis on salinity and waterlogging, and screen these in glasshouse conditions to identify stress tolerances of each plant type (Marcar & Termaat 1990; Bell & van der Moezel 1991; Farrell *et al.* 1996a; 1996b). Testing clonal lines within these candidate species ensures that genotypes can be selected for transplanting in the field with some confidence in their success (Farrell *et al.*, 1996a). The point is often raised however, that these screening methods are time-consuming, as they are constrained by the need to compare relative growth rates (RGRs) after a lengthy propagation period. A further restriction is that the greatest variation in stress tolerance usually occurs in those species with lower tolerances overall. This also suggests that genetic manipulation may ultimately be found to have an upper success limit; genotypic adaptation to osmotic stress may possess inherent limitations imposed by numerous interacting environmental factors.

1.9.3 The need for rapid screening

Plant physiologists have searched for characters in plants which signify their tolerance abilities in field conditions, in order to expedite screening (Aslam *et al.*, 1993). The most obvious potential character is morphological variation. Leaf thickness, root/shoot ratios, leaf expansion and xeromorphic features (Blake, 1981) are a few examples which have been shown to be inadequate pointers to tolerance abilities (Krishnaraj & Thorpe, 1996). Most have simply revealed normal intraspecific variation (Hester *et al.*, 1996), growth-stage variation (Ashraf, 1994), or adaptation to unknown (or unrelated) environmental factors (Shannon, 1985). Physiological variation can be used only where salt stress effects can be causally delineated from photosynthetic inhibition, which has proved difficult (Ayala & O'Leary, 1995), and tissue-ion relations show only tenuous taxonomic links to tolerance abilities (Greenway & Munns 1980; van der Moezel *et al.* 1989a; Morabito, Jolivet, Prat & Dizengremel 1996). It is important however, that physiological features considered singly or together, often indicate tolerance of a wide variety of stress types, and it is becoming increasingly evident that salinity tolerance may merely be one such stress (Yeo & Flowers, 1989). In other words, salt tolerance may be only one constitutive property of a widely-tolerant plant, although it should be noted that halophytic physiologies are not strongly associated with xeromorphic traits.

The limitations of tissue culture have been mentioned; these relate to the increasing awareness that stress tolerance is a whole-plant strategy which is rarely dependant on a wholly cellular response (Daines & Gould 1985; Tal 1985). Nevertheless, information on the cellular basis of tolerance continues to increase, and an increasing understanding of tolerance mechanisms will certainly lead to more expedient screening methods (Tal 1985; Flowers & Yeo 1995; Olmos & Hellin 1996).

Amino acids and carbohydrates have been observed to increase in quantity in photosynthetic tissues of plants exposed to osmotic stress. There is an ongoing debate over their place in osmotic adjustment, but Flowers and Yeo (1986) have presented evidence that some of these, notably proline, glycinebetaine and simple sugars, are present in sufficient quantity in water-stressed leaves of some species to significantly lower water potential (and stabilise cytosol/vacuole osmotic gradients in other species which accumulate ions). Further evidence that these substances are an osmotic stress response includes:

a) they are non-polar, uncharged (hence do not affect electrochemical gradients across tonoplast or plasmalemma while maintaining osmotic balance) and do not impair (in fact may help maintain [Hare & Cress, 1997]) cytosolic enzyme activity,

b) differences in proportions or absolute quantities of each of these substances are found between plant taxa, and show taxonomically-associated accumulation responses to different types of water stresses applied (eg, drought [Ali Dib, Monneveux, Acevedo & Nachit, 1994], antagonistic nutrient deficiency

[Al-Karaki, Clark & Sullivan, 1996], and salinity [Martinez, Maestri & Lani, 1996]), and

c) they are usually present in small quantities in unstressed plants, but can be caused to increase in quantity by either imposing an actual osmotic stress (drought, or high solute concentration 'physiological drought') (Ali Dib *et al.*, 1994), or imposing a simulated osmotic stress such as the application of abscisic acid (ABA) (Cachorro, Martinez, Ortiz & Cerda, 1995).

Evidence that increases in these substances are due to secondary influences of osmotic stress includes:

a) proportions and accumulation rates of each substance differ between genotypes, but it is the osmotic contribution of all substances together which is significant, rather than one substance (eg, proline) alone (Ali Dib *et al.*, 1994),

b) they are usually components of essential synthetic pathways; if such cycles are compromised, these substances can be produced in excess, perhaps preventing feedback inhibition of the end-product and thereby contributing to their own propagation, and

c) increases in concentrations of these substances are often temporary, and may lower a plant's water potential at the expense of other essential pathways (hence a potential benefit to the plant may be realised in neither the short-term nor the long-term).

Munns (1993) proposes that plant response to salinity is bi-phasic; an immediate and prolonged (weeks) reduction in growth due to water deficit which is often

reversible, followed by a period of necrosis and death (showing first in older leaves), which is due to the toxicity of absorbed salts. If this model is correct, rapid screening techniques would demonstrate the level of a plant's ability to tolerate osmotic stress, but not its ability to tolerate exposure of tissue to salt. Munns (1993) supports this model by referring to the relatively long periods required to separate salt-tolerance abilities of closely related species by observable differences in growth.

These considerations notwithstanding, there is certainly a case for utilising neutral organic osmotica as a marker of salt-tolerance ability. Wyn Jones & Storey (1978a; 1978b), in examining glycinebetaine and proline accumulation in water-stressed barley, provide support for the argument for their use as halophytic markers. They conclude that under culture conditions, ionic adjustment is the major osmotic response, but that increases in proline levels are quantitatively related to the stress levels applied. They also found that glycinebetaine levels usually exceeded those of proline within a subset of two families, Chenopodiaceae and Poaceae. Within this subset proline levels increased dramatically at the onset of osmotic shock, often to levels exceeding those of glycinebetaine, while the latter increased incrementally as stress increased. Beyond this subset however, this relationship was less apparent. Aspinall & Peleg (1981) provided evidence that proline levels increase with stress applied, but they could not support a quantitative link with halophytic ability. Sundaresan and Sudhakaran (1995) state that "convincing evidence in

support of ...(correlation of proline levels with osmotic stress tolerance abilities)... is lacking”.

Yet the assaying of proline levels remains an attractive approach. Correlation is apparently found within certain subsets of plant taxa, so this method may increase the efficiency of screening techniques, at least within restricted boundaries. This project examined proline levels at a range of salt concentrations in the roots and shoots of a grass with well-documented stress tolerances, to examine any quantitative relationship.

1.10 A Database of Salt Tolerant Herbaceous Species

Databases of plants with halophytic ability have been mentioned previously. These are the result of screening programs which, for Australian woody species at least, often take into account commonly-associated environmental factors such as waterlogging and pH extremes. Plants can be grouped according to both absolute and relative stress tolerances; a significant improvement on broad groupings as either halophyte or glycophyte. Creation of such databases has been undertaken on trees and shrubs due to their obvious value in both salt/waterlogging remediation and conservation. This review found no similar efforts toward construction of a database of tolerances of Australian herbaceous species.

Many quantitative studies on salt effects/tolerances have been undertaken on crop species (Dvorak, Noaman, Goyal & Gorham 1994; Krishnaraj & Thorpe 1996; Maiti, Amaya, Cardona, Dimas, De La Rosa-Ibarra & Castillo 1996), and coastal (Hester *et al.*, 1996) and arid-land grasses. The information gathered is usually utilised in physiological studies, and tolerances to a range of stresses are rarely recorded. Databases of crop and forage/pasture plant tolerances are available from North America, but generally exclude information on stresses other than salinity or drought, and are mainly useful only in a uniquely American agronomic context.

Grasses and ground-cover vegetation are often overlooked in the light of the immediacy of agricultural concerns. Yet herbaceous species are of proven importance in revegetation of industrial sites and chemically-contaminated soils in many types of applications (Freer, 1978). The Standing Committee on Agriculture (SCOA, 1993) strongly advocates an integration of economic and ecological research foci, with a view to long-term sustainability.

In Australia, introduced grasses have been widely used in revegetation programs, making use of their known stress-tolerance abilities. The African species *Urochlea mosambicus*, for example, has recently been shown to grow aggressively in the extreme environments of Queensland's Bowen Basin, where it dramatically reduces erosion around coalmine sites. It was chosen after extensive field-trialling of over 60 species. Legal requirements for revegetating disturbed areas are met by these approaches, but as mentioned, there is a growing

awareness of the desirability of indigenous Australian species in fulfilling these roles.

Tall wheatgrass *Agropyron elongatum* (introduced from Asia Minor in 1935 [Lamp, Forbes & Cade, 1990]; classification of tall wheatgrass from Hnatiuk [1990]), is well-established in Australia, following six decades of use in reclamation of saline/waterlogged and alkaline soils in many applications (Lamp *et al.*, 1990).

1.11 Experimental Aims

The environmental laboratory at Boddington Gold Mine (BGM) proposes to revegetate areas within the mine surrounds using herbaceous plant varieties. The soils there are adversely affected by mining activities, and are commonly saline, alkaline and subject to waterlogging. The company intends to stabilise soils, particularly where the original topsoil has been removed or disturbed, and improve the aesthetic qualities of the site. Soil improvement prior to revegetation has been considered by BGM. Strategies such as the application of gypsum or topsoil translocation were believed to be financially impractical. Revegetation programs at the site must therefore utilise stress-tolerant plant species.

Tall wheatgrass has been used successfully in Australia in revegetation of salt-crusted, compacted soils, duplex alkali soils and calcareous sands wherever water is plentiful. The species' hardiness and limitations are well known from anecdotal evidence, and from decades of field testing. BGM therefore proposed

its use in their revegetation program. The company considered that testing of the species' stress tolerances under controlled conditions was a prerequisite to revegetation efforts.

The company also proposed the testing of stress-tolerances of indigenous herbaceous species observed to grow naturally near minesites, since these were considered ideal candidate species from an ecological viewpoint. If these plants proved to be tolerant of adverse soil conditions to some degree, they could be utilised in place of, or in conjunction with, tall wheatgrass.

Data gathered on all species' tolerances to stress could contribute to a database of specific properties. Data relating to tall wheatgrass would be relevant to Australian soil conditions, and since the species' hardiness is already known, the data could be analysed to more fully characterise its halophytic physiology. Data relating to Australian plants would be useful both in comparison with tall wheatgrass, and in the current and future searches for plants able to be used in revegetation of degraded soils. Currently, most candidate herbaceous species are exotics for which stress-tolerance data is available. Addition of Australian species to lists of candidates would appear essential to conservation efforts.

This project aimed to provide stress-tolerance data on an introduced grass and indigenous herbaceous species proposed for use in revegetation of mine sites in southwestern Australia.

The project examined, under glasshouse conditions, the growth responses of *Agropyron elongatum*, and the Australian species *Podolepis gracilis* and *Danthonia caespitosa* to a range of rootzone NaCl concentrations, and to waterlogged conditions.

Screening of plants for tolerances to salinity has undergone an increase over the past decade, as land degradation becomes an increasingly immediate threat. Screening trials could be expedited if a physiological indicator of tolerances could be identified. Proline accumulation is one such potential marker, though plant physiologists are divided as to its worth in this regard. Analysis of proline accumulation in tall wheatgrass could provide evidence either for or against a relationship between levels of accumulation and stress applied.

This project examined proline accumulation in *A. elongatum* following exposure to a range of salinities.

CHAPTER 2. TALL WHEATGRASS *AGROPYRON ELONGATUM*

(POACEAE)

2.1 Introduction

Tall wheatgrass is most commonly called *Agropyron elongatum* in the literature. Its taxonomic treatment has, however, varied in the past according to a range of different criteria (Walsh & Entwisle, 1994). An examination of the various treatments of tall wheatgrass is not relevant here, but it is worthwhile to note some of those encountered in the literature (showing addenda where known): *Agropyron (agro-) pyronoides*, *Agropyron elongatum auctorum non* (Host) P. Beauv 1812, *Elymus elongatus* (Host) Runemark ssp. *ponticus* (Podpera) 1978, *Elytrigia pontica* (Podpera) Holub, *Lophopyrum ponticum*, *Thinopyrum ponticum*, *Thinopyrum elongatum*. In addition, there are a number of recognised cultivars that have been developed, including 'Largo', 'Alkar' and 'Tyrell'.

In its native habitat of southern Europe, Asia Minor and south-eastern Russia, the species grows near the coast and in saline marshes where flooding may occur (Walsh & Entwisle, 1994). It is a tussock-forming perennial which spreads by seeding, and may grow to 2m in height (Lamp *et al.*, 1990). Growth rate may be dependent on season, as it has been observed to suspend growth in Mediterranean winters. Growth in spring, summer and autumn is maintained provided there is sufficient moisture or a shallow water table. Flowering occurs

in summer; germination and early vigour is dependent on soil characteristics. The species appears to be relatively intolerant of drought (Walsh & Entwisle, 1994).

The species' salt tolerance has been measured in the past. Yeo & Flowers (1989), for example, state that a 50% yield reduction occurs at an electrical conductivity of 19.5dS/m; threshold conductivity (salt level at which growth is reduced) has been quantified at 7.5dS/M. Such figures are usually described as relative to those species with which it is being compared, and as being related to environmental conditions, soil type and propagation practices. Hence the species is variously described as being moderately salt-tolerant to tolerant.

The use of tall wheatgrass internationally as a forage species suitable for planting on saline land has been well-documented (Walsh & Entwisle, 1994). In Australia, this grass has been used for six decades to revegetate saline, waterlogged and alkaline soils, mainly in temperate zones, and has shown little evidence of any tendency to invade adjacent areas. Hence the species would appear ideal as both a pioneer species and, since it can become a dominant or co-dominant in suitable conditions, as an endpoint species in some situations.

Although relative salt tolerance figures are available from North America, where it is compared to local species in American environmental conditions, data relevant to Australia is scarce. Most information regarding the plant in this country is anecdotal and unquantified. Information on tolerance to waterlogging is largely derived from field sampling. Established plants appear to be relatively

unaffected by soil hypoxia. Information on germination and early growth in waterlogged conditions is lacking. Tolerance of the species to high soil pH is also determined largely from field observation.

Worsley Alumina, acting as manager for BGM, required quantified data on the ability of tall wheatgrass to revegetate mine surrounds, including mine residue heaps. Such revegetation would comply with government requirements for rehabilitation and aesthetic improvement. BGM required data relevant to soil conditions found at the mine, from screening trials in controlled conditions.

