The agronomy of *Distichlis spicata* cv. yensen-4a (NyPa Forage) and its potential role as a forage plant on saline land

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A thesis submitted in total fulfilment
of the requirements for the degree of
Doctor of Philosophy

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March 2009

Dedication

I would like to dedicate this thesis to the memory of Dr. Nicholas Yensen, who first inspired me in the fascinating world of halophytes. Nick's original vision was to develop the underutilized saline resource that is present in many regions to help provide food security for those in developing countries. I hope the research contained within this thesis can be used to help achieve Nick's original vision.

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Abbreviations

ADF	Acid detergent fibre
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CRC	Cooperative research centre
DAP	di-ammonium phosphate
DSE	Dry sheep equivalent
E	Transpiration rate (mol m^{-2} s^{-1})
EC	Electrical conductivity (dS m^{-1})
EC_e	Electrical conductivity of saturated paste (dS m^{-1})
e_{an}	Vapour pressure of water out of the chamber (mbar)
EM38	Electromagnetic 38
e_s	Saturated vapour pressure at leaf surface temperature (mbar)
ICBA	International centre for biosaline agriculture
IVDMD	In-vitro dry matter digestibility
LSD	Least significance different
N	Nitrogen
P	Phosphorus
p	Atmospheric pressure (mbar)
PAR	Photosyntheticaly active radiation
PVC	Polyvinyl chloride
r_b	Boundary resistance to H_2O (chamber constant = 0.32 m^2 s mol^{-1})
ϖ%	Gravimetric water content

List of publications

Refereed scientific papers

Sargeant M., Sale P. and Tang C. (2006). Salt priming improves establishment of Distichlis spicata under saline conditions. *Australian Journal of Agricultural Research* **57**, 1259-1266.

Sargeant M.R., Tang C. and Sale P.W.G. (2008). The ability of Distichlis spicata to grow sustainably within a saline discharge zone while improving the soil chemical and physical properties. *Australian Journal of Soil Research* **46**, 37-44.

Conference proceedings

Sargeant M., Tang, C. and Sale P. (2006). Eight years of Distichlis spicata growth improves soil properties in a saline discharge zone. "Ground-breaking Stuff" 13th Australian Society of Agronomy Conference. 10-14 September, Perth WA.

Sargeant M., Sale P. and Tang C. (2006). Salt priming improves establishment of Distichlis spicata under saline conditions. "Ground-breaking Stuff" 13th Australian Society of Agronomy Conference. 10-14 September, Perth WA.

Sargeant M.R., Sale P.W.G. and Tang C. (2007). Salt concentration is more critical to the performance of Distichlis spicata var. yensen-4a (NyPa Forage) than light levels. ASA-CSSA-SSSA Conference. 4-8 November, New Orleans, USA.

Sargeant M.R., Tang C. and Sale P.W.G. (2007). Distichlis spicata var. yensen-4a improves the soil physical properties when grown in saline discharge zones. ASA-CSSA-SSSA Conference. 4-8 November, New Orleans, USA.

Summary

Distichlis spicata cv. yensen-4a is a halophytic pasture grass that was developed in the USA and introduced into Australia in the mid 1990s. It has since been used on a trial basis to assess its suitability for forage production within saline discharge zones in southern Australia. During this time, it has been shown to be able to produce palatable forage throughout the summer months, when other green forage is scarce. This thesis investigates in more detail some of the mechanisms of salt tolerance, establishment and management factors to maximize production and feed quality along with the sustainability of such a farming system.

The establishment of *D. spicata* has been problematic since it was introduced into Australia, and a number of glasshouse and field experiments were conducted to further investigate this. It was concluded by both glasshouse and field trials, that high concentrations of salts within the root-zone hinder establishment. However, it was found that if the vegetatively established plants are exposed to salts prior to planting, then establishment and subsequent dry matter production is increased significantly. It was also observed during the glasshouse experiment that overcast conditions appeared to coincide with higher death rates of *D. spicata* when grown at high salinities. However, a subsequent glasshouse experiment did not find any relationship between light levels and salt tolerance. In fact, high salt excretion appears to be linked to transpiration, rather than light intensity.

The management of *D. spicata* was also investigated in terms of clipping or "grazing", nutrient inputs and salt levels. This was achieved by a combination of glasshouse and field experiments. It can be concluded that higher rates of nitrogen and phosphorus maximize plant survival, and increase the feed value by altering the plant morphology. Grazing management is also important and can further maximize feed quality.

A farming system that includes *D. spicata* in saline discharge zones also appears to be sustainable. A field survey conducted in Western Australia concluded that there was no evidence to suggest that salt had accumulated within the root-zone. There were also significant improvements in the soil chemical and physical properties where *D. spicata* had been growing for 8 years.

Statement of authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

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Mark R. Sargeant

Acknowledgements

There are many people that deserve recognition for providing assistance, encouragement and support to enable me to get to the point of submitting this thesis. Firstly, I would like to thank Dr. Peter Sale and Dr. Caixian Tang, my supervisors at La Trobe University, who provided guidance and encouragement throughout all aspects of the PhD. I would also like to thank Mr. John Leake from NyPa Australia for providing the genetic material and allowing this research to occur. I am hopeful that this research will enable a more productive use of saline discharge sites throughout Australia to the benefit of farmers.

I also would like to thank all the industry partners who came together to support this research. These industry partners are NyPa Australia Ltd., Elders Ltd, Buloke Park and the Department of Primary Industries Victoria. In addition, I would also like to thank Steve Williams from Donald and the Elford family from Manangatang for allowing me to conduct field research on their properties.

There are also many people I would like to thank within the Department of Agricultural Sciences at La Trobe University. In particular, I'd like to thank Joe Edwards for assistance with various bits and pieces in the laboratory, Julie Francis for putting up with my continual occupation of the glasshouses, and Rob Evans who was so accommodating of my space on the "farm". I would also like to acknowledge Gary Clark for our many long and interesting discussions which helped me keep my sanity. Sometimes these discussions were even about our research…. sometimes even about mechanisms.

In addition, I would like to thank Raymond Matthews from Wickepin in Western Australia, for putting up with me digging up his paddock and *Distichlis* plants (all in the name of science), and the many weeks of accommodation over the years. I thoroughly enjoyed the many conversations and bouncing ideas of each other to try and work out how to improve our management of this grass.

My wife Sarah Sargeant probably also deserves to get a PhD out of this work. She has dug up, planted and watered more *Distichlis* than most people. She has also read, corrected, re-read, re-corrected many drafts. Thanks heaps!

Lastly, I would like to thank God our creator, for this amazingly complex world, and creating such interesting and complex things to research. How little we know about how it all works!

Chapter 1

General introduction

Soil salinity is a major soil constraint that limits the growth of conventional agricultural species. While there are some salt tolerant conventional species, they are not able to grow productively in highly saline soils, such as those that occur at saline discharge sites in southern Australia. These areas are typified by shallow saline groundwater, salt crusted surfaces, and hot dry summers. Areas such as these are often left abandoned within the farming system, as there are very few options to utilize these areas productively. If these areas do support vegetation, it is often indicator species such as sea barley grass (*Hordeum marinum*), which may provide some poor quality feed during the winter and spring period when good quality feed is available elsewhere on the farm.

Halophytes are a group of very salt tolerant plants that are able to grow productively when exposed to high concentrations of salts within the root-zone. Halophytes are now being considered as potential crop and pasture species due to their ability to grow productively in these hostile environments, producing food (Yensen *et al.* 1985), forage (Barrett-Lennard 2003b; Leake *et al.* 2002), fibre (Lieth and Lohmann 2000) and as a source of biofuels (Hendricks and Bushnell 2008). The utilization of halophytes has the potential to increase food and energy produced from a world with dwindling fresh water resources. Within Australia, the adoption of halophytes within the agricultural system has predominantly been limited to *Atriplex* spp.

While *Atriplex* is the most widely utilized halophyte within Australia it is not suited to saline soils that are waterlogged (Barrett-Lennard 2003b). Such soils are quite common in saline discharge sites. There are also issues with the feed quality of *Atriplex* species, with high concentrations of salts being found within the leaf tissue (Norman *et al.* 2002; Norman *et al.* 2004). A high dietary salt intake for sheep will depress feed intake, and consequently reduce growth rates (Masters *et al.* 2005). Supplementary feed is required to ensure acceptable growth rates are achieved when grazing an *Atriplex* pasture (Barrett-Lennard 2003b; Franklin-McEvoy *et al.* 2007). Despite the long-term use of *Atriplex* spp. on saline soils in Australia, there have been sustainability concerns raised about the increasing chloride concentrations being measured in the soils where *Atriplex* is grown (Barrett-Lennard and Malcolm 1999). Despite these issues, *Atriplex* pastures are still widely grown on saline soils in southern Australia.

Distichlis spicata is another halophyte that is being considered as a forage grass for growing on saline discharge soils in southern Australia. This halophytic C4 grass has a number of advantages compared with *Atriplex* species. The first is its ability to excrete salts that have been taken up by the roots on to the leaf surface (Hansen *et al.* 1976). From the leaf surface, the salts are then presumably blown or washed away. This ensures that the salt concentrations in the plant tissue are not excessive and this has metabolic benefits for livestock that ingest *Distichlis* forage. A second advantage for saline discharge sites is its tolerance to waterlogged soils. *Distichlis spicata* possesses arenchyma tissue throughout the root system that allows oxygen flow to the waterlogged roots (Hansen *et al.* 1976). Both adaptations give *Distichlis spicata* an advantage over other halophytes, such as *Atriplex,* as a forage plant for saline discharge sites.

Distichlis spicata cv. Yensen-4a has been grown in Australia for about 10 years. During this time, it has shown promise as a forage grass for saline discharge sites (Leake *et al.* 2002; Sargeant 2003). The area under production has steadily grown, and the species is now being grown on farms in Western Australia, Victoria and South Australia. Despite this, very little research has been conducted on the agronomy of *D. spicata* swards. No information is available on how to best manage this grass for forage production, in terms of nutrient or grazing management. The establishment of the grass has also been problematic, with initial plantings only achieving a 50% strike rate (Leake *et al.* 2002). There is a real need to undertake research to establish guidelines for establishing and managing this grass for forage production.

The research documented in this thesis aims to provide some initial guidance in these areas by undertaking a number of glasshouse and field experiments. Three major areas of research will be investigated, including (1) the sustainability of a *Distichlis spicata* sward, (2) establishment techniques and (3) how to manage a *Distichlis spicata* sward for maximum production and nutritional value. The sustainability of a *D. spicata* sward will be investigated with a soil survey at an established site in Western Australia. Establishment techniques and management guidelines will be researched by undertaking field trials in the Mallee and Wimmera regions in Victoria, and with glasshouse pot experiments undertaken at La Trobe University.

Chapter 2

Literature review

2.1 Introduction

Soil salinity is a major form of soil degradation within Australia and throughout the world. According to the Food and Agriculture Organisation of the United Nations, there were approximately 397 million hectares of saline land throughout the world in 1990, and in excess of 76 million hectares of this area was caused by human activities (Oldeman *et al.* 1991). The problem of soil salinity is often thought of in terms of lost agricultural production. However, there are also large social, environmental and infrastructure costs associated with soil salinity. On a national level, it was estimated in 1999 that the annual costs associated with damage to infrastructure in Australia was $100 million, and a further $40 million of environmental assets were also lost annually (Anonymous 1999). The National Land and Water Resources Audit (2001) estimated that 5.6 million hectares were currently at risk to, or affected by salinity, in 2001 and that a further 17 million hectares of land will be at risk to salinity by 2050. The areas of land affected by, or at risk to salinity, in the states of Australia are listed in Table 2.1.

Table 2.1. The area at risk of, and affected by salinity, for each Australian State and Territory in 2000, and the projected areas in 2050 (National Land and Water Resources Audit 2001).

State	1998/2000 (ha)	2050 (ha)
New South Wales	181 000	1 300 000
Victoria	670 000	3 110 000
Queensland	n.a.	3 100 000
Western Australia	4 363 000	8 800 000
South Australia	390 000	600 000
Tasmania	54 000	90 000
Total	5 658 000	17 000 000

The common perception is that agriculture is to blame for Australia's salinity problems. However salt has always been part of the Australian landscape. Aboriginal mythology records these conditions from the Pleistocene times:

"Gumuduk was a tall, thin, medicine man, who belonged to the hill country. He owned a magical bone of such power that he could use it to make the rain fall in seasons, the trees bear much fruit, the animals increase and the fish multiply. Because of such good fortune the hill people always had plenty of food.

However, the tribe that lived on the fertile plain below the Kiti range captured the medicine man and his bone, convinced that they, too, would in the future have more food.

But instead of bringing them prosperity, the theft resulted in a calamity which totally destroyed their country. For the medicine man escaped and was so angry over the indignity that he had suffered that, plunging his magical bone into the ground, Gumuduk decreed that wherever he walked in the country of his enemies salt water would rise in his footsteps.