The current trials examined growth responses of tall wheatgrass in a range of external salt concentrations, waterlogging and high soil pH. The effects of waterlogging on growth were examined both separately and in conjunction with soil salt. The effects of high pH on growth were examined separately. Proline accumulation was measured following growth in a range of salt concentrations.

A range of different salt and waterlogging treatments were applied in the first experiment (Experiment 1.). Those considered to be less relevant were omitted from subsequent trials. In addition, results from several parameters in the first experiment were included in this report. These were useful in determining which were most relevant in analyses of plant responses to these conditions. Subsequent experiments present only those parameters considered most appropriate.

2.2 Materials and Methods

2.2.1.1 Plant material

Agropyron elongatum seed was from one seedlot (cultivar unknown), supplied by Boddington Gold Mine in March, 1997. Seed was germinated in 180mm pots for approximately six weeks, at which time their numbers were thinned to three or five plants of similar size per pot. Plants were watered daily, and fertilised (half strength Thrive™) every fourth day until treatments began. All germination and plant propagation was performed in a glasshouse, with temperatures ranging from 20 - 35° C.

2.2.1.2 Pots and soil

180mm pots were used in all experiments, with a single 10mm hole at the base which could be sealed using a rubber bung. The soil was a 1:1 ratio mixture of fine and coarse pasteurised white sand. Each pot contained 4kg of soil.

2.2.1.3 Saline solutions

Nutrient/salt solutions comprised NaCl in multiples of 1.461g/L for each 25 mM increase in concentration, half-strength Thrive™ fertiliser (0.889g/L) and Hoagland's solution strength Ca(NO₃)₂ (0.656g/L) (Hoagland, 1920) to avoid potential effects of sodicity, in deionised water. The control solution comprised

the same additives with the exception of NaCl. Control treatments are referred to as 0 salt, or 0 mM NaCl, but Thrive™ contains KNO₃ (9.0%), Na₂B₄O₇ (0.005%) and Na₂MoO₄ (0.002%).

Salt solutions are described in units of electrical conductivity (EC). Soil salt solutions are measured in deciSiemens per metre (dS/m), from extracts of saturated soil solution. In these salinity trials, treatments are described as concentrations of NaCl in millimoles per litre (mM). However, the nutrient solutions contained other ions, hence solution conductivities are given here as a general guide: control solution - 1.2dS/m, 25 mM - 3.9dS/m, 50 mM - 6.5dS/m, 100 mM - 11.5dS/m, 200 mM - 21.9dS/m, 300 mM - 30.5dS/m, 400 mM - 39.6dS/m. Conductivity was measured with an Orion™ Model 140 conductivity meter. The pH of control and salt solutions was 5.00 ± 0.02 at 24°C prior to application to pots.

2.2.2 Experiment 1. Salt and waterlogging

Each pot contained five plants of similar size, treatments comprised three pots (total 15 plants per treatment). Salt treatments began in mid-June, and consisted of control, 25 mM NaCl, 50 mM NaCl, 100 mM NaCl, and 200 mM NaCl solutions. Solutions were applied at daily increments of 25 mM, until the target concentration was reached. Sufficient solution was added to the pots until the conductivity of the solution entering was equal to that of the solution exiting the drainage hole.

Eight days after the treatments began, three pots treated with control solution, and three with 100 mM NaCl, were sealed with a rubber bung before flushing was complete such that the solution filled the pot to a depth of approximately 1cm over the soil surface. These were the 0 salt/100% waterlogged, and 100 mM salt/100% waterlogged treatments respectively.

Another three control pots and three 100 mM NaCl pots were sealed before flushing was complete. A 1mm hole was drilled in the pot halfway between the base and the soil surface, such that waterlogging occurred in the lower half of the soil column. These were the 0 salt/50% waterlogged and 100 mM NaCl/50% waterlogged treatments respectively.

The experiment therefore comprised a control treatment, four salt treatments (25 mM, 50 mM, 100 mM and 200 mM NaCl), two non-saline/waterlogged treatments (50% and 100% waterlogged) and two saline/waterlogged treatments (100 mM NaCl/50% waterlogged and 100 mM NaCl/100% waterlogged). Pots were well-spaced and randomly rearranged daily. Waterlogged (100%) pots were watered using deionised water, to 1cm above the soil surface. Waterlogged (50%) pots were watered by weight with deionised water. All other pots were maintained at field capacity by watering to weight daily with deionised water.

Plants were harvested 50 days after the treatments began. They were separated into roots and shoots and their fresh weights measured. They were dried at 70^o C for six days then their dry weights measured.

2.2.3 Experiment 2. High salinity

Each pot contained three plants of similar size. Each treatment comprised four pots (total 12 plants per treatment), though only three pots (total 9 plants per treatment) were used in the salinity trial, with the extra single pot per treatment being used in analysis of proline accumulation. The experiment began in early November. Treatments were control, 100 mM NaCl, 200 mM NaCl, 300 mM NaCl and 400 mM NaCl. Solutions were applied in daily increments of 25 mM as in Experiment 1., such that the final concentration (400 mM) was reached on day 16. Pots were sealed with a rubber bung on day 17, and watered by weight daily, using deionised water. Pots were well spaced and randomly rearranged each day. Plants from all pots were harvested 44 days after treatments began, separated into roots and shoots and their fresh weights measured. Three pots from each treatment were dried at 70^o C for six days, and their dry weights measured. Plants from the remaining pots were analysed for proline content.

2.2.3.1 Proline analysis

Samples of leaf material were taken from those pots set aside for proline analysis in Experiment 2. One pot was chosen at random from the four pots in each treatment. Replication was four samples from a single plant in each of the five pots. Proline levels were analysed using the procedure of Troll & Lindsley (1954), modified by Bates, Waldren and Teare (1973).

2.2.4 Experiment 3. Alkalinity

2.2.4.1 Alkaline treatments

Nutrient solutions were made up, comprising half-strength Thrive™ fertiliser in deionised water. The pH of the solutions was adjusted to the appropriate level by adding 1M NaOH.

Pots contained three plants of similar size, five pots per treatment (total 15 plants per treatment). Treatments were pH 6.0, 7.0, 8.0, 9.0 and 10.0 (all values ± 0.1). The experiment began in early November. Attempts were made to equilibrate soils with the solutions by flushing in a similar manner to that performed in the salinity experiments. These were unsuccessful, the pots returning to a lower pH within a few hours of each application. It was decided that pots should be sealed and watered with pH-adjusted solutions for the duration of the experiment, similar to the method of Farrell *et al.* (1996b). Plants were watered to field capacity by weight daily, using the appropriate pH-adjusted nutrient solutions. Pots were well-spaced and randomly rearranged daily. The plants were harvested 29 days after treatments began. The pH of the soil solution in each pot was measured during harvesting. The plants were separated into roots and shoots and their fresh weights measured. They were dried at 70° C for six days, then their dry weights measured.

2.2.5 Statistical analysis

Statistical analysis was performed using SPSS for Windows Version 6.0. All data subsets were tested for normality, and transformed using natural logarithms where appropriate. Analysis of variance (ANOVA) was performed to test the validity of replication within data sets. Two-way ANOVA was performed on experiments where two factors were tested, and where significant ($P \leq 0.05$) effects were noted, a one-way ANOVA was used to test for significant differences (probabilities are presented for each parameter in parentheses) between treatments; post hoc analysis comprised Tukey's honestly significant difference multiple range test (Tukey's hsd) at a significance level of 0.05.

2.3 Results

2.3.1 Experiment 1. Salt and waterlogging

2.3.1.1 Salinity trials

In most parameters, growth in low salt concentrations was greater than in non-saline conditions, but decreased at progressively higher concentrations such that growth was reduced in 200 mM NaCl relative to the control. The decrease in growth at that concentration was significant in some parameters. Waterlogging reduced root growth, but did not affect growth of shoots or whole-plants, hence

waterlogging trial results are presented separately in Section 2.3.1.2. Survival rate was 100%.

Mean fresh weights of shoots (Figure 2.1a) differed from that of the control ($P=0.0010$). Lowest shoot growth occurred in 200 mM NaCl. Mean fresh weights of roots (Figure 2.1b) in 25 mM and 50 mM NaCl were significantly higher than those in the control ($P=0.0000$). Mean whole-plant fresh weights (Figure 2.1c) reflect the component results, clearly showing an initial increase in growth at lower salt concentrations, followed by a progressive decrease in growth in increasing concentrations ($P=0.0000$). Mean fresh weights of whole plants were lowest in 200 mM NaCl.

Mean shoot dry weights (Figure 2.2a) showed a similar trend to shoot fresh weights ($P=0.0045$). Salt concentrations to 100 mM had no significant effect, but a significant decrease occurred in 200 mM NaCl. Growth increases were shown more markedly by dry weights of roots (Figure 2.2b; $P=0.0000$). Mean whole-plant dry weights (Figure 2.2c) showed results similar to those obtained for fresh weights, except that growth was significantly lower in 200 mM NaCl relative to the control ($P=0.0000$).

Significant decreases in relative growth rates (RGR), measured as a percentage of mean control dry weights, were apparent in 200 mM NaCl for shoots (Figure 2.3a; $P=0.0045$) and roots (Figure 2.3b; $P=0.0000$). Mean RGRs of shoots in saline treatments to 100mM were comparable to that of the control, while roots

showed a marked increase in growth in those treatments. Relative growth rates of whole plants (Figure 2.3c) illustrate the initial increase at low concentrations, and progressive decrease thereafter, seen in both fresh and dry weight parameters ($P=0.0002$). A marked decrease in RGR in 200 mM NaCl is apparent from the chart, but is not significantly lower than the control.

Fresh and dry weight parameters suggested that whole-plant growth measurements were more influenced by root weights than those of the shoots. Mean shoot/root ratios, determined from dry weights (Figure 2.4) show the relationship more clearly ($P=0.0000$). Saline treatments to 100 mM caused an increase in root growth relative to shoots. In 0 salt and 200 mM NaCl, root growth was markedly lower in comparison. The mean shoot/root ratio of almost 1:1, seen in the 50 mM treatment, was significantly lower than that of the control.

Of the salt-only treatments, percentage water content (%WC) of the shoots was significantly lower in the control than all other treatments (Table 1; $P=0.0000$). Percentage WC of roots in the 200 mM treatment was significantly higher ($P=0.0500$). In whole plants, %WC was significantly lower in the control than in other treatments ($P=0.0003$).

There was little leaf death observed in any treatment. Chlorosis was confined to a small proportion of leaves of some plants in the control treatments. No leaf-roll was observed in any treatment.

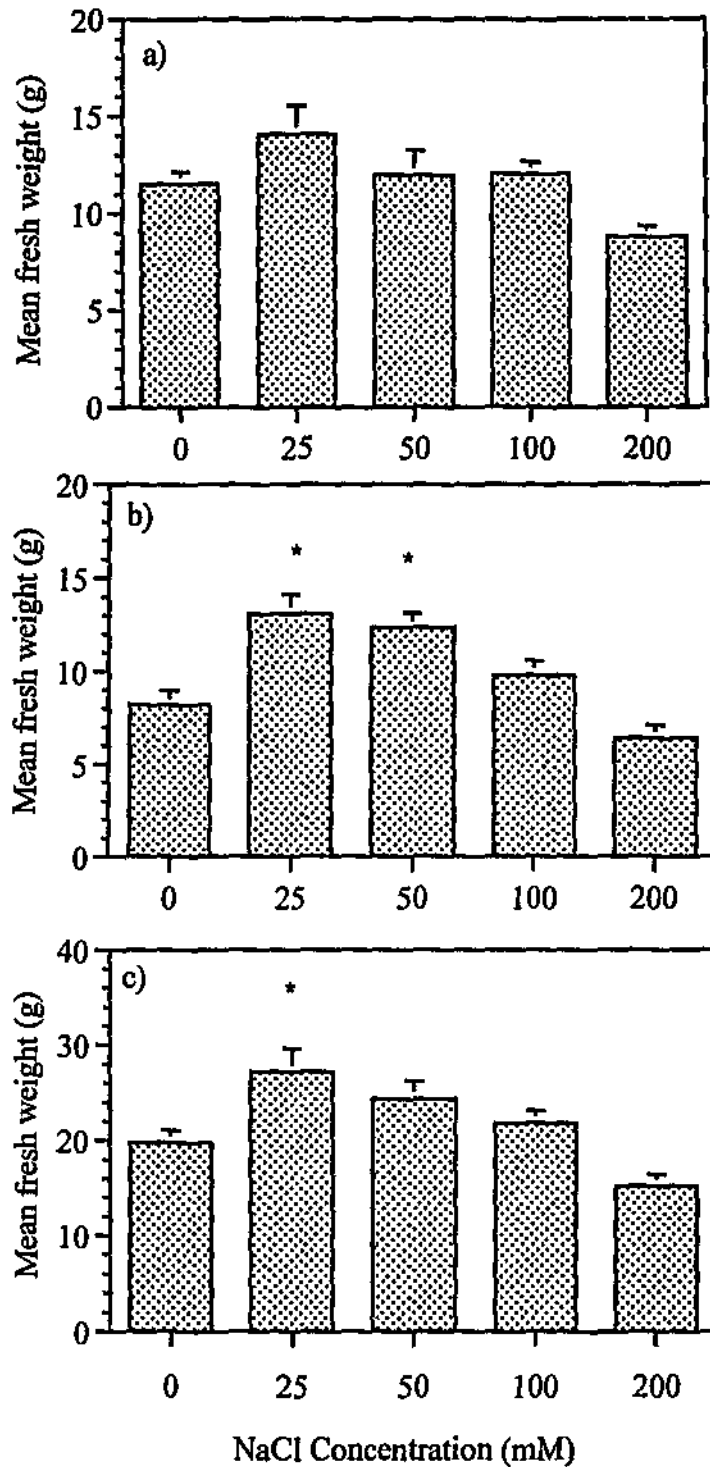


Figure 2.1. Mean fresh weights of *A. elongatum* a) shoots, b) roots and c) whole plants grown in NaCl concentrations from 0 mM (control) to 200 mM for 50 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $h_{sd_{0.05}}$) of 15 replicates compared to control.

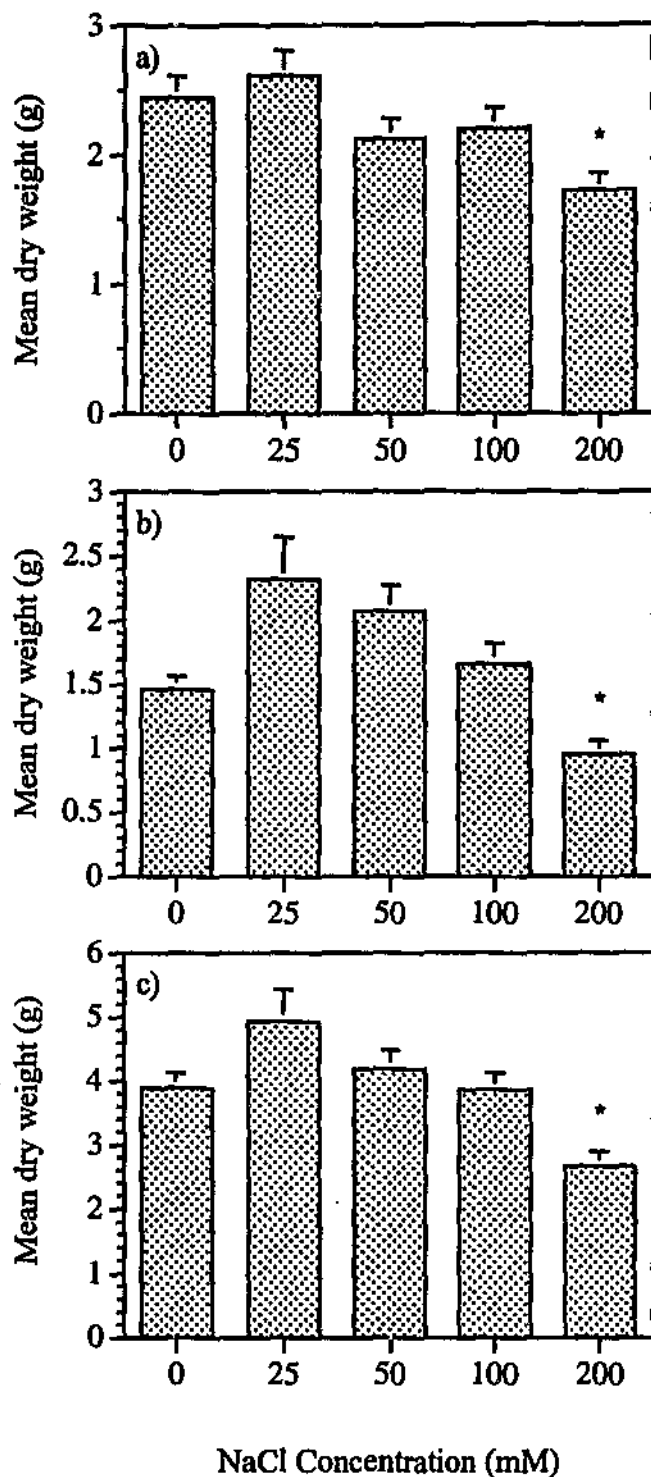


Figure 2.2. Mean dry weights of *A. elongatum* a) shoots, b) roots and c) whole plants grown in NaCl concentrations of 0 mM (control) to 200 mM for 50 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $hsd_{0.05}$) of 15 replicates compared to control.

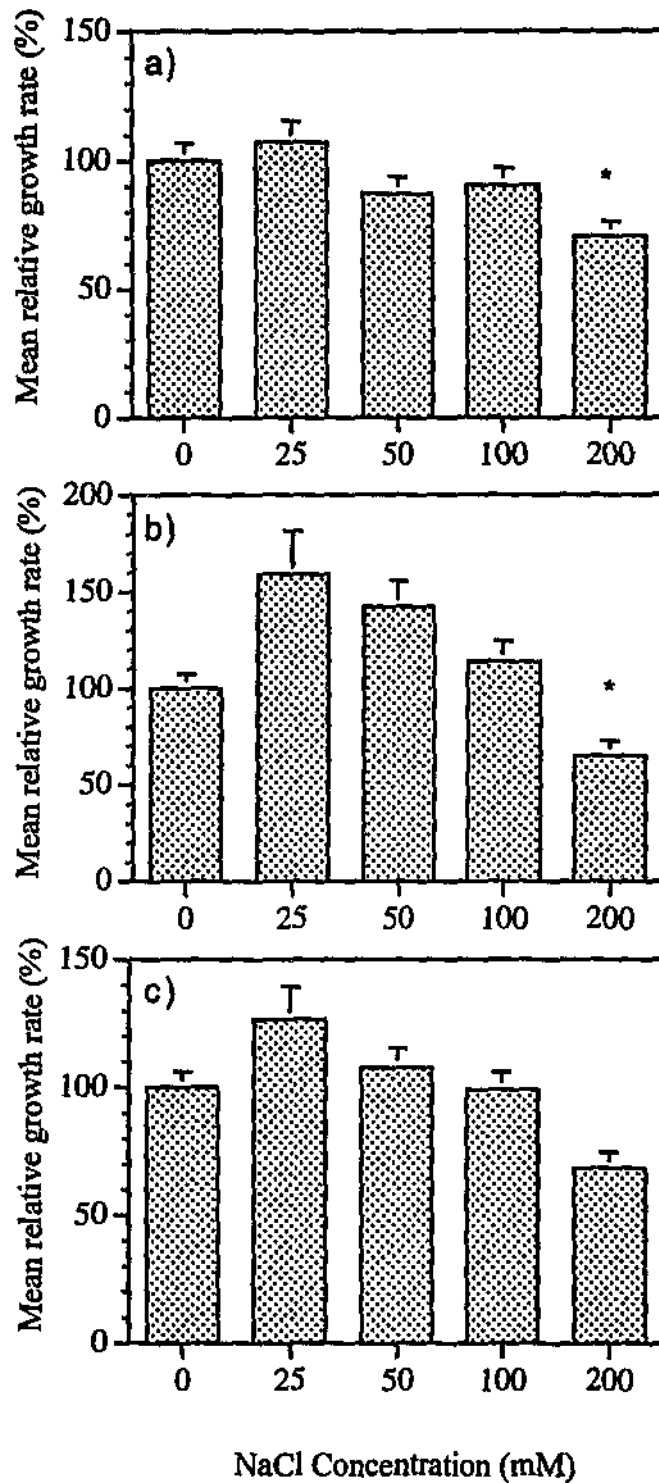


Figure 2.3. Mean relative growth rates, determined from dry weights of *A. elongatum* a) shoots, b) roots and c) whole plants, grown in NaCl concentrations of 0 mM (control) to 200 mM for 50 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $hsd_{0.05}$) of 15 replicates compared to control.

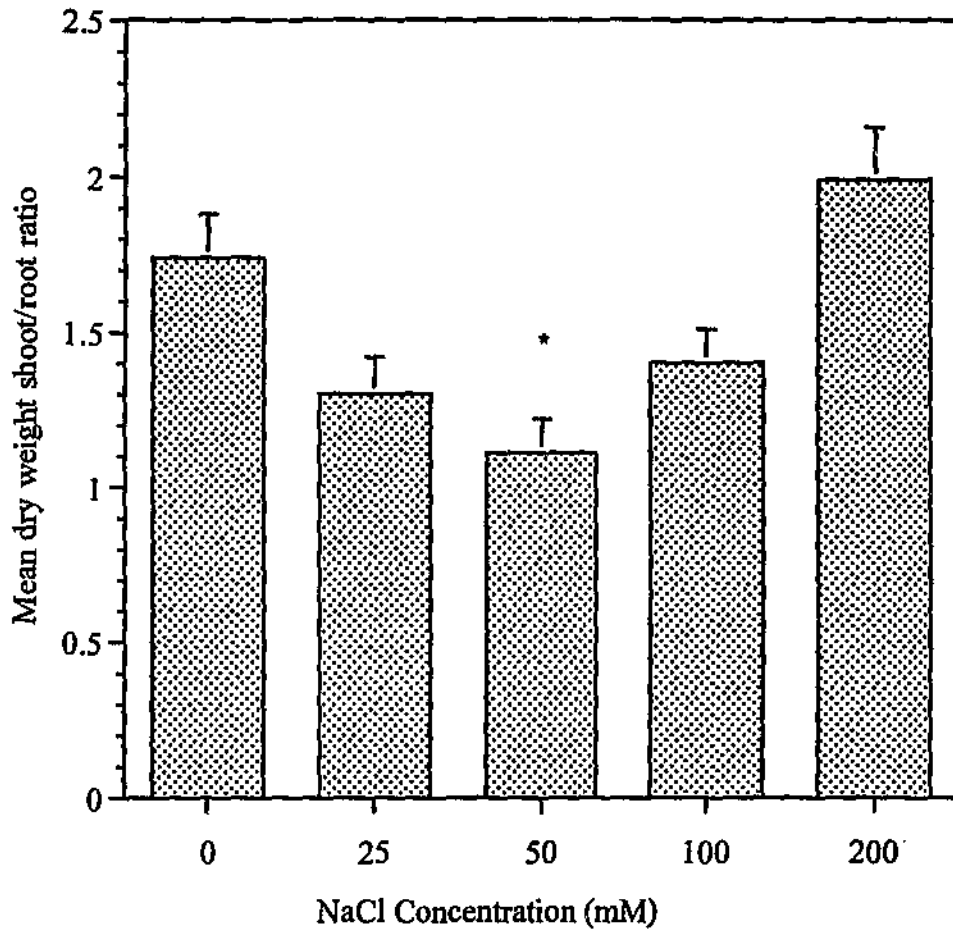


Figure 2.4. Mean shoot:root ratios calculated from dry weights of *A. elongatum* grown in NaCl concentrations of 0 mM (control) to 200 mM for 50 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $hsd_{0.05}$) of 15 replicates compared to control.

	%WC shoot	%WC root	%WC total
Control	79	82	80
25 mM	81*	83	82*
50 mM	82*	83	83*
100 mM	82*	83	82*
200 mM	80*	85*	82*

Table 2.1: Mean percentage water content (%WC) of *A. elongatum* shoots, roots and whole-plants grown in NaCl concentrations from 0 mM (control) to 200 mM for 50 days. (*) represents difference in means (Tukey's $hsd_{0.05}$) of 15 replicates compared to control in the same column.

2.3.1.2 Salt and waterlogging

Growth of whole plants was unaffected by waterlogging. However, root growth was reduced by complete waterlogging, shown by the significantly higher dry weight shoot/root ratios in these treatments (Figure 2.5) compared to the control ($P=0.0000$). 50% waterlogging did not affect growth of roots or shoots.

Mean shoot relative growth rates, determined from dry weights (Figure 2.6a), were comparable to that of the control in all waterlogged treatments ($P=0.1470$). Mean root RGR (Figure 2.6b) in the 0 mM NaCl/50%WL and 100 mM NaCl/50%WL treatments, was higher than that of the control, while markedly lower in both 100%WL treatments ($P=0.0000$). Relative growth rates of whole plants in all treatments were comparable to that of the control (Fig. 2.6c; $P=0.498$).

Percentage water content of plants in waterlogging treatments is not presented, in order to simplify analysis of the salt/%WC relationship.

Eighty percent of plants in 0 salt/100%WL, and 26.7% of plants in 100 mM NaCl/100%WL possessed upwardly-oriented roots, originating from the crown. These varied between 10 - 40mm in length, and varied in number between plants from 2 to 20 (the latter occurring in one plant in the saline/WL treatment). They were not observed on plants in 50%WL treatments, or in salt-only treatments.

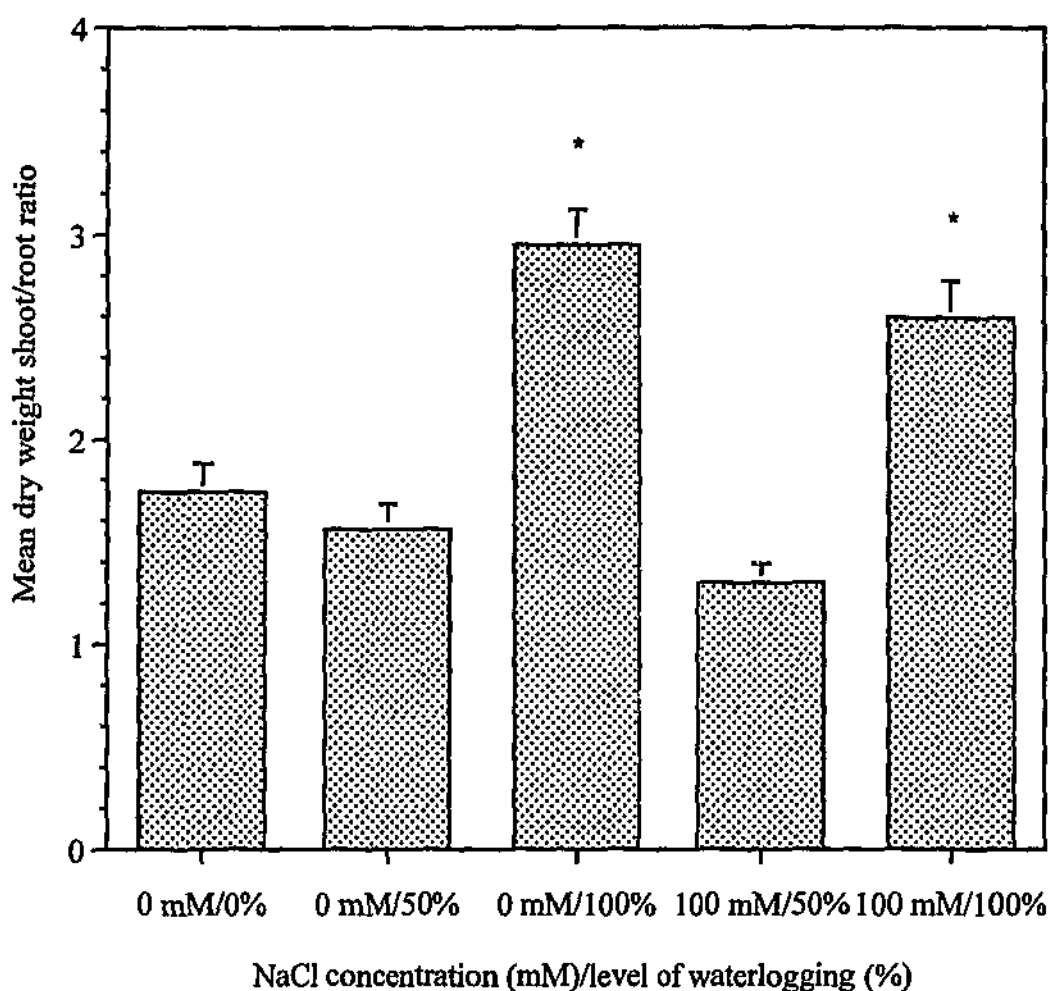


Figure 2.5. Mean shoot:root ratios calculated from dry weights of *A. elongatum* grown in 0 mM NaCl (control), 0 mM NaCl/waterlogged to 50% and 100% levels in 180mm pots and 100 mM NaCl/waterlogged to 50% and 100% levels in 180mm pots for 50 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $h_{sd_{0.05}}$) of 15 replicates compared to control.