These waters not only contaminated the rivers and lagoons, but completely inundated the tribal lands. And when these waters dried up, the whole area was changed to an inhospitable desert of salt lakes, useless to both the creatures and the aborigines" (Russ 1995).

Accounts from the early explorers in Australia also clearly indicate that there were large quantities of salt in the Australian landscape, long before European settlement. When Sturt's expedition reached the Darling River near the current site of Bourke, they found the water was too salty to drink (Mackay 1990). Salinity was also a major problem during the early days of Western Australia. Some of the first studies conducted on soil salinity were brought about by the concern for fresh water supplies for the railways. By 1905, a number of railway water supplies had become too salty for use in the boilers of steam engines (Wood 1924).

The two different types of salinity are dryland salinity and irrigation salinity. Dryland salinity occurs in areas where the water for plant growth comes from natural rainfall. Primary dryland salinity areas were naturally saline before European settlement, whereas secondary dryland salinity were caused by human activities since European settlement. Irrigation salinity is a secondary form and is caused by irrigating land, resulting in a rise in the level of the watertable, due to a lack of sufficient drainage.

It is generally accepted by the community, that dryland salinity is caused by the removal of deep-rooted native vegetation and replacing it with shallow-rooted annual species (Dumsday *et al.* 1989). Under this simple model, the new shallow-rooted agricultural species do not use all the rainfall, and instead allows excess water to 'leak' through to the ground water in the recharge zones higher up the landscape. Lower in the landscape, the watertable rises to the point where water will rise by capillary action to the soil surface, bringing with it dissolved salts from deeper soil layers. However, Russ (1995) paints a different picture in the Murray-Darling basin, where he describes a landscape that has a long history of salinisation, long before European settlement. Many areas within the Murray-Darling basin had saline top soils long before conventional agriculture was introduced. While the simplistic model that is accepted by the community may provide an explanation for the salinisation process in some regions, it must be accepted that the Australian landscape has had a long association with salt. There are a number of documented cases where this simplistic model does not fit, such as in the Heytesbury region of western Victoria, where saline sites are caused by perched water tables, rather than rising water tables (Dalhaus and MacEwan 1997). Ferdowsian *et al.* (2002) also described a one dimensional system in the Western Australian wheat belt, where water movement was primarily up and down. These two documented cases demonstrate that no simple model can explain all occurrences of soil salinity.

One of the major problems associated with salinity, is its negative effect on plant growth. Most agricultural plants were not domesticated on saline soils (Yensen *et al.* 1995), and hence cannot tolerate high salt concentrations in the soil. Efforts have been made to improve the salt tolerance of conventional crop species through genetic modification and conventional breeding, but this work has not yet led to commercially available varieties for farmers. In recent years, there has been an increased interest in using halophytes in agricultural systems where saline soils occur. Halophytes (halo = salt + phyte = plant) are plants that can complete their life cycle at high salinities (Flowers *et al.* 1977). *Distichlis spicata* cv. yensen-4a is one halophyte that has been selected for forage production on saline soils (Yensen *et al.* 1985).

2.2 Glycophytes

Soil salinity is caused by a buildup of dissolved salts in the root zone of the soil. Many cations and anions can contribute to soil salinity, but the major ones are Na^+, Ca^{2+}, Mg^+, K^+, Cl^-, SO_4^-, HCO_3^-, CO_3^- and NO_3^- (Tanji 1990). The predominant salt present in Australian soils is NaCl (Rengasamy and Olsson 1993), whereas other salts may be more predominant in other parts of the world such as in the San Joaquin Valley in California where $NaSO_4$ is common (Cervinka *et al.* 1999).

Glycophytes are plants that are not able to survive in soils with high concentrations of dissolved salts in the root zone (Yensen 2002). Most agricultural crops and pastures are glycophytes, and have an upper tolerance limit of between 1-10 dS m^{-1} (EC_e) within the root-zone when their dry matter yield begins to decline (Mass 1993). Generally leguminous crops and pastures are more salt-sensitive than monocots, with barley (*Hordeum vulgare*) being one of the more salt-tolerant crops used commercially today.

High salt concentrations within the root-zone of glycophytes present a number of problems for these plants. These problems involve: (1) extracting water from the saline soil because of the low osmotic potential of the soil water (water deficit) (Groenevelt *et al.* 2004; Munns 2002), (2) maintaining cellular activities with excessive amounts of Na^+ and Cl^- within the plant tissue (ion toxicity) (Greenway and Osmond 1972), and (3) growing in an environment that has an ion imbalance in the soil solution (nutritional disorders) (Grattan and Grieve 1992). For plants to grow successfully within saline environments, they need to be able to manage these different constraints. Different plants have different abilities to manage these constraints and this gives rise to a range of salt tolerant plants.

2.2.1 Water Deficit

A water deficit is considered to be responsible for the initial decrease in growth when a glycophyte is first exposed to salt (Munns 1993; 2002). There is an initial reduction in leaf expansion rate that is caused by a decrease of the internal osmotic potential that compensates for the lower external osmotic potential. The low osmotic potential within the soil matrix reduces water availability to the plant and so it is difficult to take up soil water. It is essential that the plants adapt to this low osmotic potential in the soil solution to maintain cell turgor. This can be achieved by increasing the concentrations of ions/molecules in the plant cells. This is the process of osmoregulation. The most efficient method to achieve this is to take up ions (e.g. Na^+ or Cl^-), which consumes 3-4 moles ATP per mole of ion, compared to 30-50 moles of ATP for synthesizing organic solutes (Raven 1985) that would achieve the same degree of osmoregulation.

2.2.2 Nutritional disorders

There are two ways that soil salinity has the potential to disrupt nutrient uptake by glycophytes. The first results from the ionic strength of the soil solution, regardless of the composition, which has the potential to disrupt nutrient uptake (Awad *et al.* 1990; Grattan and Grieve 1992). The second involves the reduction in the availability of nutrient ions due to the competition with saline ions. These may be present in concentrations that are many orders of magnitude higher (Grattan and Grieve 1992). The second of these effects is the more common of the two, as Na^+ ions can induce Ca^{2+} and/or K^+ deficiencies (Botella *et al.* 1997; Chow *et al.* 1990; Grattan and Grieve 1992; Lazof and Lauchli 1991). Bohra and Dorffling (1993) suggested that the ionic imbalance may impair the selectivity of the root membranes.

2.2.3 Ion toxicity

In salt-sensitive species, the visible effects of ion toxicity may arise after a few days of exposure to high levels of salinity. The extent of the ion toxicity will depend on the degree to which the Na^+ and Cl^- ions can be sequestered in cell vacuoles, where these ions will not interfere with cellular functions. Ion sequesteration in the vacuole was demonstrated by Binzel *et al* (1988), where cultured tobacco cells were grown in 430 mM NaCl. In this experiment, the concentration of Na^+ and Cl^- in the vacuole was 780 and 625 mM respectively, compared with a concentration of less than 100 mM in the cytoplasm. Salt toxicity damage is often seen first in the older leaves which have been transpiring for a longer period than the younger leaves. Concentrations of Na^+ and Cl^- are higher in these leaves (Colmer *et al.* 1995), and salt injury occurs in the leaves when the accumulation of Na^+ and Cl^- within the leaves exceeds the ability of the cells to sequester the ions into the vacuoles. When this occurs, the ion concentrations increase rapidly in the cytoplasm which can inhibit enzyme activity. Alternatively, the ions can build up within the cell wall which causes the cell to dehydrate (Flowers and Yeo 1986; Munns and Passioura 1984).

2.2.4 Waterlogging

While not directly related to soil salinity, waterlogging is often a problem in saline soils due to shallow water tables, or decreased surface infiltration due to the dispersion of soil colloids from sodicity (Ghassemi *et al.* 1995; Qureshi and Barrett-Lennard 1998). Waterlogging causes a condition called hypoxia (low oxygen concentrations in the soil), which arises due to the low rate of diffusion of oxygen through water-filled pores. It has been estimated that the diffusion coefficient of oxygen is approximately 10,000 times less in water than through air (Grable 1966). Another consequence of growing in waterlogged conditions is the reduced capacity of roots to generate ATP. Barrett-Lennard (2003a) have estimated that a decrease of 95% in ATP generation can be attributed to waterlogged-conditions.

A comprehensive review of the interactions between waterlogging and salinity in plants has been provided by Barrett-Lennard (2003a). This author cites different research findings that support the view that waterlogging interacts with salinity to increase the concentrations of Na^+ and Cl^- in the plant shoots. This was first shown by John *et al.* (1977) who reported that the concentrations of Na^+ and Cl^- were higher in shoots of plants that were grown under saline waterlogged conditions, compared to those grown under saline conditions. It appears that this increase is caused by an initial increase in the net uptake of Na^+ and Cl^- to the shoots, and then a subsequent decrease in shoot growth (Barrett-Lennard 2003a). Barrett-Lennard (2003a) postulated that the concentrations of the saline ions would most likely be regulated by H^+ gradients across the plasma membrane of the cortical cells. These H^+ gradients would be maintained by H^+- ATPase activity, which would presumably be impaired by hypoxia. Ion toxicity is already an issue for plants growing in saline soils, but it can be seen that waterlogging exacerbates this effect.

2.3 Halophytes

Halophytes are characterized by having high growth rates in saline soil, with many species having optimal growth in solution culture with concentrations of 100-200 mM NaCl in the solution (Flowers *et al.* 1977). Halophytes occur throughout the world from inland desert regions, to coastal sand dunes (Yensen 1998; 1999a; b), and are found on all continents except Antarctica (O'Leary and Glenn 1994). There has been a considerable amount of research conducted on halophytes during the past 50 years, with many authors concluding that there is a role for halophytes in farming systems, and they have a range of potential uses. These include food sources for humans and livestock (Glenn and Felger 1994; Glenn *et al.* 1994b; Khan and Duke 2001; Lieth and Lohmann 2000; Yensen *et al.* 1995; Yensen 1995), carbon sequestration (Glenn *et al.* 1994a; Glenn *et al.* 1993), environmental remediation (Yensen *et al.* 1999) and more recently for biofuel production (Lal and Pimentel 2007). All of these roles depend upon unique salt-tolerance mechanisms which enable halophytes to grow in saline soils.

2.3.1 Salt tolerance mechanisms of Halophytes

Halophytes have a range of different mechanisms to manage the high salt concentrations in the root zone. Different approaches have been used to categorize these mechanisms. Gorham (1996) has used the approach of defining the mechanisms in terms of physiological and morphological adaptations, while Levitt (1972) used categories such as salt avoidance and tolerance. Flowers (1977) has reviewed the mechanisms of salt tolerance in halophytes with more specific categories and details. For the purposes of this review, the different mechanisms used by halophytes to successfully grow in saline environments can be allocated into three different strategies. These are to regulate ion uptake into the plant, to decrease the osmotic potential in plant tissue by osmoregulation, or to remove salt from the plant.

2.3.2 Regulating ion uptake

In glycophyte species, minimizing the uptake of Na^+, in favor of K^+ is a critical factor in the salt tolerance, which Hajibaghera *et al.* (1989) demonstrated in Maize (*Zea mays*). Bradley and Morris (1991) have also demonstrated that ion exclusion at the root level is an important mechanism of salt tolerance in the halophyte *Spartina alterniflora*. However, Glenn *et al.* (1992) found that the salt tolerance of *Atriplex canescens* did not depend on the preferential uptake of Na^+ or K^+ in the plant tissue. Glenn (1987) goes further in suggesting that the accumulation of Na^+ in the plant tissue is necessary for the osmotic adjustment in this species. These different views on the importance of regulating or restricting uptake of Na^+ or Cl^- ions are related to the species being studied.

There is also a further mechanism to regulate saline ion uptake that has received very little attention in the salt tolerance literature of halophytes. This mechanism involves the closing of stomata to reduce transpiration, thereby reducing the transport of water and saline ions to the shoots via the xylem (Robinson *et al.* 1997). These authors indicated that this strategy was a major mechanism for salt tolerance in the halophyte *Aster tripolium*, where high concentrations of Na^+ were found to restrict stomatal opening.

2.3.3 Osmoregulation and Compatible solutes

Osmoregulation is a major mechanism that halophytes employ to enable them to grow successfully in saline environments. Osmoregulation occurs at the plant and cellular level, and involves the accumulation of solutes within plant tissue to enable water uptake by the roots to continue and for the shoots to transpire. Distilled water has an osmotic potential of zero MPa, and this potential becomes negative when the salt concentration increases. Sea water for example has a potential of approximately -2.5 MPa (Jefferies 1981). As moisture only moves to more negative potentials, the plant needs to maintain a more negative gradient from the roots to the leaves to

allow transpiration to occur. Osmoregulation achieves this, with sap potentials in the range of -2.0 to -5.0 MPa being recorded in different halophytes (Flowers *et al.* 1977).