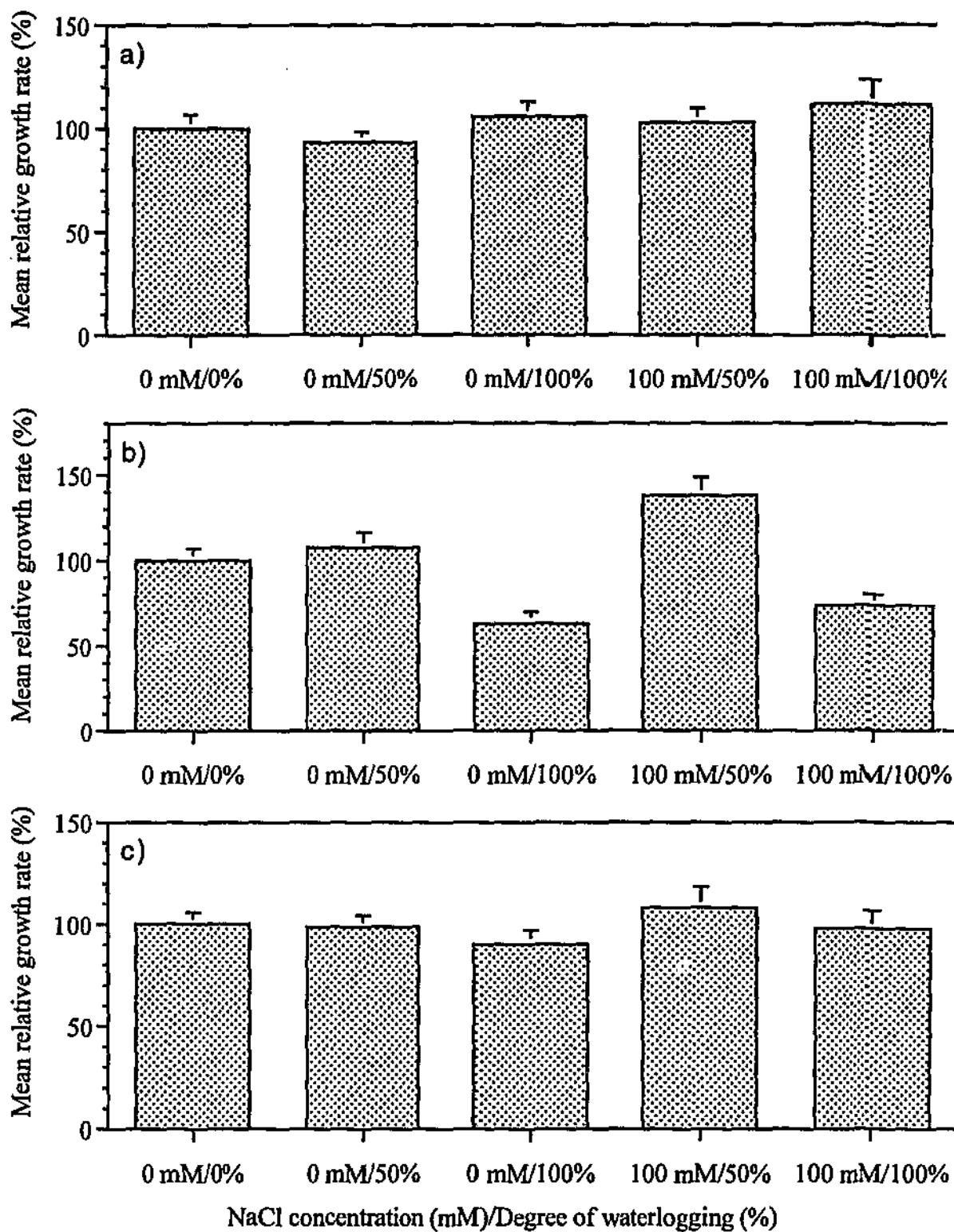


Figure 2.6. Mean relative growth rates (%) calculated from dry weights of *A. elongatum* a) shoots, b) roots and c) whole plants, grown in 0 mM NaCl (control), 0 mM NaCl/waterlogged to 50% and 100% levels in 180mm pots and 100 mM NaCl/waterlogged to 50% and 100% levels in 180mm pots for 50 days. Vertical bars represent standard errors of the mean.

2.3.2.1 Experiment 2. High salinity

Fresh and dry weights were higher in this experiment than in the corresponding treatments (control, 100 mM and 200 mM NaCl) of Experiment 1. Growth response trends, however, were similar. In most parameters, growth decreased in progressively higher salt concentrations, such that a significant decrease relative to the control was first observed at 200 mM NaCl. Growth decrease from 100 mM to 400 mM NaCl was near-linear. Growth in the control and 100 mM treatments was comparable.

Mean shoot dry weights (Figure 2.7a) showed an almost linear decrease at increasing salt concentrations, the 200 mM, 300 mM and 400 mM treatment means differing significantly from the control ($P=0.0000$). Mean dry weights of roots (Figure 2.7b) showed a similar decrease with increasing salt concentrations, though that in the 100 mM NaCl treatment was higher than the control ($P=0.0000$). The net result was a near-linear decrease in whole-plant dry weights (Figure 2.7c) at increasing salt concentrations, and significant differences between the control mean and that of the 200 mM, 300 mM and 400 mM treatments ($P=0.0000$).

There was little difference between 100 mM NaCl and the control in mean RGR of whole plants, calculated from fresh weights, although relative growth rates decreased markedly at higher concentrations, with significant decreases in the 300 mM and 400 mM NaCl treatments (Figure 2.8a; $P=0.0000$). Relative growth

rate determined from dry weights (Figure 2.8b) showed a similar trend, except that the 200 mM treatment mean also differed significantly from that of the control ($P=0.0000$).

Percentage WC of the roots did not differ significantly between treatments (Table 2; $P=0.3086$). However, %WC of the shoots was lowest in the control, and significantly higher in the 100 mM, 200 mM and 300 mM treatments ($P=0.0000$). In whole plants, %WC was lowest in the control, and significantly higher in the 200 mM treatment ($P=0.0055$).

Shoot growth relative to the root, calculated from dry weights (Figure 2.9), was significantly higher in the 400 mM treatment than in the control and lower in the 100 mM treatment ($P=0.0000$). The trend was an increase in shoot/root ratio at increasing salt concentrations from 100 mM NaCl. Ratios in the control, 100 mM and 200 mM treatments were similar to those recorded in the corresponding treatments in Experiment 1.

Control plants were paler in colour than those of other treatments, though not chlorotic. Leaf death was minimal in all treatments. Most affected were the 300 mM and 400 mM treatments, both containing an estimated 5% dead leaves or leaf tip death in older leaves. No leaf roll was observed in any treatment.

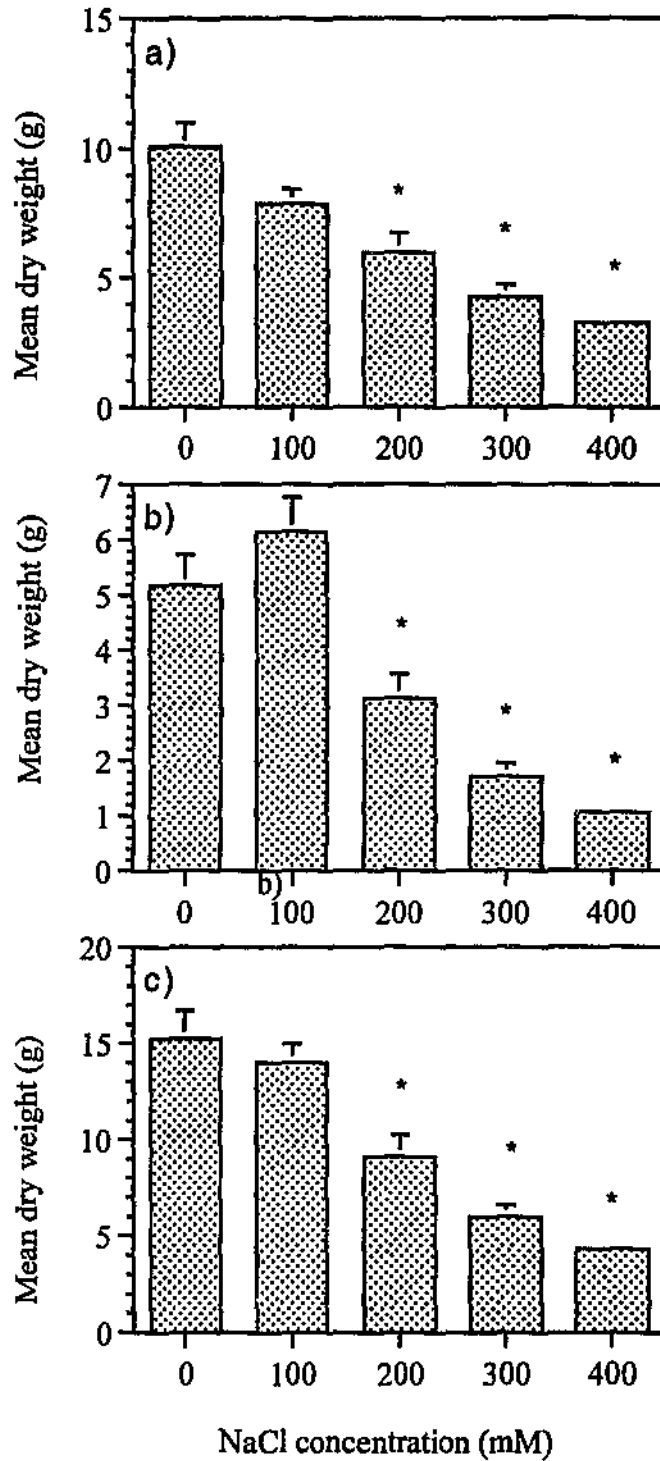


Figure 2.7. Mean dry weights (g) of *A. elongatum* a) shoots, b) roots and c) whole plants grown in NaCl concentrations of 0 mM (control) to 400 mM for 28 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $hsd_{0.05}$) of 9 replicates compared to control.

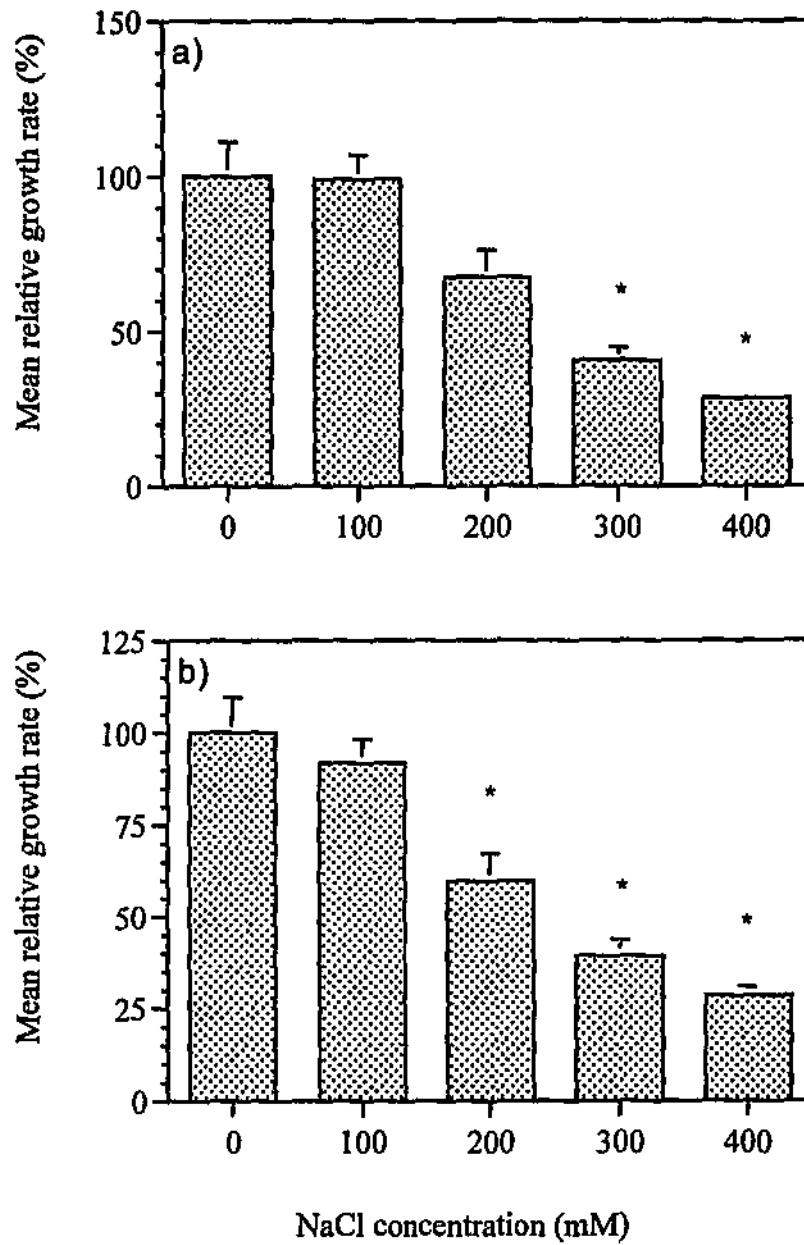


Figure 2.8. Mean relative growth rates (%) of *A. elongatum* calculated from a) fresh weights and b) dry weights following growth in NaCl concentrations of 0 mM (control) to 400 mM for 28 days. Vertical bars represent standard errors of the mean. (*) represents differences in means (Tukey's $hsd_{0.05}$) of 9 replicates compared to control.

	%WC SHOOT	%WC ROOT	%WC TOTAL
control	75	78	77
100 mM	80*	76	78
200 mM	79*	78	79*
300 mM	78*	75	78
400 mM	77	76	77

Table 2.2. Mean percentage water content (%WC) of *A. elongatum* shoots, roots and whole-plants grown in NaCl concentrations from 0 mM to 400 mM for 28 days. (*) represents difference in means (Tukey's $hsd_{0.05}$) of 9 replicates compared to control in the same column).

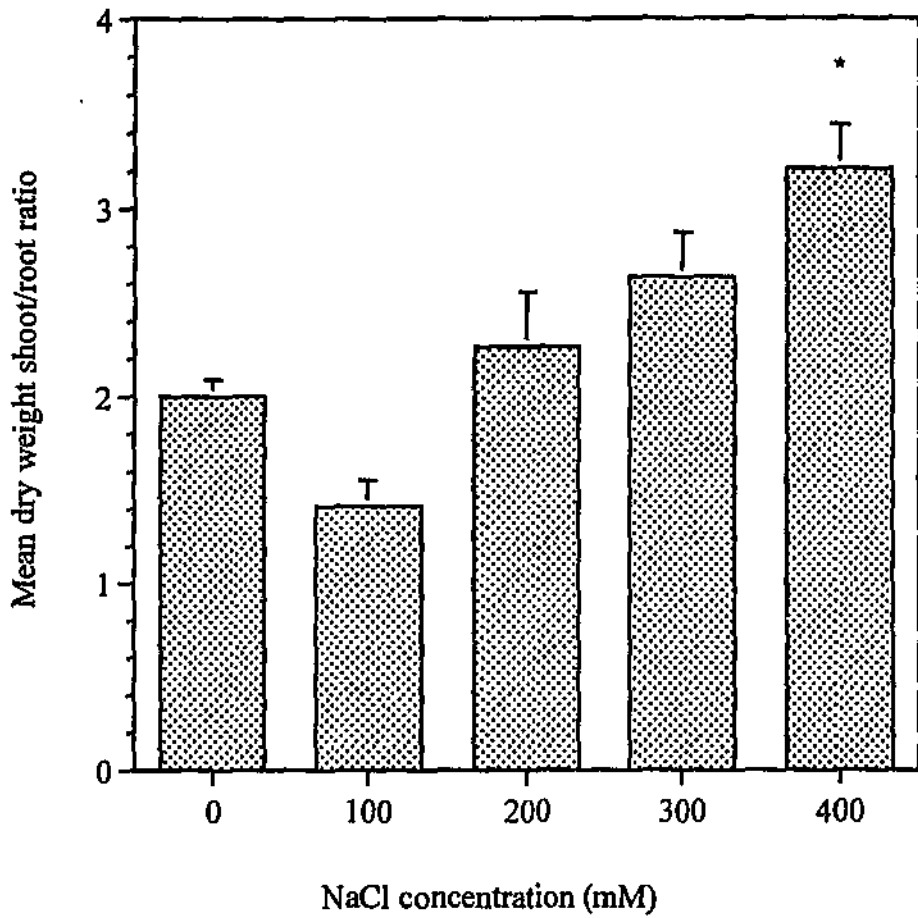


Figure 2.9. Mean shoot:root ratios, calculated from dry weights, of *A. elongatum* grown in NaCl concentrations of 0 mM (control) to 400 mM for 28 days. Vertical bars represent standard errors of the mean. (*) represents difference in means (Tukey's $h_{sd_{0.05}}$) of 9 replicates compared to control.

2.3.2.2 Proline accumulation

Increased proline in higher salt concentrations was dramatic (Figure 2.10). Accumulation of proline in the shoots increased markedly in salt concentrations of 200 mM, compared to both the control and 100 mM treatments ($P=0.0000$). Proline levels in these latter treatments were both relatively low. There was a further steep increase in mean shoot proline levels at higher salt concentrations. Mean root proline levels were approximately half those of corresponding shoots, except in 200 mM NaCl, where levels were similar, but showed the same trends in accumulation levels ($P=0.0000$). Within each treatment, mean shoot and root proline levels were comparable ($P=0.0632$).

2.3.3 Experiment 3. Alkalinity

Plants in all treatments except those in three of the four pots in pH 8 showed leaf-rolling and leaf death within 14 days of the commencement of the experiment. At day 20, leaf rolling was observed in a second pot at pH 8, leaving only two pots which contained healthy plants. Leaf rolling varied between pots from an estimated 40 to 100%, with no apparent relationship between the extent of this response and the treatment applied. Water use in these treatments, determined during watering, was estimated at approximately one tenth that of the two unaffected pots. Water use by plants in all pots at pH 10 and one pot at pH 9 increased in the week before harvesting. Black soil fungus and a blue fungal

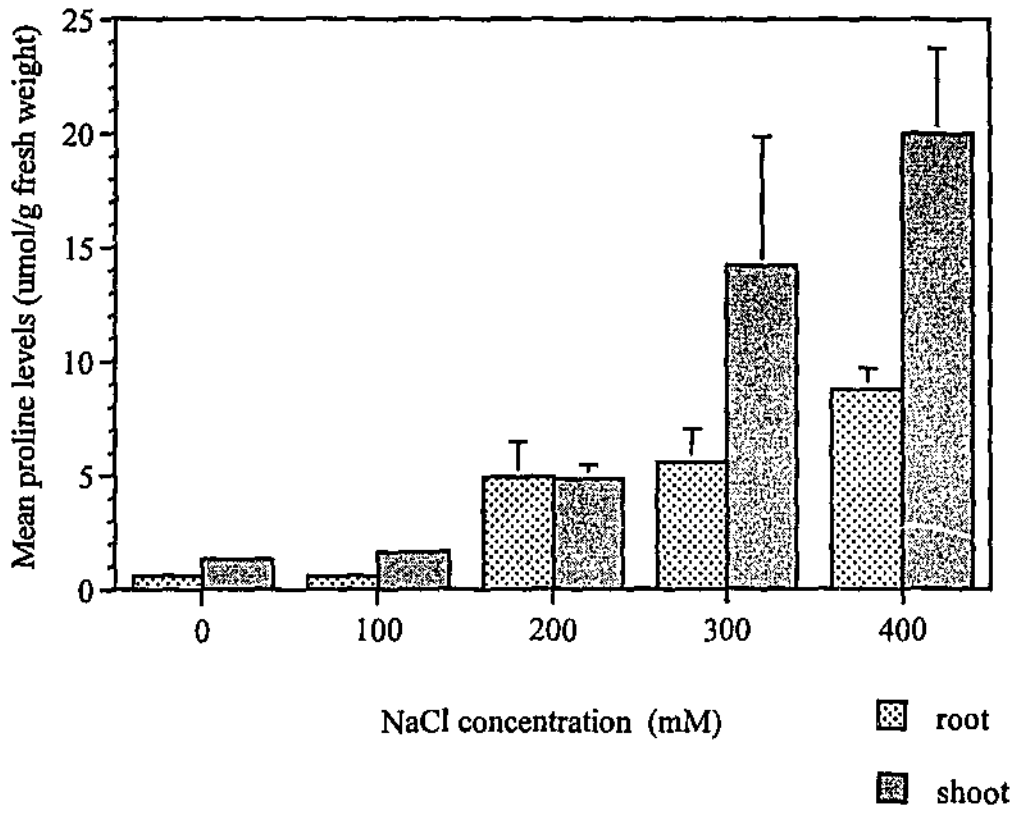


Figure 2.10. Mean proline levels ($\mu\text{mol/g}$ fresh weight) in shoots and roots of *A. elongatum* after growing in NaCl concentrations of 0 mM (control) to 400 mM for 28 days. Vertical bars represent standard errors of the mean.

growth at the plant crowns were observed in all pots except those containing apparently healthy plants at pH 8.

Roots in affected pots were damaged and deformed, though a small amount of apparently healthy root tissue was observed in most of these plants.

The pH of the soil solution of all affected pots was 3.5 ± 0.5 ; pH in the two unaffected pots was 7.8 ± 0.1 .

ANOVA revealed replication by individual plants was not valid in any data subset ($P > 0.05$), hence growth responses could not be statistically analysed. However, mean dry weights of shoots (Figure 2.11a), roots (Figure 2.11b) and whole-plants (Figure 2.11c) are presented.

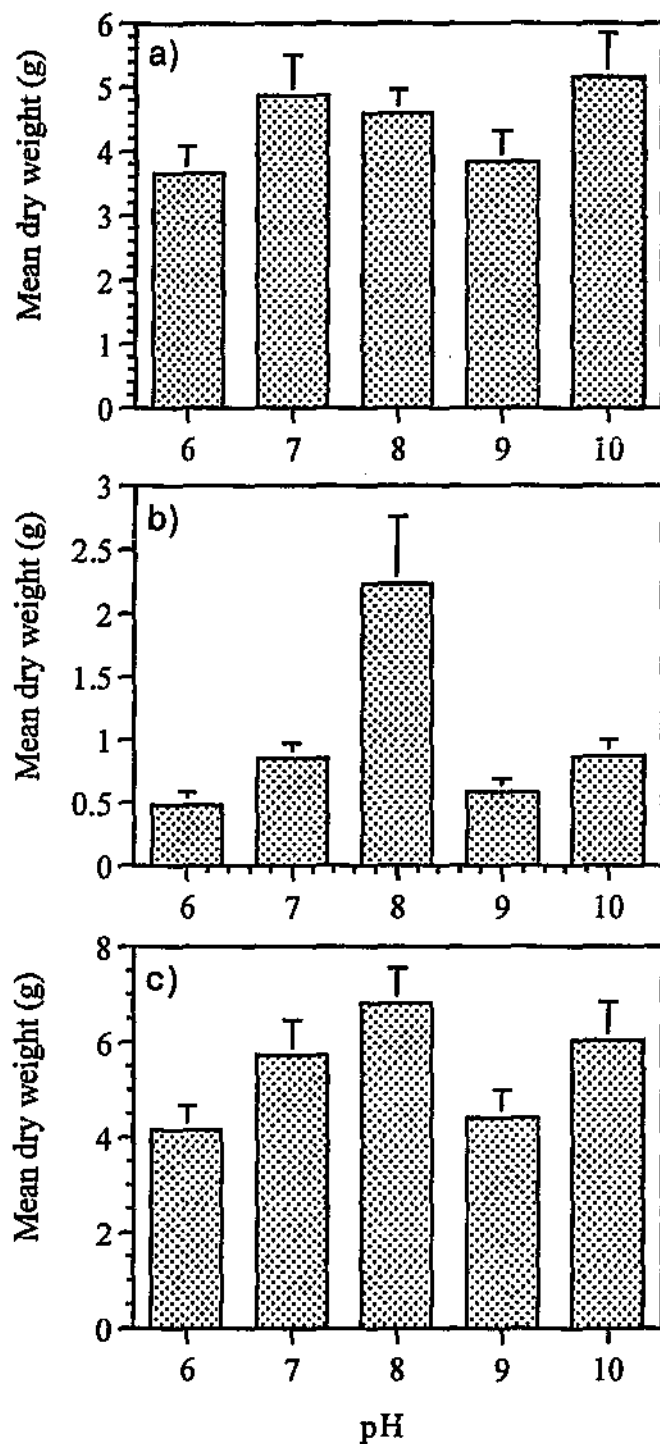


Figure 2.11. Mean dry weights (g) of *A. elongatum* a) shoots, b) roots and c) whole plants, following growth for 29 days in nutrient solutions adjusted to a range of pH values from 6 to 10. Vertical bars represent standard errors of the mean.

2.4. Discussion

2.4.1.1 Salt and waterlogging

These trials have confirmed the high tolerance of tall wheatgrass to salt and waterlogging. Growth rate was shown to be dependent on salt concentration and independent of any level of waterlogging. Tissue death was minimal across the range of NaCl concentrations from 0 to 400 mM. Root growth decreased at higher salt concentrations, but root tissue showed no apparent damage. Shoot growth showed similar trends in growth responses, but to a lesser degree, providing a compensatory effect on total yields.

Of most interest was the apparent euhalophytic curve shown by the results. There was an initial increase in growth at lower concentrations, and a progressive decline in growth as concentrations increased, such that growth in 200 mM NaCl was significantly reduced.

Increased growth in low salt concentrations is not usually associated with grasses. As for several other monocotyledonous plants tested, it has been proposed that grasses avoid Na⁺ toxicity by limiting its uptake. This is usually associated with osmotic adjustment through a decrease in tissue water, and a subsequent decrease in turgor and growth. These responses are seen consistently in tests on monocots, leading to the proposal that a monocot 'physiotype' exists. This experiment could not support that hypothesis.

Decrease in growth was not strongly associated with tissue damage, though leaf death was slightly higher in the 300 mM and 400 mM treatments (data not presented). Thus the growth decrease is typical of salt-including halophytes, and probably represents a progressive increase in energy expenditure at the roots as ion uptake is increased (Flowers *et al.*, 1977).

A rise in percentage water content of the leaves in all salt concentrations to 300 mM possibly represents compensation for a decrease in osmotic potential in the protoplasm, though this should not be confused with the tendency toward succulence shown by many dicotyledonous species (Glenn & O'Leary, 1984). As mentioned, a decrease in water content would be expected in a grass species. Plants in 400 mM NaCl did not show a significant increase in water content.

In agreeance with Yeo & Flowers (1989), yield was reduced in Experiment 1. to almost 50% in 200 mM NaCl (EC ~ 19.5d/Sm) relative to the highest yield (obtained in 25 mM NaCl), and to approximately 60% of the highest yield (obtained in the control) in Experiment 2. Growth at higher salt concentrations was higher than that given for a hypothetically typical monocot halophyte (Greenway, 1973), but within a reasonable error range.

The most noticeable effect of waterlogging was to increase shoot/root ratios while only marginally affecting total yield. Shoot growth was therefore compensatory to growth of the whole plant. The aerial roots observed on some

plants may account for tolerance to waterlogging in both these plants and others within the same pots, although adventitious root growth at the soil surface is also observed in flood-affected plant species with no tolerance to waterlogging (Setter & Belford, 1990). Slow growth, allowing time for development of aerenchyma within existing roots is perhaps a more likely strategy in tall wheatgrass, particularly given that this response is well documented in woody species (van der Moezel *et al.*, 1989b) and in the more closely-related crop grasses (Setter & Belford, 1990).