At the plant level, osmoregulation results from the accumulation of ions such as Na^+, Cl^-, K^+, Ca^{2+}, PO^{3-}_4 and NO^{-3} (Flowers *et al.* 1977). However, in reality, most of these ions contribute very little to the osmoregulation within halophytes. In *Salicornia rubra* for example, it was found that Na^+ and Cl^- contributed 75- 93 % of its osmoregulation, while the soluble K^+, Ca^{2+}, PO^{3-}_4 and NO^-_3 contributed less that 1% (Harward and McNulty 1965). Work by Zimmermann (1978) also supports this finding.

Although halophytes need to accumulate Na^+ and Cl^- ions to achieve osmoregulation, there are enzymes within halophytes that are just as sensitive to salt as the enzymes in glycophytes. Greenway and Osmond (1972) demonstrated that four enzymes in the halophyte *Atriplex spongiosa* and *Salicornia australis* were just as sensitive to the damaging effects of NaCl as they were in the salt sensitive *Phaseolus vulgaris*. The cells of halophytes overcome this, by partitioning the Na^+ and Cl^- ions into the vacuole of the cells, which make up approximately 90 % of the cell volume (Jefferies 1981). In halophyte cells, the solute concentration in the protoplast and vacuole are in osmotic equilibrium, while the concentration of salts in the vacuole can be as high as 700 mM (Jefferies 1981). Concentrations this high in the cytoplasm, would cause significant disruptions to metabolic activities. The accumulation of Na^+ within the vacuole is achieved by a salt-inducible Na^+/H^+ antiporter enzyme which transports the Na^+ into the vacuole, protecting the cytoplasm from high concentrations of the ion (Apse *et al.* 1999; Hassidim *et al.* 1990).

In order to maintain an osmotic equilibrium between the cytoplasm and the vacuole, organic solutes referred to as "compatible solutes" accumulate within the cytoplasm (Rhodes and Hanson 1993; Stewart and Lee 1974). Glycinebetaine and proline are the typical compatible solutes accumulated in grasses, although this is somewhat dependant upon the species (Colmer *et al.* 1996; Khan *et al.* 1998; Marcum 1999; Sakamoto and Murata 2002; Stewart and Lee 1974; Storey and Wyn Jones 1977). Marcum (2006) has presented evidence more recently that suggests that glycinebetaine and not proline acts as the major compatible solute in grasses that he studied.

2.3.4 Salt Removal from the Plant

Removal of salt from the plant is one of the major mechanisms that some halophytes employ to manage the salt load within the plant tissue. This can be achieved in two ways. The first is to accumulate salt in salt glands, which are then excised from the plant. The second strategy is to excrete salt from salt glands. The removal of salt allows the plant to osmoregulate to the degree that is required, and to then excrete excess salts to prevent toxic levels building up within the tissue.

2.3.4.1 Salt bladders

Salt bladders are specialized trichomes that are located on the leaf surface of many halophyte species (Batanouny 1993; Jou *et al.* 2007). According to Schirmer and Breckle (1982), the salt removing function of salt bladders was first recorded by Berger-Landefeldt (1959), although the development of salt bladders in *A. vesicaria* and *A. nummularia* had been described earlier by Black (1954) who did not know what their specific function was at the time. They continually initiate and develop throughout the life of the leaf.

Salt bladders consist of a stalked vesicle, which bears a swollen bladder cell about 80-200 μm in diameter. They contain a thick cytoplasm that is rich in mitochondria, a dense endoplasmic reticulum and numerous small vesicles. However, unlike salt glands, they are connected to adjacent bladder or mesophyll cells by plasmodesmata (Batanouny 1993). *Atriplex* species are the best known plants possessing salt bladders, with almost all species in the genus possessing these structures. The bladders vary quite markedly in size and density, and both of these parameters appear to be quite independent of the salt levels in the environment in which the plant is growing (Schirmer and Breckle 1982).

There have been many theories that explain the function of salt bladders in halophytes. It appears that at the very least, they assist the plants to cope with high salt concentrations, by removing salt from the plants tissue (Jou *et al.* 2007; Schirmer and Breckle 1982). Some researchers have raised questions as to the effectiveness of salt bladders in removing salt from the plant tissue due to differential response of different species that possess bladders, and to limitations in some of the research that has been conducted (Schirmer and Breckle 1982; Waisel 1972). However convincing evidence is presented by Mozafar and Goodin (1970) who found that when *Atriplex halimus* was grown in media with increasing salt levels, the salt concentration in the salt bladders increased significantly, whereas the salt concentration in the leaf sap did not increase. Furthermore, salt bladders can contain appreciable quantities of salt, as Pallaghy (1970) reported that up to half of the total salt content of the leaves in *Atriplex spongiosa* was found in the salt bladders. It has also been observed that as the leaf ages, bladders increasingly collapse or drop off

the leaves in all *Atriplex* species (Schirmer and Breckle 1982). A number of mechanisms that initiate the removal of the salt bladders have been proposed. These include the build up of salt concentrations within the bladder, reaching a critical concentration that triggers the removal (Batanouny 1993), and that new emerging bladders can burst existing bladders leaving a protective reflective coating of salt on the leaf surface (Batanouny 1993; Sharma 1982).

2.3.4.2 Abscission of Plant Parts

The abscission of salt-loaded plant parts and organs is another mechanism used by some halophytes to reduce high salt concentrations within the plant tissue (Batanouny 1993). One of the most notable examples are salt bladders, mentioned above. However, this strategy goes beyond salt bladders with some plants sacrificing leaves and stems to remove excess salts from the plant. One strategy that is used is the shedding of the stem cortex in the halophyte genera *Arthrocnemum* (Batanouny 1993), and *Allenrolfea, Halocnemum* and *Salicornia* (Chapman 1968). The cortex tissue is fleshy and high concentrations of salts can accumulate within it. The removal of the cortex also removes the photosynthesizing tissue and stomata, which in turn reduces the transpiring surface area, and salt transport to the stem through the xylem. Other authors have also concluded that the senescence of leaves is also a process to assist in the partial desalination of *Clerodendrum inerme* (Joshi and Mishra 1970), and with the *Salsola* and *Suaeda* genera (Batanouny 1993).

2.3.4.3 Salt Excretion

Salt glands are specilised secretory glands used by many halophytic dicots and monocots (Fahn 1988; Liphschitz and Waisel 1982; Waisel 1972). Salt glands are generally found on both surfaces of the leaves of plants. However they can also be found on stem surfaces in some species, such as *Statice pruinosa* which has a greater density on the stems than on leaves (Waisel 1972). Salt glands vary greatly in their structure between species, and range from simple to quite complex structures. The simplest glands, which are found in the *Poaceae*, consist of two cells: a basal cell collects the saline ions and a cap cell excretes the ions. These glands have been reported in over 30 species across a number of tribes (Amarasinghe and Watson 1989; Liphschitz and Waisel 1982). The cells contain a dense cytoplasm and a prominent nucleus, but lack a central vacuole (Liphschitz and Waisel 1982).

More complex salt glands also consist of basal cells and cap cells, but vary in the numbers of these cells. The more complex salt glands are found in members of the *Tamaricaceae* family. These are composed of eight cells, two of which are basal cells, while the remaining six are cap cells (Liphschitz and Waisel 1982). These authors provide a comprehensive list of plants that possess salt glands, including many that are not naturally found growing in saline soils.

While there are simple and more complex salt glands that contain many cells, there are also more subtle differences within the same type of glands. Differences have been shown to occur in the basal and cap cells, and also in the form of the salt glands (Liphschitz and Waisel 1982). The two main forms of salt glands are narrow trichome-like glands, and the semi-sunken glands which occur in grasses from the *Sprorobolus* and *Distichlis* genera. These sunken glands have been shown to have greater efficiency (salt excreted relative to the leaf salt content) in excreting salts from the leaf compared to the narrow trichome like glands (Liphschitz and Waisel 1982). These authors also found a positive correlation between the basal cell dimensions and excretion efficiency, with salt glands containing larger sunken basal cells being able to excrete more efficiently.

Excretion of salts from salt glands is generally selective, meaning that certain ions are excreted in preference to others. However there are a number of species, where secretion is not selective, resulting in ions being excreted in similar relative concentrations that occur in the external media. An example of non-selective secretion occurs in mangroves (Scholander *et al.* 1962), where the ionic ratios of the secreted solution are similar to those in sea water in which the mangroves are growing. However, halophytic grasses are generally more selective with their salt excretion, with most species excreting more Na^+ and Cl^- ions relative to other ions (Pollak and Waisel 1979; Rozema *et al.* 1981).

The process of salt excretion from salt glands is not well understood. The presence of many ribosome's and large nuclei and numerous organelles such as mitochondria within the cytoplasm of the secretary glands suggests that excretion is an energy dependant process (Fahn 1988). Various factors have been found to affect the rate of ion excretion from salt glands including light (Drennan and Pammenter 1982; Pollak and Waisel 1970), metabolic inhibitors (Kobayashi *et al.* 2007), relative humidity (Ramadan 1998), temperature (Pollak and Waisel 1979) and salinity in the growing media (Drennan and Pammenter 1982). However, this work has not identified the mechanisms of salt excretion, but rather identified what factors influence the salt excretion from salt glands. Pollak and Waisel (1979) concluded that high light intensity increased salt excretion due to the indirect effect of increasing transpiration. This theory makes sense, and could also be expanded to explain how temperature affects excretion rates. However, high relative humidity

was also found to increase salt excretion (Pollak and Waisel 1979), which can not be attributed to increased transpiration.

While the precise mechanisms of salt excretion are not yet known, the evidence points to at least a number of mechanisms being involved. As previously mentioned, there are the indirect passive mechanisms that affect the transpiration stream such as temperature and light, and therefore affecting the salt load within the leaves. However, Fahn (1988) suggested that a more active process of excretion was at work. If salt excretion was primarily an active process, then it would be expected that any increase in salt load for the plant would reduce dry matter growth as extra energy would be required to excrete the excess salt. However, the growth of some halophytes is stimulated in the presence of salt (Greenway 1968). *Distichlis spicata* is one such halophyte in which growth was stimulated by the presence of salt; maximum growth occurred in lysimeters in full sunlight when irrigated with saline water at 30 dS m^{-1}, compared to those that were grown at 2, 6, 10, 16 and 24 dS m^{-1} (Shannon *et al.* 1998). However, Sargeant (1999) found that salt had no stimulation effect on the same species when grown in growth cabinets.

2.4 Halophytes in the farming system

The utilization of halophytes for agricultural production makes sense in order to achieve some productive use from saline land. Halophytes are currently used in agricultural systems in different countries to differing degrees. There is a history of using halophytes, with reports of the Cocopa Indians collecting seed from the halophyte *Distichlis palmeri* for human consumption (Yensen *et al.* 1985). A comprehensive list of halophytes that are used by humans, the way they are used, and regions where they are being used, has been provided by Leith and Lohmann (2000). An expanded summary is provided in Table 2.2.

Table 2.2. A list of halophytes that are currently used for agricultural production, landscaping and ornamental purposes.

Name	Country	Use	Reference
Aster tripolium	Belgium, Netherlands, Portugal & Pakistan	Used as a vegetable (in salads), cash crop and ornamental flower	(Lieth and Lohmann 2000)
Atriplex spp.	Australia	Livestock grazing	(Barrett-Lennard 2003b)
Avicennia germinans	Pakistan & Columbia	Used as a cash crop	(Lieth and Lohmann 2000)
Avicennia marina	Pakistan, Columbia, United Arab Emirates & Eritrea	Used as a cash crop and wildlife habitat	(Cribb 2004; Lieth and Lohmann 2000)
Batis maritime	United Arab Emirates	Used for roadside plantings	(Lieth and Lohmann 2000)
Distichlis palmeri	USA & Mexico	Used as a grain by the Cocopa Indians	(Yensen *et al.* 1985)
Distichlis spicata	Australia, Spain, USA, United Arab Emirates	Used for livestock grazing and as a turf	(Yensen 2004)
Maireana brevifolia	Australia	Livestock grazing	(Barrett-Lennard 2003b)
Paspalum vaginatum	Australia	Livestock grazing	(Barrett-Lennard 2003b) (Lieth and Lohmann 2000)
Salicornia fruticosa	Europe	Vegetable oil production	(Lieth and Lohmann 2000)
Sesuvium portulacastrum	Morocco & United Arab Emirates	Ornametal plan used for roadside plantings	(Lieth and Lohmann 2000)
Siedlitzia rosmarinus	Arabia	Leaves used for detergent	(Lieth and Lohmann 2000)
Tamarix amnicola	Africa	Wood fuel	(Lieth and Lohmann 2000)

A major initiative within Australia to assist landholders to make productive use of saline soil has been the series of conferences with the title 'Productive Use and Rehabilitation of Saline Lands (PUR$L)'. These were held across Australia between 1990 and 2005. These conferences involved a network of people who were actively involved in saline land management. Papers presented at these conferences have demonstrated that saline land can be used productively (Badawy N *et al.* 1994; Bathgate and O'Connell 2001; Darling 1999; O'Connell and Young 2002). In addition the conferences have given indications of the management practices required for profitable agricultural production on saline land.

A more recent initiative has been the formation of the Co-operative Research Centre (CRC) for Plant Based Management of Dryland Salinity, and its successor, the Future Farm Industries CRC. The CRC's aim is to increase the understanding of the agricultural landscape, and how to provide new plant-based systems to address the problem of salinity, and to improve the viability of agricultural businesses that are affected by salinity in rural Australia. There are a number of publications including "Focus on perennials" and "Salt magazine" put out by the CRC that outline recent research and profiles of leading landholders and their efforts to combat salinity problems on their farm.

Internationally, one of the major centers of salinity work is being conducted at the International Centre for Biosaline Agriculture (ICBA) at Dubai in the United Arab Emirates. The centre was established to generate new knowledge and technology involving the use of saline water for irrigation, and to spread this information to farmers who rely on saline water for crop production. The research includes screening for salt tolerance, the storage of genetic material, field demonstrations within neighboring countries and training of local managers and farmers (ICBA 2007).

2.4.1 Benefits of using halophytes in the farming system

A survey of landholders was conducted in Western Australia to assess how salinity was impacting on communities (McLarty and I'Anson 2002). These authors reported that the major impacts of salinity included loss in production, loss of farm income, damage to infrastructure, loss of spending power, poor aesthetics, decreasing population, depression, loss of employment and businesses and a loss in the native flora and fauna. Thus there were a series of social, environmental and economic impacts. The survey found that the revegetation of saline land with halophytes, and salt tolerant glycophytes, has delivered many positive outcomes. These go a long way towards reversing some of the damage caused by salinity, both socially, environmentally, and in terms of farm production and farm profitability (Heuperman 1999; Payne 2002; Walsh *et al.* 2002).

Initially it was thought that the adoption of halophytes within the farming system would result in improved farm production. However one of the major benefits from this successful management of saline land involves personal satisfaction, confidence and pride gained from the successful management (Bennett and Price 2007). The improved social benefits include, but are not limited to, the improved visual amenity of the area, an enhanced feeling of satisfying social responsibilities and contributing to overall catchment health, and achieving personal and family goals in farm management (Bennett and Price 2007).

There are also real benefits for the environment from the revegetation of saline land with halophytes. One of the benefits is the lowering of the watertable. It had been suggested by Barrett-Lennard (1999) that the use of halophytic stands should lower watertables, after it was shown that small blocks of *Atriplex* species used significant amounts of ground water. The development of more appropriate statistical methods has also shown that *Atriplex* species are capable of lowering the groundwater below the capillary fringe (Ferdowsian *et al.* 2002). This is an important finding, as the capillary fringe is the depth at which soil water can rise to the surface by capillary action, bringing dissolved salts with it. Therefore, by reducing the water to below this depth, the soil water and dissolved salts cannot reach the soil surface. Research conducted at Tammin in Western Australia has also shown that basal respiration of soil microbes, and total soil microbial mass both increased greatly where *Atriplex* had been established, compared to adjacent unimproved saltland. Improved biodiversity has also been observed at a serial biological concentration scheme near Shepparton in Victoria. This site was established with *Atriplex* and *Eucalyptus* species and irrigated with saline ground water. After 5 years, over 50 native bird species had been observed at the site (Cornwall *et al.* 2002).

Despite the environmental and social benefits from revegetating saline land, there are also potential economic gains to be made by landholders with the adoption of halophytes and salt tolerant glycophytes. Numerous case studies of farms with significant saline areas have shown increased carrying capacities where adoption of saltland pastures has occurred (Bruce 2003; Kilminster *et al.* 2002; Payne 2002). Generally this increased carrying capacity arises from bringing land that was abandoned back into production with salt-tolerant forages. There is also the added benefit of out-of-season forage production in southern Australia, making the forage even more valuable (Payne 2002). This occurs from the summer growth of the saltland pastures during the summer period. Bathgate and O'Connell (2001) point out that feed produced during scarce times can be 4-10 times more valuable than when feed is in abundance. This out-of-season forage production can reduce the need for supplementary feeding of stock throughout the summer months, which can be quite costly and is a major financial driver for the adoption of saltland pastures (O'Connell *et al.* 2006). Numerous case studies of farmers who have adopted saltland pastures have indicated that this out-of-season forage has meant that carrying capacities of the farm have been increased as a result. One case study points out how a farm in the low rainfall region of Western Australia (310 mm annual rainfall) increased their carrying capacity from 3.4 DSE per hectare prior to the establishment of saltland pastures to 6.2 DSE per hectare after investing in establishment and good management of these pastures (Kilminster *et al.* 2002). Another case study at Dunkeld in the higher rainfall region of Victoria found that the adoption of

saltland pastures not only increased the carrying capacity due to the out-of-season forage, but also allowed better pasture management through spelling of paddocks (Payne 2002).

There has also been some anecdotal evidence that the meat from sheep that graze on saltbush pastures is tastier than that grazed on non-saline pastures. In response to this view, a supply chain has been created to supply meat from sheep grazed on saltbush pastures to restaurants throughout Australia (Pearce 2006). However, research does not support this anecdotal evidence, with no improvement in flavour or acceptance of the meat from sheep grazed on saltbush pastures (Pearce 2006). While there were no measurable differences detected by tasting pannels, the carcasses from sheep grazed on saltbush pastures were more hydrated, had a lower fat deposition and the meat maintained an acceptable red colour longer than that from sheep grazed conventional pastures (Pearce 2006; Pearce *et al.* 2004). It was also noted that a diet of saltbush alone would not produce adequate carcus weights, and that supplementary feeding was required to ensure adequate weights (Pearce 2006).

2.4.2 Selecting suitable salt tolerant plants

The selection of salt-tolerant species for a particular saline site requires careful consideration. Issues to consider include the severity of the salinity, whether the site is permanently waterlogged or seasonally waterlogged, and the intended use of the site. Many salt tolerant species are extremely salt tolerant such as the *Atriplex* spp., but are not tolerant of waterlogged conditions, while others such as balansa clover (*Trifolium michelianum*) are more tolerant of waterlogged conditions, but not so tolerant of salinity. A matrix of the salinity and waterlogging tolerance (Fig. 2.1) of a number of salt-tolerant plants used in Australia has been developed by Barrett-Lennard (2003b). This provides a guide to species that are tolerant to both waterlogged and saline conditions. One of the more promising halophytes that is highly tolerant of waterlogged conditions and saline soils is *Distichlis spicata*.

2.4.3 Distichlis spicata

Distichlis spicata is a C4 halophytic grass that has been identified as a suitable species for forage production, and has subsequently been the subject of a program to select more suitable forage cultivars (Yensen 1997). The grass has been described as an important pioneer plant in the revegetation of saline soils and has many attributes that ensure that it is successful for this task (Hansen *et al.* 1976). These include morphological and anatomical adaptations such as sharp pointed rhizomes which are high in silica-containing cells near the tip. These rhizomes also contain an aerenchyma network, running the length of the rhizomes, which enables gas exchange to occur through the root system, enabling growth in waterlogged soils (Hansen *et al.* 1976). The spread of rhizomes enables the grass to colonize adjacent soil and to transport water, air and nutrients to new tillers or plants growing in less favorable conditions. The two major salt-tolerance mechanisms used by *D. spicata* are salt excretion from salt glands in the leaves and stems, and the ability to osmoregulate (Hansen *et al.* 1976; Marcum 1999). The salt glands of *D. spicata* are the sunken type, and are consequently very efficient at excreting salts from shoot tissue and are quite ion specific for Na^+ and Cl^- ions (Liphschitz and Waisel 1982).

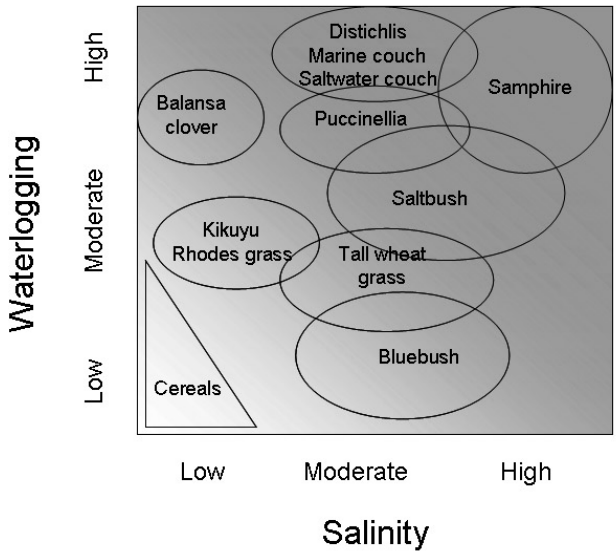


Figure 2.1. The salinity waterlogging matrix for many of the common salt tolerant plants used in Australian agriculture. (Taken from Barrett-Lennard (2003b)).

Selections of *D. spicata* were made by Dr. N. Yensen during the 1980s and resulted in the patent for *D. spicata* cv. Yensen-4a (NyPa Forage) as a forage grass. Selections were based on qualities such as soft leaves, increased growth rates and low salt concentrations in the shoots (Yensen and Bedell 1993). *Distichlis spicata* cv. yensen-4a is currently being grown in the United States of

America, United Arab Emirates, Spain and Australia. The largest areas are located within Australia (Leake *et al.* 2002; Yensen 2004).

The introduction of a new pasture species into the farming system brings with it many questions about management, and how the species might best be used in the farming system. Management questions range from nutrient and grazing management to establishment techniques. The establishment of plants on saline sites can be quite difficult due to the hostile conditions found at saline sites (Barrett-Lennard 2003b). For a successful establishment to occur, the process must occur when conditions are most conducive. There needs to be minimal competition, good soil moisture, favorable soil temperatures and minimal soil salinity stress (Malcolm *et al.* 2003). Although halophytes are salt tolerant, seed germination is suppressed by high salt concentrations (Ajmal Khan *et al.* 2002; Ajmal Khan *et al.* 2006; Barrett-Lennard 2003b; Myers and Morgan 1989). Although high salt concentrations may inhibit germination, this may be a mechanism to ensure that germination occurs during periods of higher rainfall to ensure a more favorable environment for establishment. Tobe *et al.* (2000) found that the seeds of the halophyte *Kalidium caspicum* can remain ungerminated and viable in saline topsoil. This is an important attribute, as it allows the seeds to germinate when rainfall lowers the salinity of the surrounding soil, allowing germination, and establishment. Despite this, niche seeding has been shown to be a less reliable form of establishment of *Atriplex* spp., with a 2 year study showing a 18% survival rate of seeds, compared to a 90% survival of nursery-raised seedlings that were then transplanted into the saline soil (Barrett-Lennard *et al.* 1991).

Distichlis spicata cv. yensen-4a is a male clonal selection, and consequently does not produce seed and needs to be established with vegetative cuttings. However minimal research has been conducted on the vegetative establishment of *D.* spicata cuttings in saline soils. One study by Pavlicek *et al.* (1977) demonstrated that *D. spicata* can be successfully established from rhizomes, with an optimum temperature of 25-30 °C for sprouting, and were able to tolerate a wide range of osmotic potentials to less than -22.8 bar, which is equivalent to 0.5 M NaCl. Within Australia, *D. spicata* cv. yensen-4a has been established by planting bare rooted plantlets that were harvested by hand (Sargeant 2003). This technique is both time consuming, expensive and has had mixed success, presumably due to the large spatial variation in soil salinity found in saline discharge sites (Semple and Koen 2004).

Management practices to optimize the forage production from *D. spicata* have not been developed. Few management recommendations are provided by Yensen (1997), apart from mentioning that high nitrogen fertiliser is useful. Suggested application rates of 100 kg N ha^{-1} yr^{-1} would be expected to increase yields significantly. This recommendation is also supported to

work by Smart and Barko (1980), who found that repeated application of nitrogen fertiliser increased dry matter production . Sullivan (1981) also found that nitrogen application to *D. spicata* growing in a salt marsh increased initial dry matter production in unclipped plots, and also the re-growth in clipped plots. Clearly more information is required about the nutrient management of this species if it is to be successfully used as an out-of-season forage and to ensure that production is maximized.

The feed value of *D. spicata* is also another area where there is little published information available. Yensen (1997) provided limited information about the chemical composition of *D. spicata* cv. yensen-4a and reported that yearling calves grew as well on a diet consisting of *D. spicata* compared to a conventional formulated diet. However, no data were provided on the diets, or on the way the performance of the yearling calves was measured. Leake *et al*. (2002) also provides some insight into the feeding and nutritive value of *D. spicata*. He reported that crude protein concentrations were in the range of 5-17%, *in-vitro* dry matter digestibility values in the range of 45-61% and metabolisable energy in the range of 6.2 – 7.5 MJ ME kg^{-1} DM. Leake *et al*. (2002) also reported that the range in nutritive value was quite wide, ranging from very low to moderate nutritive values. Clearly a greater understanding is required on the impact of management on nutritive value, if successful animal production is to be achieved from this halophytic forage grass.