2.4.1.2 Proline analysis

A clear relationship was shown by the increased proline levels in plants at higher salt concentrations. The initial significant increase at 200 mM NaCl supports a link between proline accumulation and a response to stress (Hare & Cress, 1997), since significant growth decreases occurred at this concentration in Experiments 1. and 2. This, and the further significant increases in proline at 300 mM and 400 mM NaCl, is evidence that proline levels in tall wheatgrass are quantitatively related to the degree of osmotic stress.

2.4.2 Alkalinity

Results from this experiment were included in this report, despite the effects of fungal infection, for two reasons. Firstly, all plants except those in two pots at pH 8 exhibited leaf rolling and severe root growth reduction, yet mean whole-

plant weights appeared to be within a range expected of healthy plants. Secondly, soil solution pH of pots unaffected by fungus was similar to the treatment solution, while soil solution of affected pots was strongly acidic.

The presence of a small proportion of undamaged roots may both account for the later increase in water use shown by some plants in the pH 9 and pH 10 treatments, and tentatively rule out a sustained effect of fungus on growth.

The flushing of pots prior to the commencement of the experiment may have damaged root tissue, providing opportunity for fungal infection and subsequent deformation of roots (pH of control and saline solutions in Experiments 1. and 2. was close to 5, and caused no discernable root damage).

It must be concluded that fine analysis of growth responses to pH would be inappropriate in this instance, but that soil reaction up to pH 10 appeared unlikely to have affected plant survival. The experiment was also probably useful in clarifying appropriate methods of adjusting pH in a confined rootzone.

2.4.3 Procedural limitations and further development

A comparison of the results of Experiments 1. and 2. shows that absolute figures cannot be integrated into a single range of relative growth rates in salt concentrations up to 400 mM. Dry weights were considerably lower in Experiment 1. compared to those in the corresponding treatments in Experiment

2. The former comprised treatments with five plants per pot. Competition for light and nutrients was presumably more intense in this experiment. There exists also the possibility that nutrient uptake by five plants in the first days of the experiment may have caused depletion of one or several elements more quickly than would three plants, leading to growth responses that were both treatment and nutrient related. However, this seems unlikely given that only the control plants in both experiments were observed to be slightly pale in colour. This observation does, however, raise the possibility that growth of control plants in both trials was retarded by a nutrient deficiency, leading to an artificially-induced euhalophytic-type increase in growth at low salt concentrations. It should be noted that despite these considerations, growth response trends were well-correlated with those found in the literature. Certainly, plant numbers per pot should be carefully considered in future trials of this type.

This point also raises the issue of comparison of a halophyte's growth responses in salt with a non-saline control. It would seem more appropriate to compare growth of plants in higher concentrations with plants in an optimum environment (where Cl^- is not limiting [Greenway & Munns, 1980]), in this instance at 25 mM NaCl. The procedure was, however, appropriate in these experiments, since the known halophytic ability of tall wheatgrass was to be compared with the unknown properties of Australian plants, which required the use of salt-free control treatments.

Time restrictions prevented a search of roots in waterlogged treatments for aerenchyma. This simple microscope analysis should have been undertaken, and the absence of this data must be blamed on inadequate time-management.

The procedural problems experienced in the pH trials have been mentioned. Plant roots will regulate proximal soil chemistry through exudates (Brady, 1990), but the sustained application of pH-adjusted solution in this experiment presumably placed excessive energy demands on root cell membranes, leading to tissue death. If so, hydraulic resistance would have increased, and stomatal aperture would pose an immediate threat to the plants' water balance. Hormonal mediated leaf-rolling was the visible physiological response. Leaf death may have been due to diversion of essential elements away from older leaves, possibly in response to the inability of the damaged roots to take up ions.

It is apparent that soil reaction has attributes not shared with soil salinity, and should be applied differently. Farrell *et al.* (1996b) applied pH increases to *Eucalyptus camaldulensis* during gravimetric watering in increments of 1 or 2 over 38 days, obtaining treatment-related growth responses. This suggests that the current trial was procedurally correct, but was affected by the early attempts to rapidly adjust pH.

CHAPTER 3. AUSTRALIAN PLANTS

3.1 Introduction

Herbaceous species have been used extensively on degraded lands in Australia, often as a component of mixed communities (Mitchell & Wilcox, 1994).

In the past, this usually entailed using exotic species, whose broad hardiness was well known, for pasture-land improvement. Some of these introductions spread widely (for example, some couch varieties), many being listed as weeds (Lamp *et al.*, 1990). More recently, trials of candidate species have been directed at determining specific tolerance characteristics relevant to the site under study. Mining and industrial sites are often rehabilitated with herbaceous plants or shrubs able to rapidly stabilise topsoils and residue heaps communities (Mitchell & Wilcox, 1994). Suitable plants often originate from overseas, where their specific tolerances have been either tested or inferred from their usual habitats. Indigenous Australian species with appropriate tolerance abilities may be found for these purposes.

Podolepis gracilis (Lehm.) (Asteraceae) is endemic in Australia, occurring over much of the southwestern corner of the continent. It is an annual herb with a branching habit which flowers in spring to summer. It is a 'strong' annual, indicating perhaps a wide genetic variation in seed store, given the unpredictability of rainfall in its habitat. The plant occurs naturally in sands and lateritic soils, most commonly in open jarrah and marri woodlands (Marchant *et al.*, 1987). A search of the literature found no reference to the species' relative

tolerances to environmental extremes, and no mention of any deliberate seeding on degraded lands, although this occurs in mine operations throughout the southwest of the state (Scott, pers. comm.). *P. gracilis* is found growing naturally in the woodlands surrounding minesites southeast of Perth, thereby suggesting itself for possible use in revegetation of the adjacent degraded land.

Common wallaby grass *Danthonia caespitosa* Gaud. (Poaceae) is endemic in Australia, and is widespread in temperate regions. In Victoria it is considered a useful pasture grass, and is often used in nutrient-poor, compacted soils where more valuable pasture species will not persist (Walsh & Entwisle, 1994). In Western Australia, it is common in grasslands and open woodlands, and is noted to be a dominant in some areas, particularly in the southeast of the state (Mitchell & Wilcox, 1994).

There has been some debate regarding this plant's taxonomic treatment. Confusion has been caused through the anatomical similarity of several *Danthonia* species, in particular that of *D. setacea* (Lamp *et al.*, 1990). In addition, two forms of *D. caespitosa* have been noted which differ only in leaf thickness and attitude (Walsh & Entwisle, 1994).

No reference to the species' tolerances to environmental extremes could be found in the literature. Deliberate seeding has been performed on soils described as poor, but usually in a context of pasture yield optimisation rather than revegetation of degraded soils. This practice does, however, occur on minesites

in southwestern Australia (Scott, pers. comm.). The species has been observed to grow naturally in the vicinity of mine sites in southern Western Australia, suggesting itself for inclusion as a candidate for deliberate planting in such areas.

The current trials examined the growth responses of *P. gracilis* and *D. caespitosa* to a range of soil salinities and waterlogging conditions. This was done to determine their tolerances in relation to conditions found at Boddington Gold Mine, southeast of Perth, in order to test their suitability for revegetation at that site. The trials also enabled comparison of tolerances in these species with tall wheatgrass.

3.2 Materials and Methods

3.2.1.1 Plant material

Podolepis gracilis and *Danthonia caespitosa* seed was from single seedlots, both supplied by Boddington Gold Mine in March, 1997. Seed was germinated in 180mm pots for approximately ten weeks, at which time their numbers were thinned to three plants of similar size per pot. Plants were watered daily, and fertilised (half-strength Thrive™) every fourth day until treatments began. All germination and plant propagation was performed in a glasshouse with temperatures ranging from 20-35°C.

Approximately 2% of *D. caespitosa* showed a comparatively higher growth rate, and a broader leaf anatomy. In these plants, leaves were oriented almost vertically, in contrast to the remainder, in which leaves appeared less rigid, and drooped under their own weight. These faster-growing plants were not included in the experiment.

3.2.1.2 Pots and soil

180mm pots were used in all experiments, with a single 10mm hole at the base which could be sealed using a rubber bung. The soil was a 1:1 ratio mixture of fine and coarse pasteurised white sand. Each pot contained 4kg of soil.

3.2.1.3 Saline solutions

Nutrient/salt solutions were made up as described in Chapter 2.

3.2.2 Experiment 4. *P. gracilis*

Each pot contained three plants of similar size, treatments comprised three pots (total 9 plants per treatment). The experiment began in mid-September. Treatments were control, 25 mM NaCl, 50 mM NaCl, 100 mM NaCl, 200 mM NaCl and 0 salt/100% waterlogging. As described in the *A. elongatum* salinity/waterlogging experiments, solutions were applied in daily increments of 25 mM, requiring 8 days to reach the highest concentration (200 mM). The pots in the waterlogged treatment were sealed then flooded with control solution to approximately 1cm above the soil surface on day 8. Waterlogged pots were watered as required, using deionised water, to 1cm above the soil surface. All other pots were watered to field capacity by weight daily, using deionised water. All pots were well spaced and randomly rearranged daily. Plants were harvested 36 days after the treatments began. They were separated into roots and shoots and their fresh weights measured. They were dried at 70^o C for 6 days and their dry weights measured.

3.2.3 Experiment 5. *D. caespitosa*

Each pot contained three plants of similar size, treatments comprised three pots (total 9 plants per treatment). Treatments began in mid-September. They were control, 25 mM NaCl, 50 mM NaCl, 75 mM NaCl, 100 mM NaCl, 125 mM NaCl, 150 mM NaCl, 175 mM NaCl, 200 mM NaCl, 0 salt/100% waterlogged and 100 mM NaCl/100% waterlogged. Solutions were applied as described in Experiments 1., 2. and 4., except that each increment was included as a separate treatment. The final concentration (200 mM) was reached on day 8. The two waterlogged treatments were flooded to approximately 1cm above the soil surface on day 8. Spacing and watering regimes were as mentioned above. Plants were harvested 36 days after treatments began, separated into roots and shoots and their fresh weights measured. They were dried at 70^o C for 7 days and their dry weights measured.

3.2.4 Statistical analysis

Statistical analysis was performed as described in Chapter 2.

3.3 Results

3.3.1 Experiment 4. *P.gracilis*

In all parameters measured, growth was not affected by salt concentrations. Waterlogging severely decreased growth in all parameters, but survival rate was 100%.

Of the saline treatments, there were no statistically significant differences in dry weight RGRs of the shoots (Figure 3.1a; $P=0.3335$), roots (Figure 3.1b; $P=0.1059$), or whole-plants (Figure 3.1c; $P=0.2269$), although all results show a slight progressive decrease at increasing concentrations. Of the waterlogged treatments, however, significant growth decreases were seen in all parameters; shoots ($P=0.0360$), roots ($P=0.0000$) and whole-plants ($P=0.0070$).

Mean shoot/root ratios of plants in saline treatments, calculated from dry weights, did not differ significantly (Figure 3.2; $P=0.5992$). The high ratio in 100 mM NaCl is moderated by the large error. Plants in the waterlogged treatment showed a high ratio of shoot to root dry weight ($P=0.0300$).

There was almost no variation in percentage water content of plants ($P=0.2570$) over the range of saline treatments. Percentage WC of roots in the waterlogged treatment was, however, markedly lower ($P=0.0300$), probably a result of the

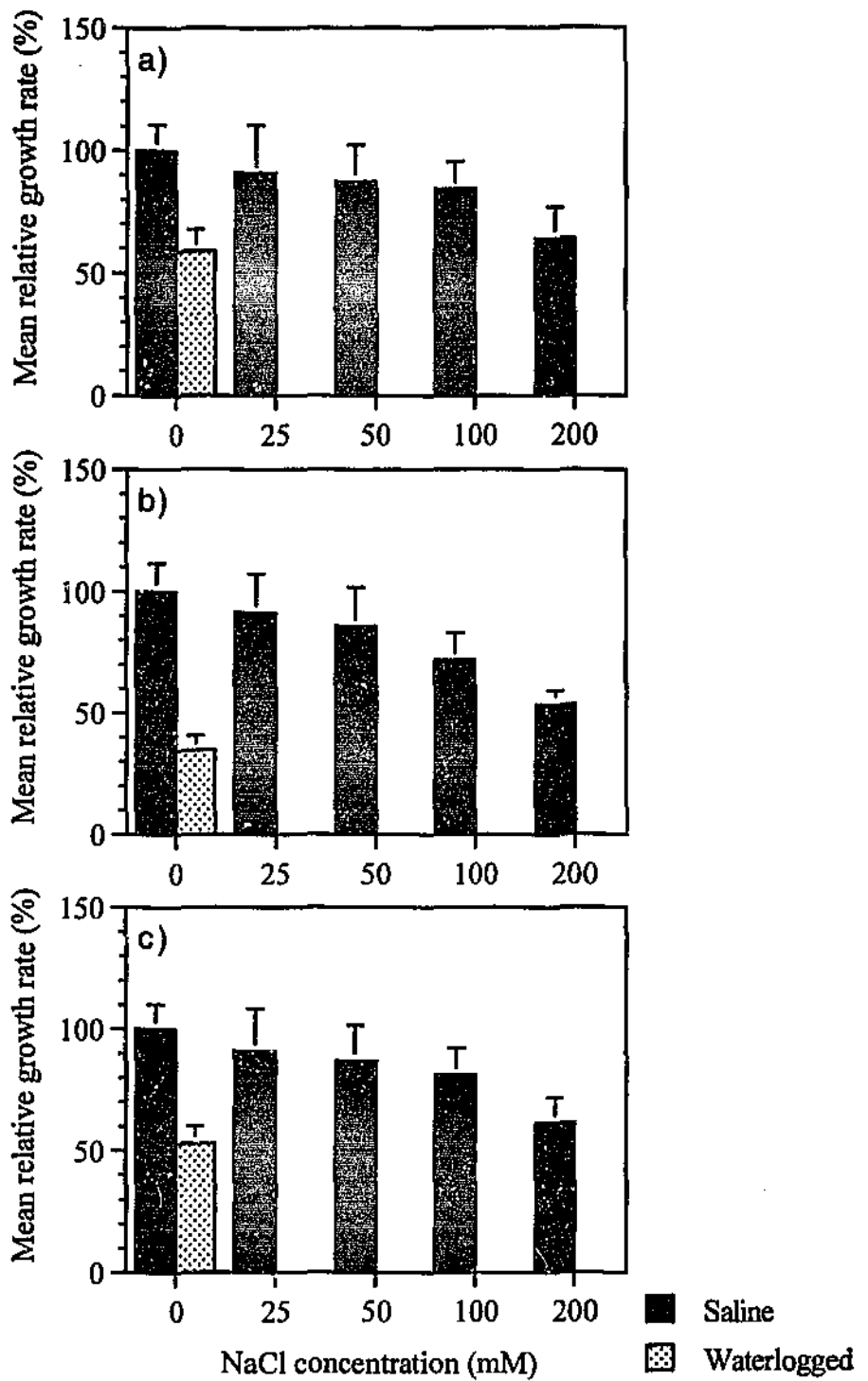


Figure 3.1. Mean relative growth rates (%) calculated from dry weights of *P. gracilis* a) shoots, b) roots and c) whole plants, grown in NaCl concentrations from 0 mM (control) to 200 mM, and in 0 mM NaCl/waterlogged conditions, for 36 days. Vertical bars represent standard errors of the mean.