When assessing the nutritive value of saltland pasture systems, care needs to be taken to ensure that the results reflect the likely animal performance from such a system. Masters *et al.* (2001) provides a comprehensive review of the errors involved in the measurement of the nutritive value of saltland pastures, with the major error involving the measure of digestibility. The error results from the overestimation of dry matter digestibility, as the soluble salts within the plant tissue 'appear' to be digestible as they dissolve in the digestion solution. Dry matter digestibility is measured by incubating a known weight of the plant material into a solution of metabolites and enzymes. This simulates the action of digestion in the rumen. However, the problem occurs when NaCl is present in the plant material, as this dissolves in the solution. The digestibility is determined by removing the plant material following the digestion process, and determining how much of the sample has disappeared. The dissolution of the salt in this manner has led to overestimations of digestibility of around 20 % in the case of *Atriplex* samples (Tiong *et al.* 2004). As metabolisable energy is calculated from digestibility values, then this value is also overestimated.

2.5 Conclusion

Soil salinity is a major constraint for conventional agricultural crops and pastures in many areas in southern Australia. Salt has been part of the Australian landscape well before European settlement, with saline water being found in many inland rivers by the early explorers. While there are many glycophyte species that exhibit some degree of salt tolerance, such as barley, there are many areas where salinity is too high for the growth of these conventional species. The use of halophytes in these situations may provide a productive system for utilizing these saline soils. As discussed earlier, halophytes have previously been used as a major food source for the indigenous Cocopa Indian population, where they collected seeds of the halophyte *D. palmeri* (Yensen *et al.* 1985).

Halophytes have also been used quite extensively within the farming system. An example is where saltbush (*Atriplex* spp.) is used for grazing. This species has proven to be quite useful in providing feed throughout the summer period in southern Australia. However, a diet of saltbush alone is not ideal for sheep performance, and supplementary feeding or access to other pasture species, are required to ensure acceptable animal performance. Saltbush pastures are also not suited to waterlogged conditions, which are common in saline soils in southern Australia.

Distichlis spicata cv. Yensen-4a is a C_4 halophytic grass that was introduced into Australia as a fodder grass for wet saline soils in the mid 1990s. Since its introduction, it has been used successfully as a fodder species in the mixed farming zone of Western Australia. Initial observations have shown that this pasture grass performs well in wet saline soils, is quite palatable, and produces a significant amount of green summer feed (Leake *et al.* 2002). However, there is very limited information available about how to best manage this new pasture species, and whether such a system is sustainable on saline soils. Information on how to best manage a *D. spicata* sward has been limited to the observations that the grass responds to nitrogen fertilizers. No information is available about how to establish this species other than anecdotal evidence from landholders, or how to manage the grazing of this species to maximise production and nutritional value.

The research documented in this thesis aims to address these gaps in knowledge. Three key objectives will be addressed. The first is to determine whether salt accumulates in the root zone of a *Distichlis spicata* cv. Yensen-4a pasture, which would threaten the long-term sustainability of the pasture. The second aim is to determine how the grass might be established in saline soils. The final aim is to develop management guidelines for grazing the *D. spicata* pasture, and for supply of nutrients to the halophytic grass to optimize pasture yield and quality. The specific

hypotheses for each experimental chapter is listed in the Table below. However the scientific rationale behind these hypotheses will be developed further in the introduction of the relevant chapters.

Table 2.3 Experimental chapter hypotheses.

Chapter	Hypothesis
3	Growth of *D. spicata* improves the chemical and physical properties of the salinised soil in which it grows
4	Salt priming improves the establishment of *D. spicata* plants in saline growing media
5	High light levels increase the salt tolerance of *D. spicata* by increasing the salt secretion from the salt glands
6	Establishment success can be improved by adopting different planting techniques and by altering the planting window
7	Rhizome fragments can initiate growth under a range of moisture and salt regimes
8	High rates of nitrogen and phosphorus will increase feed quality by altering the plant morphology of *D. spicata*
9	Grazing frequency and nutrient inputs affect plant persistence, dry matter production and feed quality.

Part I

Sustainability of *Distichlis spicata* swards on saline land

Chapter 3

The ability of *Distichlis spicata* to grow sustainably within a saline discharge zone while improving the soil chemical and physical properties

3.1 Introduction

Soil salinity is a major issue within many Australian farming systems throughout southern Australia. The area affected by or at risk to salinity, due to high or rising water tables, was estimated at 5.7 million hectares in 2000, with the potential to reach 17 million hectares by 2050 (National Land and Water Resources Audit 2001). These sites are generally regarded as degraded from a chemical and physical sense, with high concentrations of dissolved salts within the root zone, and are commonly waterlogged. There are very few ways that landholders can use these degraded areas for commercial use. In most cases they are abandoned and lost from the farming system.

Distichlis spicata cv. yensen-4a is a halophytic pasture grass that has been selected for forage production on saline land (Yensen and Bedell 1993; Yensen *et al.* 1995). The grass was introduced into southern Australia in 1994 and trialed to determine its suitability for forage production on saline farmland (Leake *et al.* 2002). During this time, research has been focused on establishment techniques and forage potential (Sargeant 2003). Since its introduction, *D. spicata* has been able to be established successfully in a seasonally waterlogged saline discharge site where it has been able to grow and continually spread.

One of the major benefits of growing *D. spicata* on saline discharge sites is its ability to produce green forage throughout the summer months when it grows actively. In the past the feed value of *D. spicata* has been questioned. However, unreplicated test strips conducted by farmers have shown that this halophytic pasture species can produce forage that is suitable as a maintenance diet for ruminants throughout the summer period, with ash concentrations ranging from 10-12%, metabolisable energy values up to 9.5 MJ ME kg^{-1} DM and crude protein up to 17%, when the sward has been well managed (personal communication R. Matthews). This feed is produced

throughout the warmer months of the year, at a time when there is very little standing green feed available, and supplementary feeding of stock to maintain live weights is common.

A number of landholders have observed that the condition of the saline soils have improved where *D. spicata* had been grown for a number of years. The soils appeared to "hold together" better, and in some cases the area has been re-colonized by less salt tolerant species (personal communication R. Matthews). A rhizocanicular effect has also been hypothesized, whereby decaying roots and rhizomes will leave a network channels throughout the soil profile which would increase water percolation (Yensen 1997).

This paper reports on a field survey that was set up to test the hypothesis that the growth of *D. spicata* improves the chemical and physical properties of the salinised soil in which it grows. An extensive field survey was carried out at a discharge site where a sward of *D. spicata* had been growing for up to 8 years. Two other discharge sites located on the property were also sampled where *D. spicata* had been growing for 5 years. Soil properties were compared between different aged *D. spicata* swards to test this hypothesis.

3.2 Materials and Methods

3.2.1 Site description

Soil sampling was conducted at three saline discharge sites on a property at Wickepin in Western Australia. These sites were established with *Distichlis spicata* cv. yensen-4a between 5 and 8 years prior to the initial sampling period. There were adjacent areas where no *D. spicata* was planted which were used as controls. These areas had been intentionally left unplanted to allow future comparisons to be made. All of these sites were established by vegetative means. The mother plot was managed as a *Distichlis* dominant pasture and grazed regularly throughout the summer when sufficient growth had occurred. The sand plain site was at the end of a cropping paddock, and was also grazed over the summer period. The Flats site was a small area within a larger saline paddock that was opportunistically grazed throughout the year.

3.2.2 Rainfall

The average annual rainfall at Wickepin is 411 mm and this predominantly falls throughout the winter months (Table 3.1 and Fig. 3.1). Since 1997, when the mother plot area was established (see below), the annual rainfall at Wickepin has generally been less than the long-term average. There were only 3 out of the 10 years when rainfall exceeded the average (Table 3.1). The farm is located approximately 25 km east of Wickepin, and so, the actual rainfall at the survey site was less than that at Wickepin. Figure 3.1 shows that prior to the first sampling period in 2005, autumn rainfall exceeded the average, while winter rainfall was below average. In 2006, monthly totals were below average in the months leading up to the sampling period.

Table 3.1. Seasonal and total yearly rainfall data for Wickepin since the *D. spicata* sward was established.

Summer rainfall for each year, including the December rainfall of the previous year, whereas yearly rainfall is for the calendar year. (Data supplied by the Australian Bureau of Meteorology).

Year	Summer rainfall (mm)	Autumn rainfall (mm)	Winter rainfall (mm)	Spring rainfall (mm)	Total yearly rainfall (mm)
1997	44	108	137	61	346
1998	0	102	205	74	395
1999	22	121	204	91	440
2000	128	41	187	24	364
2001	2	42	181	65	319
2002	46	54	120	76	277
2003	58	148	202	78	478
2004	17	48	174	82	321
2005	7	205	149	75	439
2006	140	66	125	47	375
Long term average 1912-2006	39	99	197	76	411

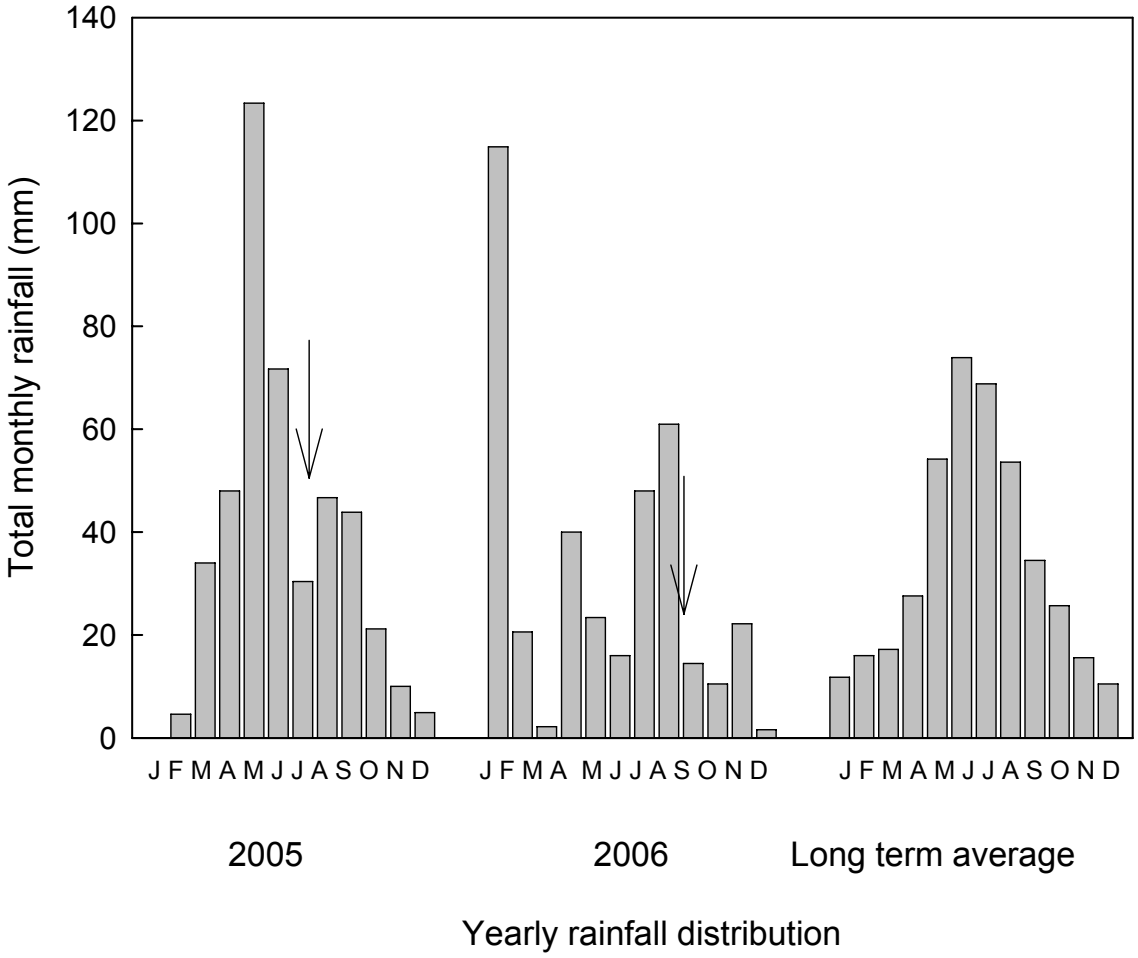


Figure 3.1. The monthly rainfall distribution throughout 2005 and 2006, along with the long-term average (1912-2006), at Wickepin. Arrows indicate when the two sampling periods occurred. (Data supplied by the Australian Bureau of Meteorology.)

Mother Plot. The mother plot site (32°42'15"S, 117°44'30"E) (Plate 3.1) was established with *D. spicata* in 1997. This site is located on the upper edge of a saline discharge site, which is half-way down a gentle slope, with a cropped paddock above the plot. A surface drain is located upslope from the site that runs along the contour of the land. The control area, where no *D. spicata* plants were established, occurred downslope of the established area. The control area was approximately 20 cm lower than the 8 year old treatment, which was 10 m upslope; it had a patchy covering (approximately 50%) of immature sea barley grass (*Hordeum marinum*). The soil textures at this site were sand in the top 10 cm, sandy loam in the 10-20 cm layer, and sandy clay loam in the 20-30 and 30-50 cm layers. Soil pH $_{(water)}$ ranged from 6.6 to 6.8 in the topsoil, and increased with depth to 6.8 to 7.0 at 30-50 cm depth.

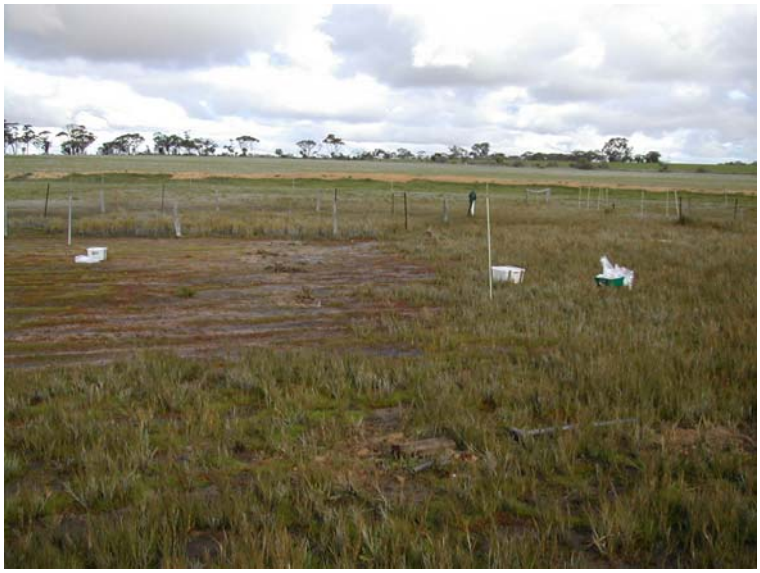


Plate 3.1. The mother plot area showing the *D. spicata* in the foreground and background, with the bare area used for comparison in the centre.

Sand Plain. The sand plain site (32°42'50"S, 117°44'54"E) (Plate 3.2) was established with *D. spicata* in 2000. The area was planted as a transect down the slope, with the adjacent area that was not planted also located down the transect. This control area also had patches of sea barley grass that covered approximately 50% of the soil surface. The soil pH $_{(water)}$ ranged from 5.5 to 6.3 in the topsoil and 5.6 to 6.1 at 20-30 cm depth. The soil had a sand texture at all depths measured (0-10, 10-20 and 20-30 cm).

Plate 3.2. Overlooking the sand plain site, showing the *D.spicata* on the left hand side, and the area on the right used for comparison.

Flats. The flats site (32°40'60"S, 117°45'0"E) (Plate 3.3) was also established with *D. spicata* in 2000. This site was planted in a square block in a discharge site. The adjacent control soil was located 10 m upslope from the established area and was covered in a patchy covering of sea barley grass that covered approximately 50% of the soil surface. The soil textures at the three sampling depths (0-10, 10-20 and 20-30 cm) were sandy loams. Soil pH $_{(water)}$ ranged from 6.3 to 6.8 in the top soil, and increased with depth and ranged between 6.6 and 7.4 at the 20-30 cm depth.

Plate 3.3. Looking across the flats site, with the *D. spicata* in the middle of the picture and the area used for comparison in the foreground.

3.2.3 Sampling Procedure

Destructive soil samples were collected from all sites in July 2005 and again from the mother plot in September 2006. The mother plot was sampled more extensively, with destructive samples taken in 2005 and 2006 from 0-10, 10-20, 20-30, 30-50 and 50-70 cm using a 50 mm diameter sampling tube, with intact cores taken in 2005 from 0-6, 10-16 and 20-26 cm depths. Obtaining samples from the 50-70 cm depth proved to be quite problematic, and hence only samples from the 8 year old treatment were collected. All sampling profiles were replicated 6 times. Soil samples were taken from three treatment areas at the mother plot. These were: (i) from the adjacent bare area where no *D. spicata* or any other significant vegetation grew; (ii) from the spreading margin of the grass where it was estimated that *D. spicata* had been growing for 2 years; and (iii) where *D. spicata* had been growing for 8 years. A second control was taken a further 5 m down slope of the main control to see if there was any major gradient in soil EC values down the slope. The Sand Plain and Flats site were only sampled in 2005 with destructive samples at 0-10, 10-20 and 20-30 cm depths, with 5 replicated profiles being sampled from the 5-year old *D. spicata* stand, and the adjacent control areas.

3.2.4 Soil Measurements

The intact cores were used for saturated hydraulic conductivity and root density measurements, while the destructive samples from 2005 were used for determination of water-stable aggregates, pH, electrical conductivity and soil carbon and nitrogen.

Saturated hydraulic conductivity measurements were carried out using the constant head method of Klute and Dirkson (1986), with cores being stored in a cool room prior to analysis. Roots were recovered from the soil cores, after the saturated hydraulic conductivity measurements were taken, by washing the sample, and then the root length was measured with a Win Rhizo root scanner. Roots were then dried at 70 °C until a constant weight was achieved. Water stable aggregate measurements involved the random selection of 10 air dried aggregates (10 mm in diameter) from each of four replicates from each treatment. These aggregates were wet sieved in distilled water at 34 rpm with a stroke length of 20 mm for a period of 5 minutes through 2, 1, 0.5 and 0.25 mm sieves. These sieves were then dried in an oven at 120 ºC for 1 h and the retained dry aggregates on each sieve were weighed to determine the mass of aggregates of different sizes. Soil texture was determined mechanically for each soil depth, and the results used to correct for the sand particles and gravel component.

The Sand Plain site did not have water stable aggregate analysis done due to difficulty in obtaining aggregates from the samples after collection. Destructive samples from 2006 were only used for electrical conductivity determination to provide a second year of comparative data.

Soil carbon and nitrogen were analyzed by dry combustion using a CNH auto-analyzer (Elementar, Vario EL). Soil was prepared by air drying, and then sieving to pass through a 2 mm sieve. Only three replicates were used for carbon and nitrogen analysis, unless large variation in the measurements was found, and then the six replicates were used. Soil electrical conductivity and pH were measured in a 1:5 soil:water solution, after 5 g of air dried soil was shaken for 1 h with distilled water. Standard errors were calculated for the mean values for each soil measurement for each soil treatment (*D. spicata* age, soil depth and site).

3.3 Results

Eight years growth of *D. spicata* resulted in a much higher root length per soil volume at all depths up to 26 cm when compared to the control and the 2-year-old swards (Fig. 3.2). Although there was no difference in root lengths between the control and 2-year-old sward in the top 6 cm, the 2-year-old sward had a greater root length at depths of 10-16 cm and 20-26 cm. The control area, where no *D. spicata* had grown, had virtually no roots at 20-26 cm. Root dry weights followed a similar pattern to root lengths at all depths, with the greatest mass of roots occurring where *D. spicata* had been growing for 8 years. Again, the greatest mass occurred in the top 6 cm of soil across all treatments.

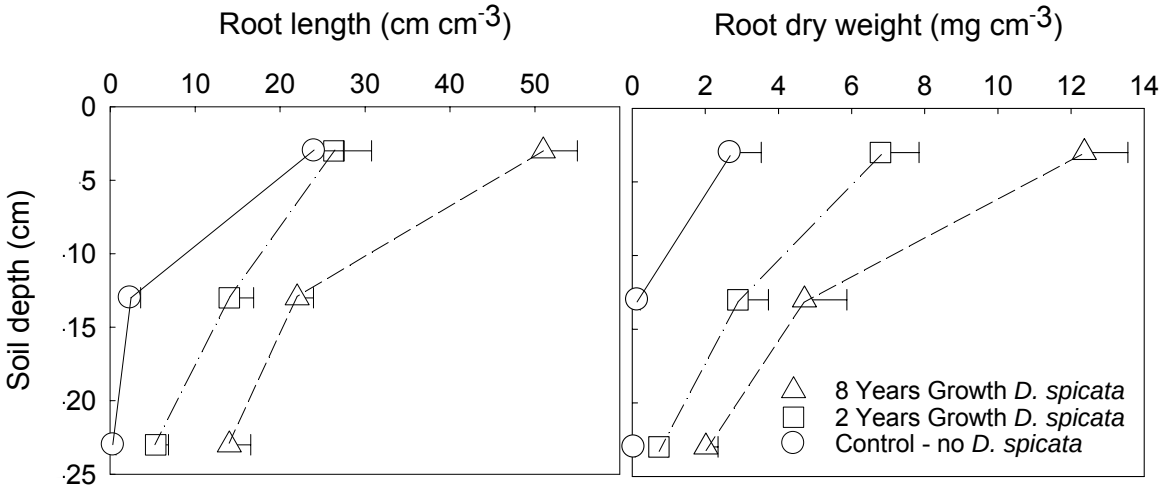


Figure 3.2. The effect of growth duration of the *D. spicata* sward in the Mother plot on plant root length and root dry weight of all plants at various depths. The measured root lengths include all roots (dead and alive) within the sample regardless of species. Error bars represent the standard error of the means (n=6).

Concentrations of soil carbon and nitrogen in the Mother plot were generally higher in the top soil, and then decreased with depth down the soil profile (Fig. 3.3). This pattern occurred regardless of treatment. However, soil carbon in the topsoil almost doubled in concentration to 0.75% after 8 years of *D. spicata* growth, compared with 0.40% for the control treatment. Smaller increases in soil carbon were also seen down the soil profile where there had been 8 years of growth. Two years of *D. spicata* showed a trend of higher soil carbon levels, compared to the control. Soil carbon concentrations tended to be higher at all depths (0-30 cm) where *D. spicata*

had grown for 5 years in the Sand Plain site. However at the Flats site soil carbon was similar within the 0-10 and 20-30 cm depths, and lower where *D. spicata* had been growing for 5 years at 10-20 cm depth (Fig. 3.3).

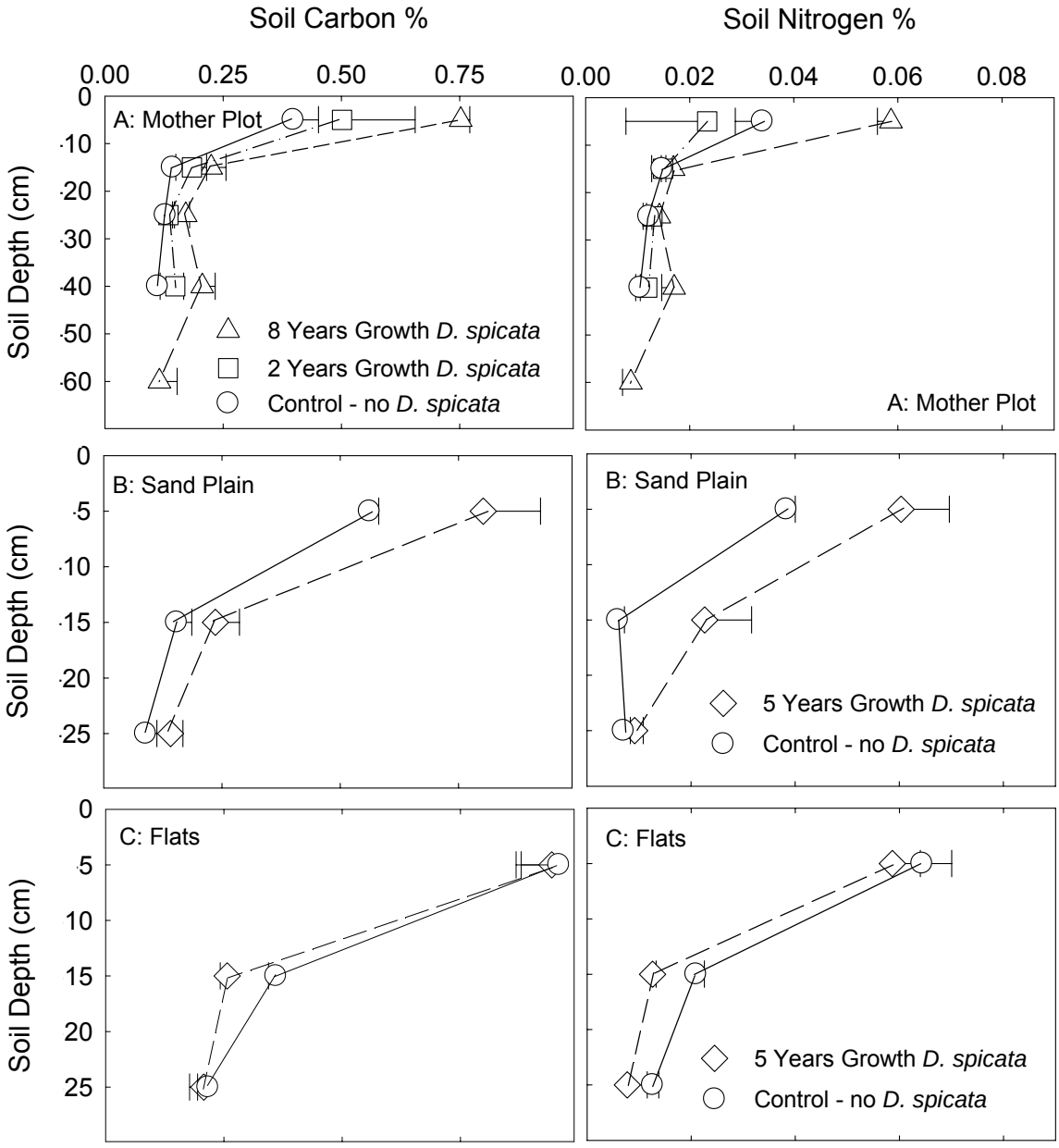


Figure 3.3. The percentage of carbon and nitrogen in soil layers where *D. spicata* has been grown for 0, 2, 5 and 8 years in a saline discharge site. Error bars represent the standard error of the means where n=3-6.