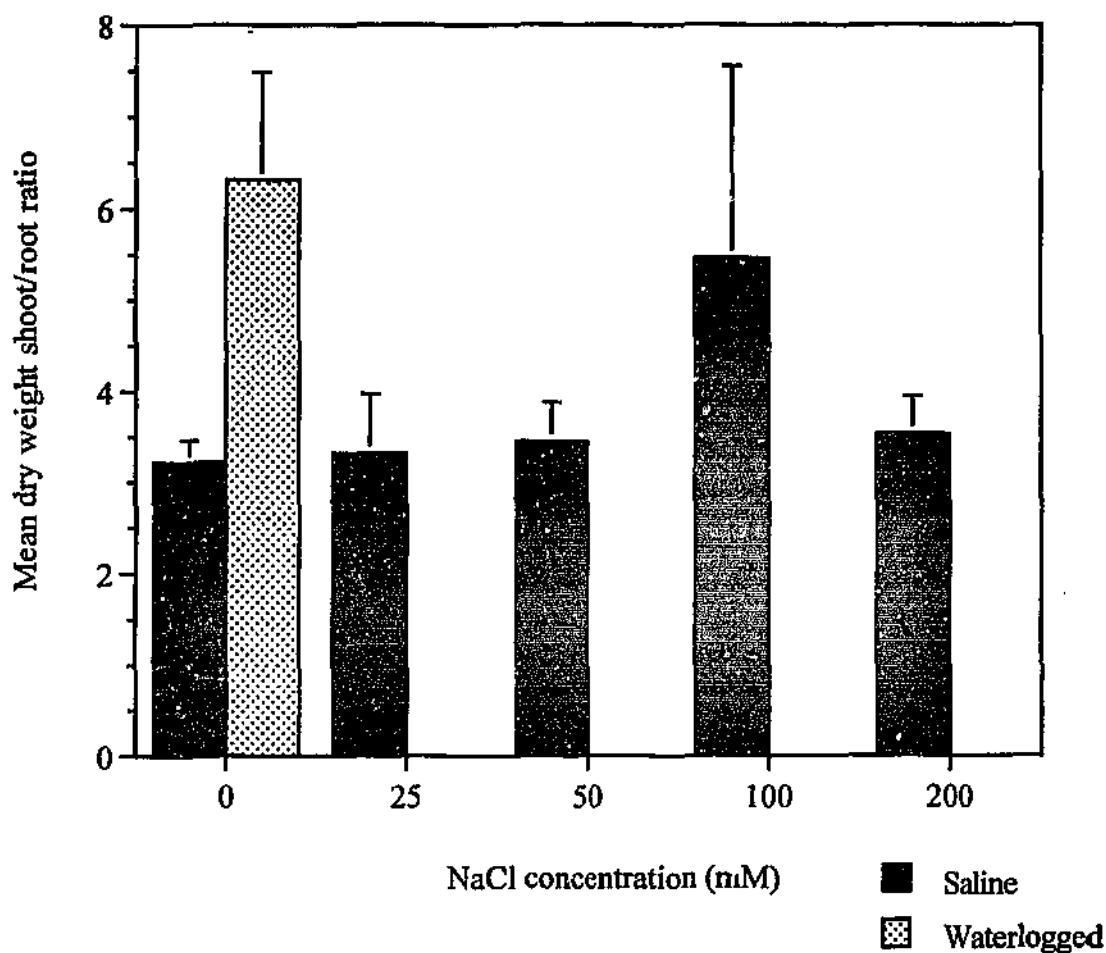


Figure 3.2. Mean shoot:root ratios calculated from dry weights of *P. gracilis* grown in NaCl concentrations from 0 mM (control) to 200 mM, and in 0 mM NaCl/waterlogged conditions for 36 days. Vertical bars represent standard errors of the mean.

high proportion of necrotic tissue observed during harvesting (data not presented).

All plants in the control treatments were paler in colour compared to the remainder, though not chlorotic. Leaf death was minimal in all treatments up to 100 mM NaCl. Plants showed approximately 10-15% death of older leaves in the 200 mM NaCl treatment, and approximately 50% in the waterlogged treatment. Flowering occurred across the range of treatments; highest flower counts occurred in the 25 mM NaCl pots, and the lowest counts in the 200 mM treatment (two flowers on one plant). Approximately 20 flowers per plant were observed in the waterlogged treatments.

3.3.2 Experiment 5. *D. caespitosa*

Growth increased slightly, in most parameters, in salt concentrations up to 100 mM NaCl. At higher concentrations, there was a progressive decrease in growth with increments of salt concentration, such that lowest growth occurred in the highest concentration (200 mM). Survival rate in salt-only treatments was 100%. Waterlogging severely affected growth in all parameters. Survival rate in the 0 salt/waterlogged treatment was 100%, and 67% in the 100 mM NaCl/waterlogged treatment. Two-way ANOVA revealed a combined effect of salt and waterlogging on shoot growth. There were no combined effects on root growth or growth of whole plants.

Mean dry weight RGR of shoots in all salt-only treatments were comparable (Figure 3.3a; $P=0.2108$). Mean shoot RGR of plants in the 0 salt/waterlogged treatment was comparable to that in the highest salt concentration, and 67% of the control mean, while mean RGR of the 100 mM NaCl/waterlogged treatment was less than 33% that of the control ($P=0.0001$).

Plants in both the 25 mM and 50 mM treatments showed a higher root RGR than the control (Figure 3.3b; $P=0.0000$). Root growth decreased at higher salt concentrations. Lowest growth was observed in 125 mM NaCl. Root RGR in both waterlogged treatments was significantly reduced ($P=0.0000$).

Whole plant RGRs reflect the component results, showing higher dry weights of plants in salt-only treatments up to 100 mM, though only fractionally so in the 75 mM treatment (Fig. 3.3c; $P=0.0344$). At higher concentrations, plant growth progressively decreased. Of the salt-only treatments, RGR was lowest in 200 mM NaCl. Waterlogging had a marked effect on whole-plant growth ($P=0.0000$); in 0 salt/waterlogged conditions the mean RGR was significantly reduced, and more markedly so in the 100 mM NaCl/waterlogged treatment.

Percentage water content (%WC) varied little between whole-plants in salt-only treatments ($P=0.1831$). Low %WC in waterlogged plants ($P=0.0000$) was likely due to a high proportion of dead tissue (data not presented).

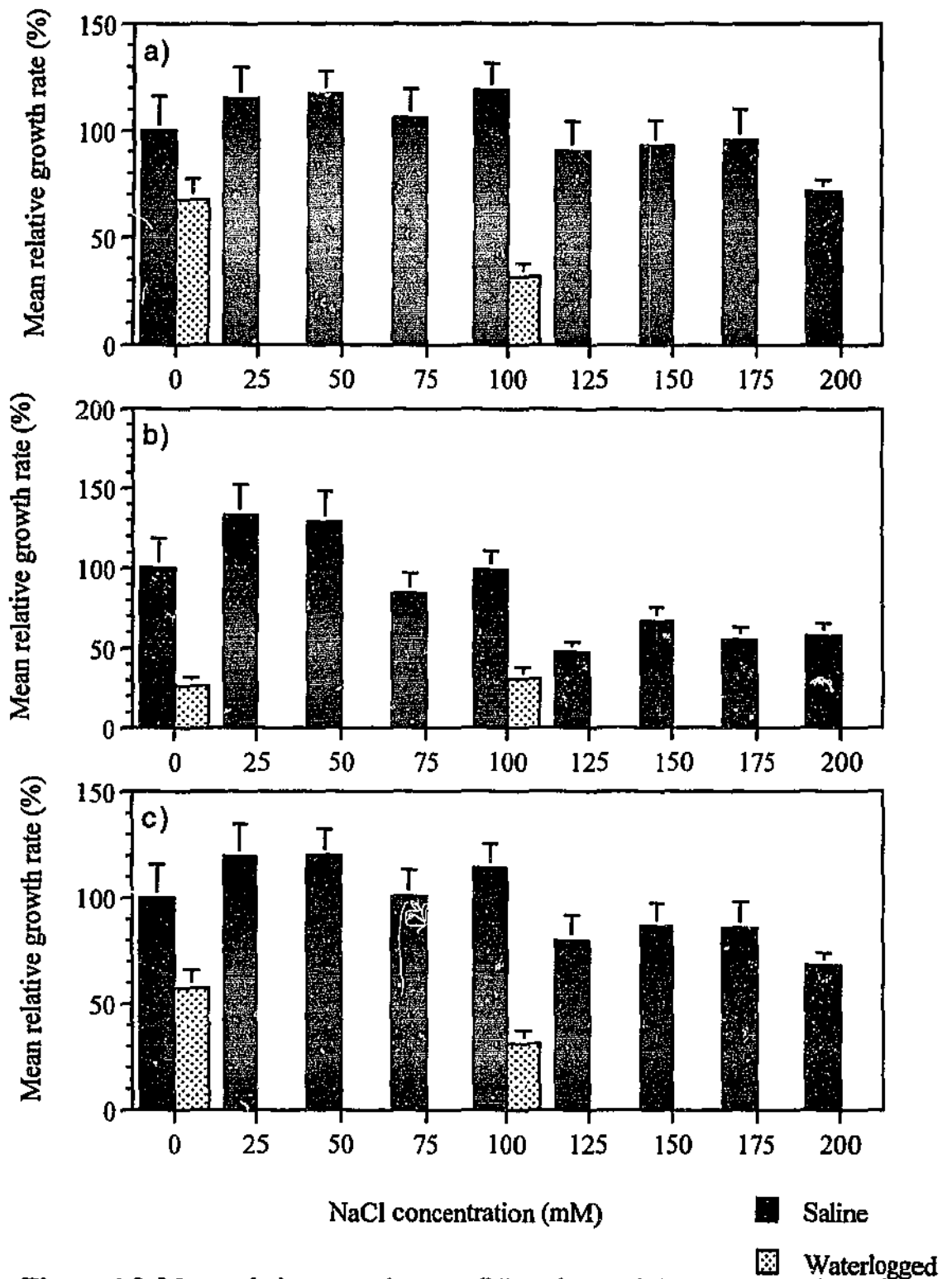


Figure 3.3 Mean relative growth rates (%), calculated from dry weights of *D. caespitosa* a) shoots, b) roots and c) whole plants grown in NaCl concentrations of 0 mM (control) to 200 mM, and 0 mM/waterlogged and 100 mM/waterlogged conditions for 36 days. Vertical bars represent standard errors of the mean.

Mean shoot/root ratios in the salt-only treatments, calculated from dry weights, were lowest in the 25 mM treatment (Figure 3.4; $P=0.0001$). There was a trend toward slightly increasing ratios at higher salt concentrations. Highest mean shoot/root ratio occurred in 125 mM NaCl. Mean shoot/root ratio of the control was between the higher and lower values. The high ratio in the 0 salt/waterlogged treatment is moderated by the large error, but also illustrated a greater effect of waterlogging on roots ($P=0.0005$) than shoots. Combined effects of salt and waterlogging on shoot dry weight were likely to have moderated the shoot/root ratio in the 100 mM NaCl/waterlogged treatment.

Leaf death was observed in all treatments. Estimates of percentage leaf death in salt treatments ranged from 5% in 50 mM NaCl to 40% in 200 mM NaCl. Survival rate in salt-only treatments was 100%. Leaf death in all waterlogged treatments was estimated at 90%. Leaf roll was not observed in any treatment.