Soil nitrogen concentrations followed a similar pattern to soil carbon at all sites, with 8 years of growth at the mother site showing increases in the top soil (Fig. 3.3). However, these differences were not evident at depths of 10-20 and 20-30 cm and there were no notable increases in soil nitrogen where *D. spicata* had grown for 2 years.

Aggregate stability in the top 10 cm of soil was increased with 8 years of *D. spicata* growth in the Mother plot (Fig. 3.4). Almost three quarters of the remaining aggregates were greater than 2 mm in diameter after wet sieving for 5 minutes. Similarly, more large aggregates greater than 2 mm occurred in the 10-20 cm soil layers, after 8 years of growth, compared to the 2 year control areas. There were no notable differences between *D. spicata* treatments in soil aggregates in the 20-30 and 30-50 cm soil layers. The Flats site (Fig. 3.5) showed similar results to the Mother plot with a trend of more aggregates greater than 2 mm in diameter remaining in the 0-10 cm layer. However there was a large amount of variation within the control site. There were no significant differences in aggregates smaller than 2 mm in diameter between treatments at any site (data not presented).

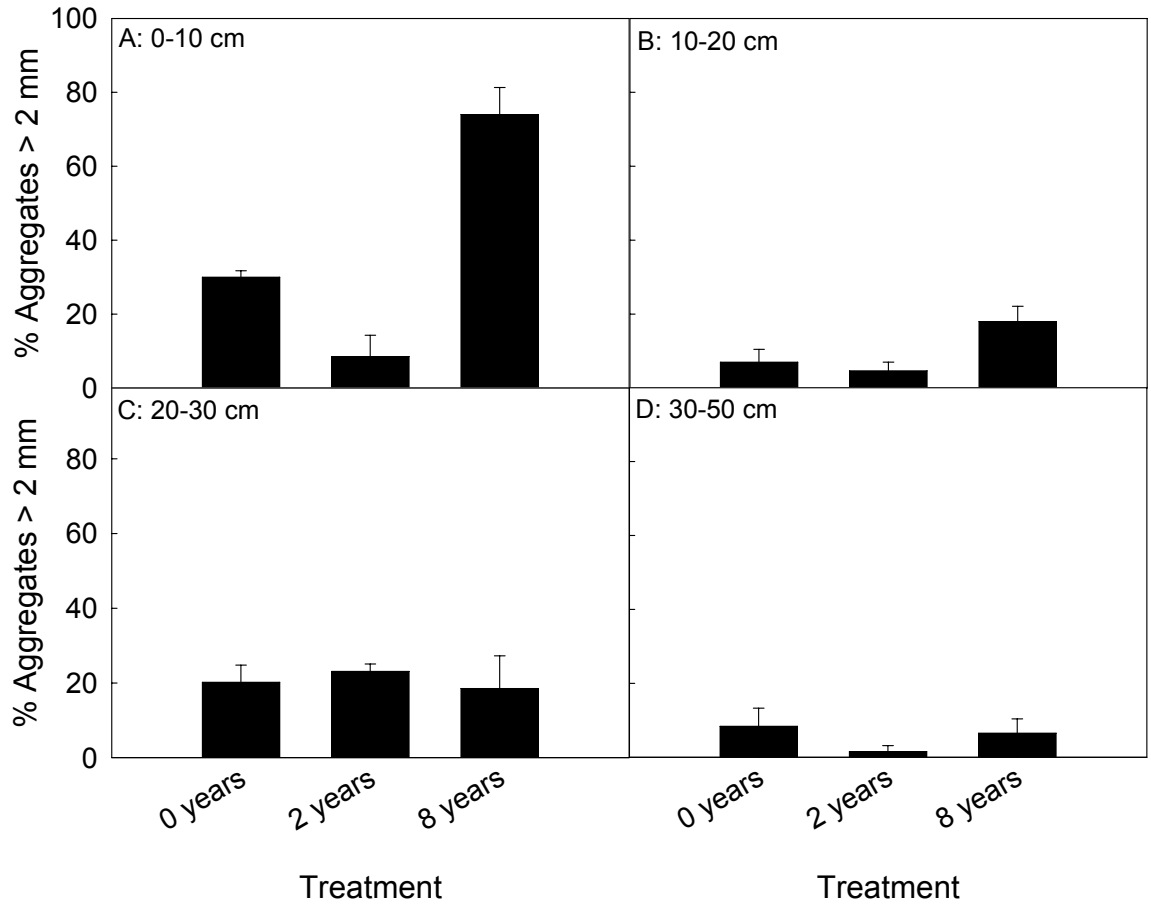


Figure 3.4. The effect of 0, 2 and 8 years of *D. spicata* growth in the Mother plot on the percentage of aggregates greater than 2 mm in diameter after wet sieving, in (A) 0-10 cm, (B) 10-20 cm, (C) 20-30 cm and (D) 30-50 cm soil layers. Error bars represent the standard error of the means (n=6).

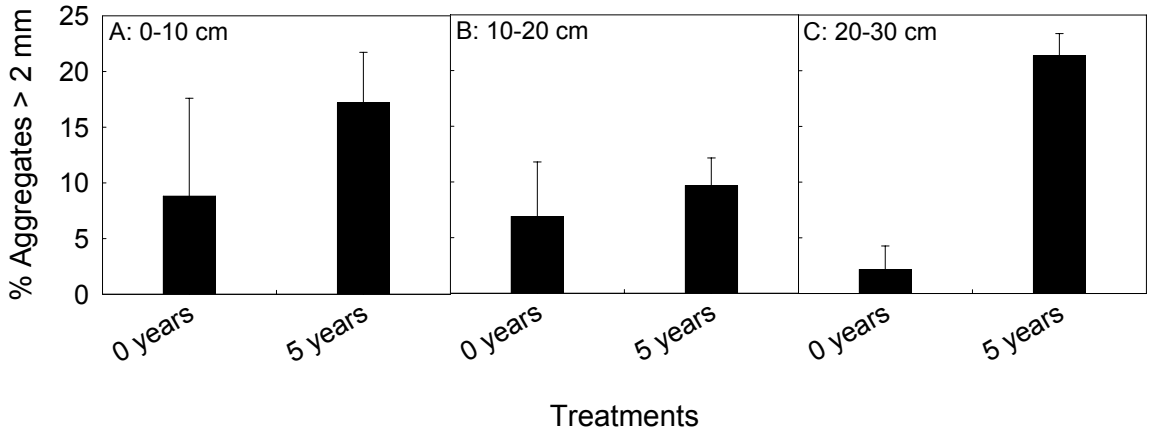


Figure 3.5. The effect of 0 and 5 years of *D. spicata* growth at the Flats site on the percentage of aggregates greater than 2 mm in diameter after wet sieving in (A) 0-10 cm, (B) 10-20 cm and (C) 20-30 cm soil layers. Error bars represent the standard error of the means (n=5).

The growth of *D. spicata* had a marked effect on the saturated hydraulic conductivity in this soil, particularly in the soil surface layer (Fig. 3.6). Where *D. spicata* had grown for 8 years in the Mother plot, the saturated hydraulic conductivity values in the surface 0-6 cm layer had increased 12 fold from 0.6 cm h^{-1} in the control to 7.4 cm h^{-1}. Two years of *D. spicata* growth resulted in more than a 3-fold increase in saturated conductivity values to 2 cm h^{-1} in the surface layer.

The effect of *D. spicata* growth on the saturated hydraulic conductivity decreased down the soil profile (Fig. 3.6). There was a 4-fold increase from 0.90 to 3.30 cm h^{-1} after 8 years of growth in the 10-16 cm soil layer, and only a small effect of *D. spicata* growth on conductivity in the 20-26 cm soil layer where values doubled from 0.5 cm h^{-1} in the control soil to 1.0 cm h^{-1} after 8 years growth.

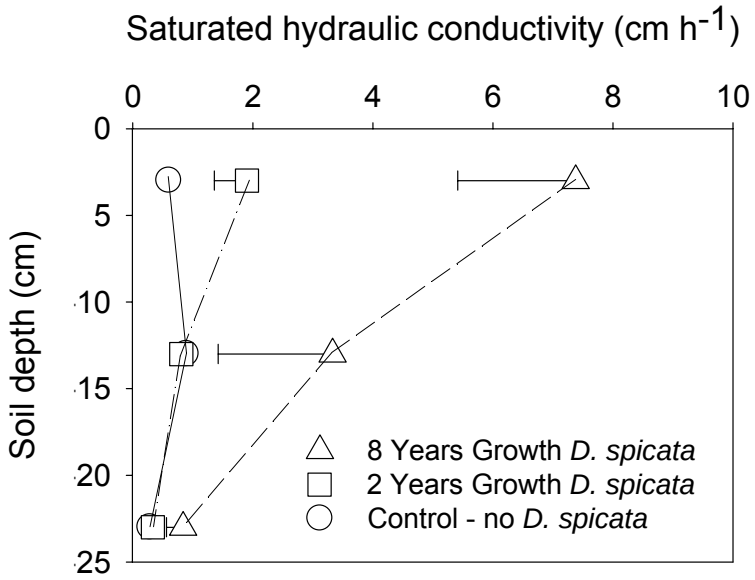


Figure 3.6. The effect of *D. spicata* growth duration on the saturated hydraulic conductivity of the soil at varying depths in the soil profile. Error bars represent the standard error of the means (n=6).

Electrical conductivity in the soil profile was also affected by *D. spicata* growth at the main sampling site in the Mother plot at the Wickepin discharge site (Fig. 3.7). In the wetter winter of 2005 there were lower electrical conductivity measurements in the top 50 cm of the profile where *D. spicata* had been growing for 2 and 8 years, compared to the control soil where no growth had occurred. The reduction in conductivity was most marked in the surface 0-10 cm layer where there was a halving in conductivity from 1.6 to 0.8 dS m^{-1}, for both *D. spicata* treatments. Conductivity increased in the 30-50 cm layer, but there were still lower conductivity values with the *D. spicata* treatments than the control treatment.

In the drier sampling period in the winter of 2006 *D. spicata* treatments continued to have lower electrical conductivity values in the 0-10 cm surface layer (Fig. 3.7) compared to the control area. The effect was greatest where *D. spicata* had been growing for 8 years, with a 50% reduction in electrical conductivity compared to the control soil. No differences in electrical conductivity occurred between treatments in the 10-20 cm and 20-30 cm layers.