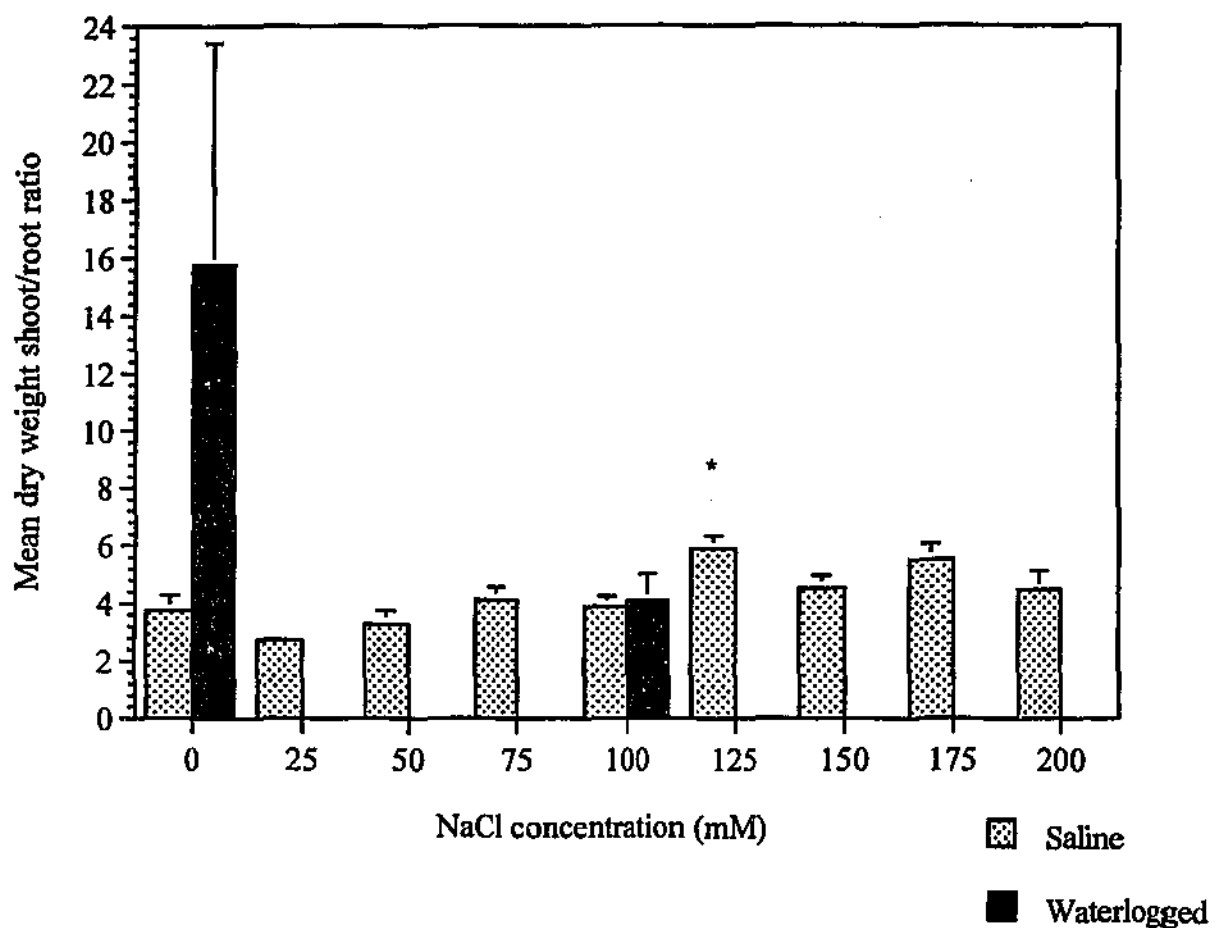


Figure 3.4. Mean shoot:root ratios calculated from dry weights of *D. caespitosa* after growth in NaCl concentrations of 0 mM (control) to 200 mM, and 0 mM/waterlogged and 100 mM/waterlogged conditions for 36 days. Vertical bars represent standard errors of the mean. (*) represents difference in the means (Tukey's $hsd_{0.05}$) of 9 replicates compared to the control.

3.4 Discussion

3.4.1 *Podolepis gracilis*

P. gracilis was found to be tolerant of salt concentrations up to 200 mM, but not tolerant of hypoxia. A progressive decline in growth rate with increasing NaCl concentrations up to 200 mM can be discerned from the results, but this was not statistically significant. Waterlogging caused reduction of growth and tissue damage, but survival rate remained high (100%).

While the results do not show a euhalophytic curve which indicates growth stimulation by NaCl, they do show a response typical of salt-excluding species with a relatively high tolerance of external salt (Greenway, 1973). *P. gracilis* might therefore be tentatively termed a miohalophyte. The growth of such species is usually decreased relatively rapidly at NaCl concentrations between 100-200 mM (Greenway & Munns, 1980), a result not seen in this experiment.

Growth of *P. gracilis* was reduced in salt-free hypoxic conditions. Root growth was affected more severely than shoot growth. Interactive effects of hypoxia and salinity were not examined, but it is likely that *P. gracilis* would be affected by ion toxicity under such conditions. Soil oxygen deficiency inhibits the energetic exclusion of ions from the roots of miohalophytes, leading to an uncontrolled uptake of salts which accumulate in the leaves (Galloway & Davidson, 1993).

Hypoxia can have similar effects on euhalophytes, but at comparatively higher salinities.

Percentage water content of shoots and roots did not vary significantly between the control plants and those in saline substrates, nor between plants in different salt concentrations. This may be further support for classification of *P. gracilis* as a miohalophyte. Glenn & O'Leary (1984) found that of twenty eu- and miohalophytic dicotyledonous species tested in saline media, miohalophytes exhibited less control of osmotic potentials through adjustment of water content. This was associated with a slight decrease in tissue water in saline conditions.

3.4.2 *Danthonia caespitosa*

D. caespitosa showed salinity-related growth responses similar to tall wheatgrass, suggesting it may have halophytic properties. There was an increase in growth at salt concentrations to 100 mM, and a progressive decrease in growth thereafter with increasing concentrations. However, differences in growth were not statistically significant.

Waterlogging decreased growth significantly; roots were affected more than shoots. The interactive effects of salt and waterlogging severely reduced root and shoot growth.

Leaf death was high in comparison to the two other species tested; tentatively associated with salt concentrations, but clearly associated with waterlogging. Of the salt treatments, none were found to differ significantly in growth responses from the control. There were, however, significant differences between the higher growth rates seen in lower salt concentrations and the lower growth rate in 200 mM NaCl.

The apparent similarity in relative growth rates to those of tall wheatgrass is not, however, conclusive support for an overall comparison. Monocotyledonous euhalophytes tend to accumulate cations, usually potassium, and produce organic counter-osmotica. This is generally associated with adjustment of cellular osmotic potential through a decrease in vacuolar water (Glenn & O'Leary, 1984), as discussed previously. In this experiment, *D. caespitosa* was found to have a significantly reduced shoot water content at the highest salt concentration, and to have made little or no water content adjustment at lower concentrations (although a high proportion of dead leaf tissue may account for both of these observations). In this respect, *D. caespitosa* differed from tall wheatgrass, but supported the idea of a salt-tolerant monocot physiotype.

3.4.3 Procedural limitations and further development

Further tests on the tolerances of *P. gracilis* and *D. caespitosa* are required, to concentrations of at least 400 mM, to examine the true extent of the halophytic properties shown in the current trials. As in the tall wheatgrass trials (Chapter

2.), plant numbers per pot should be carefully considered, as the increase in growth at lower salt concentrations seen in both that species and *D. caespitosa* may indicate a procedural error.

A requirement of plant species proposed for revegetation endeavours is that they germinate and grow at a reasonable rate. This is also important if trials of stress tolerance levels are to be performed. *P. gracilis* germinated and grew in sufficient quantity (ie; allowing random selection of similar-sized plants) for use in all treatments except saline/waterlogging. *D. caespitosa* grew in numbers exceeding requirements, and was therefore tested experimentally in saline increments of 25 mM, requiring more treatments (the species' salt tolerance was under-estimated during the planning of these experiments, hence the surplus plants were not utilised in trials to 400 mM NaCl). Thus, both species showed attributes valuable to revegetation programs.

Besides these species, twenty Australian seed species were proposed for these trials. The majority did not germinate, or germinated in insufficient numbers for inclusion in experiments. These included: *Ptilotus manglesii*, *P. drummondii*, *Stackhousia huegelii*, *Lobelia rhombifolia*, *Isotoma hypocrateriformis*, *Stipa campylachne*, *Tripterococcus brunoni*, *Waitzia paniculata*, *Wurmbea dioicea* ssp. *alba*, *Velleia trinervis*, *Themeda australis*, *Spinifex longifolius*, *Amphipogon amphipogonoides*, *Eragrostis eriopoda*, *Monachather paradoxus*, *Drosera gigantea*, *Patersonia occidentalis*, and *Triodia pungens*. The remaining species, *Neurachne alopecuroidea* and *Cyathochaeta avenacea* germinated

sufficiently but did not grow or survive sufficiently for use in these trials. It must be pointed out that germination of some of these species is not usually considered to be low (Scott, pers. comm.). Seed dormancy and timing of germination attempts should be considered.

CHAPTER 4. GENERAL DISCUSSION

Tall wheatgrass was found to be exceptionally tolerant of salt and waterlogging stress. Its survival rate was 100% in all treatments. Growth rate was reduced in higher concentrations of NaCl, presumably as a result of increased energy expenditure in maintaining low cellular water potentials. However, physiological responses to the 'physiological drought' caused by salinity that may be expected in a less-tolerant plant, such as leaf-roll or necrosis, were minimal.

Waterlogging caused a reduction in root growth with an associated compensatory increase in shoot growth, such that growth of whole plants was statistically associated with salinity, but independent of waterlogging. The species supported the notion that halophytic properties are associated with tolerance of a broad range of stresses. Survival rate in fungus-infected substrate at low and transiently high pH levels was also 100%, and despite marked leaf-rolling and necrosis, many plants showed indications of recovery.

Growth responses of tall wheatgrass to salt were clearly typical of a halophyte, but not of halophytic grasses, and the results do not support the case for a halophytic monocot 'physiotype' (Greenway & Munns, 1980). Statistically significant increases in %WC in concentrations up to 300 mM is not in accordance with the findings of Glenn (1987), who found that water content decreased in halophytic grasses under saline conditions. However, increased water content at salt concentrations to 300 mM and a decrease in 400 mM NaCl

might suggest that osmotic adjustment occurs in stages in tall wheatgrass. In other words, that at low to moderate external salt concentrations, adjustment is primarily through an increase in cellular ions and carbohydrates, permitting subsequent minor adjustment with increased water. This model would also help to explain the increased growth at concentrations to 100 mM seen in these trials (increase in turgor - cell wall extension - increase in organic carbon; though this model of growth is not supported by tests on succulent species, which continue to grow when dehydrated [Glenn & O'Leary, 1984]).

At higher salt concentrations, a high ion uptake and concentration may be unavoidable, enforcing a levelling-out in absolute quantities (and relative to external ions) through a decrease in water content, thus avoiding the toxic effects of monovalent cations. This model suggests that decreased growth observed in 200 mM and 300 mM would therefore be due to the energetic requirement of ion regulation exceeding the potential for growth through increased turgor. Specific ionic mass is often proportionately high relative to specific organic mass in NaCl-accumulating halophytes grown in saline conditions (Flowers *et al.*, 1977). Elemental analysis, and the testing of organic mass vs ionic mass, should be performed if these trials are repeated. Of interest would be the organic/ionic mass ratio at lower salinities.

It should be noted that Glenn (1987) found that highest growth rate occurred in salt-free treatments, while Na⁺ uptake increased, and water content decreased in 180 mM and 540 mM NaCl, in a study of salt-sensitive and salt tolerant grasses.

In that experiment, carbohydrates and K^+ were found to be unimportant in whole-plant osmotic adjustment. Tall wheatgrass, however, has been shown to accumulate K^+ in preference to Na^+ . Affinity for K^+ may be twenty times higher than for Na^+ in low concentrations (Flowers *et al.*, 1977). *A. intermedium*, another wheatgrass, though less salt-tolerant, showed similar tendencies (euhalophytes take up K^+ in the absence of Na^+ , though this is directly limited by small additions of the latter [Flowers *et al.*, 1977]). Hence plants of this genus may differ from the majority of grasses studied so far; certainly in these trials growth responses to salinity more closely approximated those of dicotyledonous euhalophytes than halophytic grasses.

P. gracilis showed growth responses to salinity typical of a miohalophytic dicot, tolerating moderately high salinities through salt exclusion (Glenn & O'Leary, 1984). Growth rate decreased in increasing salt concentrations, but survival rate was 100%. Growth rates to 200 mM were higher than those of a hypothetically typical miohalophyte, described by Greenway (1973). Trials to 400 mM, or perhaps higher, should be undertaken on this species. The species was not tolerant of waterlogging, however, probably limiting its usefulness in revegetation of permanently inundated soils. Whole-plant death and severe necrosis of surviving plants occurred within 28 days under waterlogged conditions, clearly suggesting that the plant could not find alternative access to oxygen through physiological/morphological adjustment.

However, *P. gracilis* should be included in considerations for revegetation of contaminated soil, given its high tolerance of salinity in comparison to glycophytes. Its halophytic properties were found in these trials to exceed those of tall wheatgrass in most parameters.

Genotypic diversity is likely to be high in this species as it is a strongly recurring annual occurring over a wide geographical area, and dependant on a seed store for regeneration. Natural growth occurs most commonly on sandy soils and duplex laterites. Such soils are likely to be well-drained, possibly explaining the species' intolerance of waterlogging as it is endemic to these areas with, therefore, a probable evolutionary link to environmental conditions. Salt-tolerant annuals are considered to be an important component of plant communities on saline land wherever livestock grazing occurs (Barrett-Lennard & Galloway, 1996). In contrast to halophytic perennials, such plants contain considerably less stored salt, and may be grazed preferentially.

Having mentioned that *P. gracilis* is likely to be of little value in revegetating persistently inundated land, it must also be pointed out that if waterlogging occurs to a maximum level below the soil surface, the species may be able to complete its growth and reproduction without exposure to hypoxia. Grown in such sites as a 'companion' species to other halophytes, the plant may also contribute to evapotranspiration, thus assisting in control of groundwater recharge.

D. caespitosa was also found to be tolerant of salinity, despite a relatively high leaf death at higher concentrations. This plant may be (temporarily at least) classified together with *P. gracilis* as being of comparable value in revegetation projects. Both species were shown to be sensitive to excess soil water, but robust in saline conditions. *D. caespitosa* showed growth responses in saline conditions seen in other halophytic monocots. The latter feature, as mentioned, suggests a relatively broad tolerance of environmental extremes.

4.1 Concluding Remarks

These trials set out to determine the tolerances of selected herbaceous species to a range of applied stresses in order to determine the feasibility of their use in revegetation of degraded soils at a southwestern Australian gold mine site. The two Australian species tested were found to be at least as tolerant of salinity as tall wheatgrass, a known halophyte. Further tests should be done on these species, such as exposure to higher salinities, to more fully characterise their stress-tolerance abilities, but results gained in this project are certainly cause for optimism.

A lack of tolerance to waterlogging restricts the application of these plants in the sites targeted for revegetation in this project, but may have little bearing on their use in revegetation elsewhere. In addition, sensitivity to waterlogging may not affect their ability to colonise sites where waterlogging is transient or contained below rootzones. *P. gracilis*, for example, is a strongly recurring annual, but is

endemic in a region of unpredictable hydrology. Propagation strategies in this species, while not conferring increased stress tolerances in themselves, are likely to give the species an added resilience.

It is evident that specific tolerances to individually applied stresses under controlled conditions will provide valuable information, especially where a given species is considered most appropriate for a particular revegetation program (such as tall wheatgrass at BGM). However, it is further evident that a species will have limitations and attributes that must be considered in conjunction with experimental data. Tall wheatgrass appears ideal for revegetation of saline and waterlogged minesites, but it is an introduced species. Of the two Australian species tested in this project, both were found to be tolerant of salinity, a soil condition common in Australia. Further investigations into tolerances of other Australian herbaceous species could prove to be invaluable.

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