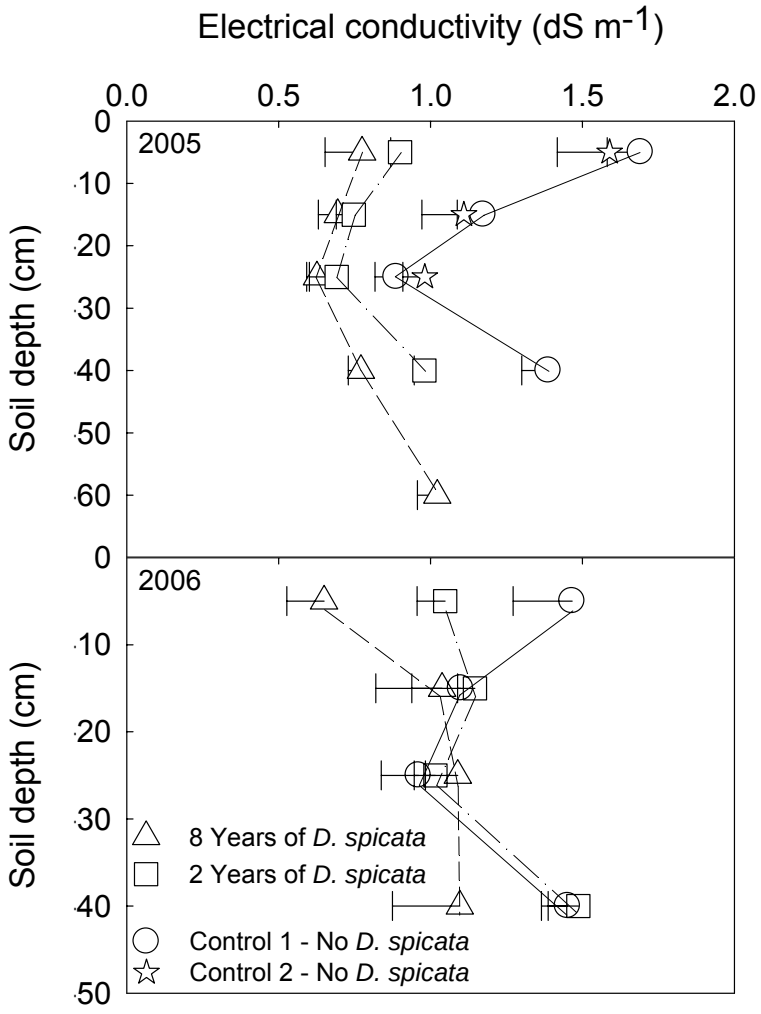


Figure 3.7. The effect of 0, 2 and 8 years of *D. spicata* growth in the Mother plot on the electrical conductivity (1:5, soil:water) at increasing soil depths, in 2005 and 2006 at the main sampling site at Wickepin discharge site. Control 2 was located 5 m down slope of control 1. Error bars represent the standard error of the means (n=6).

The soil electrical conductivity measurements at the additional sites show somewhat differing results (Fig. 3.8). At the Flats sites (A), the soil EC under the *D. spicata* was similar to the control in the top soil, but was higher than the control below 10 cm. At the Sand Plain site (B) electrical conductivity in the top soil under the *D. spicata* was lower than the control, but was similar in the 10-20 and 20-30 cm layers.

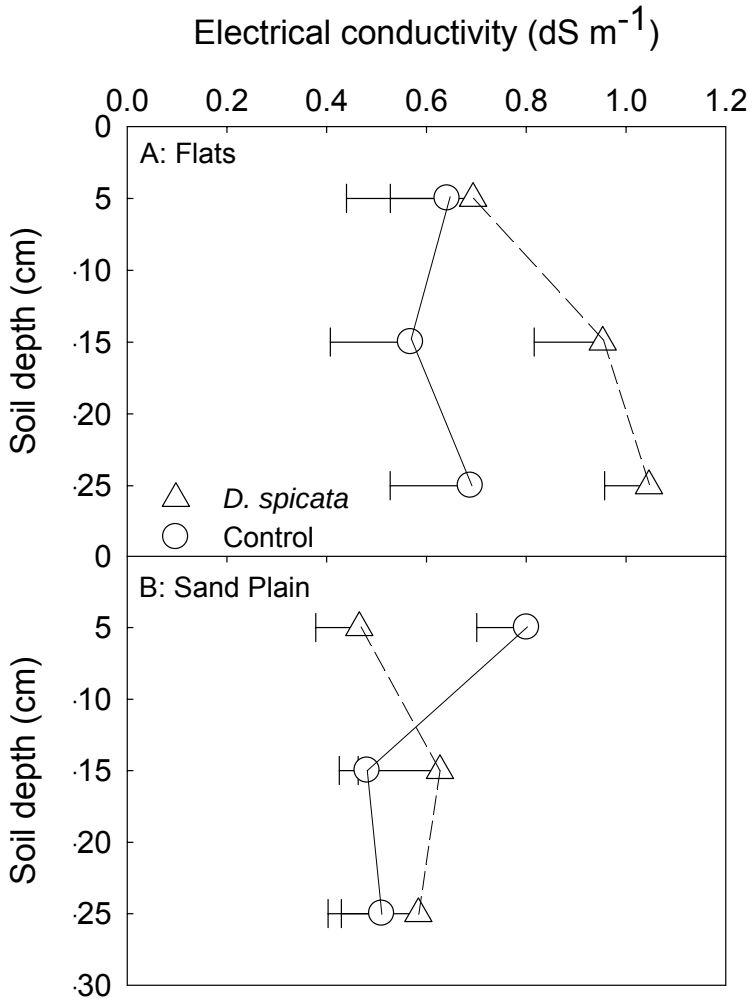


Figure 3.8. The effect of 5 years of *D. spicata* growth on electrical conductivity (1:5; soil:water) at increasing soil depths, at (A) the Flats site and (B) the Sand plain site at the Wickepin saline discharge area. Error bars represent the standard error of the means (n=5).

3.4 Discussion

The present field survey has indicated that *Distichlis spicata* cv. yensen-4a has the ability to actively grow and expand in area in saline and seasonally waterlogged soils in the survey area within the wheat-belt of Western Australia. Commercially available species that are suitable for agricultural production in saline areas within Australia are limited to *Atriplex* spp. (salt bush), *Puccinellia ciliata* (puccinellia), *Thinopyrum ponticum* (tall wheat grass) and *Distichlis spicata* cv. yensen-4a (NyPa Forage). Of these species, *P. ciliata, T. ponticum* and *D. spicata* are all suited to saline waterlogged soils. However, as *D. spicata* is the only C4 species, it is the most suited to the high temperatures and high radiation regimes during the warm summer months of southern Australia. This species not only has the ability to grow and spread in saline waterlogged soils, but also produces valuable green feed in moist saline discharge soils during the summer period. Such forage has real value for a mixed farming system in the region where conserved fodder or grain are required to maintain sheep live weights through the summer period (Leake *et al* 2002).

The present field survey has confirmed that *D. spicata* has the ability to improve the soil chemical properties at this saline site, as initially suggested by the landholder. Improvements occurred particularly in the surface soil, but to a lesser extent in subsoil layers. Most of the root growth occurred in the top soil (Fig. 3.2), with 8 years of growth producing the highest root length density and root dry matter. These roots periodically die, and over time add organic matter to the soil matrix. The subsequent breakdown of this organic matter within the soil adds organic carbon and increased the nitrogen status of the soil (Fig. 3.3).

Distichlis spicata cv. yensen-4a was also shown to improve the soil physical conditions, with notable increases in saturated hydraulic conductivity and aggregate stability (Figs. 3.4, 3.5 and 3.6). These improvements in soil physical properties are most likely due to the increased root growth and turnover throughout the soil profile. It has been shown by other researchers that soil structure improves with increasing root growth (Haynes and Francis 1993; Perfect *et al.* 1990). In addition, Bruce *et al.* (1992) showed a positive relationship between soil carbon and aggregate stability. In this survey, the increase in stability of large aggregates (>2 mm) in the top 10 cm can be attributed to the increased organic matter from the root activity and associated biological activity which is consistent with the findings of Clarke *et al.* (1967), Forster (1979) and Tisdall and Oades (1982). Similarly, the increase in saturated hydraulic conductivity throughout the soil profile can be attributed to the root activity of *D. spicata*. The periodic death of roots within the profile leaves old root channels (Yensen 1997), which along with the increased aggregate

stability, contribute to the improvement in the physical structure of the soil, and hence increasing the saturated hydraulic conductivity.

The soil electrical conductivity appears to have reduced in the surface layers of the soil where *D. spicata* had been growing for 2 or 8 years when measured in July 2005 (Fig. 3.7). Fourteen months later in September 2006, when the soil was drier and less waterlogged, electrical conductivity in the top soil occupied by *D. spicata* was half that of the control soil (Fig. 3.7). However, caution is required with these measurements as there was a physical slope at the site that would enable the lateral movement of water through the soil from the area where *D. spicata* was growing to the control area. This was noted at the time of sampling, and a second set of samples from the control soil were taken further down the slope (which appear as control 2 on Fig. 3.7). It can be seen in Figure 3.6, that this second control is similar in conductivity to the first control, suggesting that any salinity gradient down the slope in minimal within the sampled area One could therefore argue that *D. spicata* has been able to reduce soil conductivity within the top soil across all years sampled at all sites (Figs. 3.7 and 3.8). Such a claim is very tentative for two reasons. Firstly, we lack baseline conductivity measurements prior to *D. spicata* being established at the site. A second reason why such a claim is tentative is due to the large spatial and temporal variation in the data and between years. Such spatial and temporal variation in soil electrical conductivity in saline discharge sites has previously been documented by Semple and Koen (2004).

The findings from this survey generally support the proposition that growth of *D. spicata* does not lead to an accumulation of salt in the subsoil. The most convincing data are presented in Figure 3.8B, where soil samples were taken from adjacent, parallel down-slope transects; one set was taken under 5-year old *D. spicata*, while the second was taken from the control area where no *Distichlis* was growing. Low EC values occurred in the topsoil layer under *Distichlis* with no accumulation in EC in the subsoil layers under *Distichlis*. The lower subsoil EC value under *Distichlis* in the mother plot, and the high EC value under *Distichlis* in the Flats, are both confounded by the positioning of the control area relative to the *Distichlis* plot. In both instances, high EC values occurred in the subsoil in the down slope plots, suggesting that salt had moved to subsoil that was lower in the landscape.

I therefore conclude, on balance, that there has been minimal accumulation of salts within the rooting zone of *D. spicata*. This contrasts with the accumulation of salts within the root zone of *Atriplex* species (Barrett-Lennard and Malcolm 1999), which raises questions about the long-term sustainability of systems that are based on *Atriplex* species. Further study is required to directly compare salt dynamics in the rooting zone under current cultivated halophyte species in the field.

In conclusion, the work presented in this Chapter demonstrates that *D. spicata* is capable of improving the chemical and physical properties of a saline and seasonally waterlogged soil within the survey area in the wheat-belt of Western Australia. These improvements are consistent with the results of research conducted in Pakistan, where the salt-tolerant Kallar grass (*Leptochloa fusca*) was shown to improve the physical properties of saline-sodic soils (Akhter *et al.* 2003; Akhter *et al.* 2004). The question about sustainability of *D. spicata* swards in saline discharge sites can also be laid to rest, with the data indicating that salt is not accumulating in the root zone. Thus, the use of *D. spicata* in saline and seasonally waterlogged sites is a productive option for landholders as a way of utilising an otherwise abandoned unproductive land. The improvements in soil physical and chemical properties, along with the summer production of green forage make *D. spicata* a very useful species for these salinised soils.

Part II

Establishing *Distichlis spicata* on saline land

Chapter 4

Salt priming improves establishment of Distichlis spicata under saline conditions

4.1 Introduction

Distichlis spicata cv. Yensen-4a has been grown in Australia on a trial basis for the past 10 years with promising results (Leake *et al.* 2002). During this time, it has been demonstrated that *D. spicata* is highly salt tolerant with maximum productivity achieved when grown in saline irrigation water of 30 dS m^{-1} (Shannon *et al.* 1998). As the plant has been selected as a male clone, establishment has to occur by vegetative means, which has been troublesome in some areas due to high soil salinity during the establishment phase, (M. Sargeant, unpublished). Initial work to determine the suitability of *D. spicata* in the mixed farming zone within Australia, first identified establishment as a limitation, with some areas only achieving a 50% strike (Leake *et al.* 2002). Further to this, an EM38 survey that was conducted on a newly established area showed a strong relationship of establishment failure coinciding with high soil salinity (personal observation). The establishment of halophytic species by seed into saline areas has also been troublesome for other species such as *Atriplex* due to poor germination (Barrett-Lennard 2003b; Malcolm *et al.* 2003).

A strategy that has successfully been used to establish glycophyte species into saline environments is 'salt priming'. This involves exposing seeds or seedlings to NaCl before or after germination (Babu and Thirumurugan 2001; Sivritepe *et al.* 1999; 2005; 2003; Umezawa *et al.* 2000). This technique has been demonstrated to improve survival, and in some cases growth of non-halophyte species when they have subsequently been exposed to saline conditions which would otherwise have led to a failed establishment. The rationale behind this approach is that exposure to NaCl prior to germination, or to low levels shortly after germination, allows the plants to adapt better to the saline conditions.

In this chapter, the hypothesis is tested that salt priming improves the establishment of *D. spicata* plants in saline growing media. The approach taken in this chapter is to grow plants in sand culture in the glasshouse and pre-treating them with 0, 2 and 4 g NaCl kg^{-1} sand, and then transplanting them into saline treatments of 0, 2, 4 and 8 g NaCl kg^{-1} sand.

4.2 Materials and Methods

4.2.1 Experimental design

The experiment was setup as a randomized block design with 3 factorial combinations of 3 pre-treatment and 4 treatments of salinity. The pre-treatment consisted of salt levels of 0, 2 and 4 g NaCl kg^{-1} sand. After this phase, the plants were transplanted into the treatments which consisted of 0, 2, 4 and 8 g NaCl kg^{-1} sand. This gave a total of 12 treatments, each of which was replicated 4 times.

4.2.2 Growing conditions

Pre-treatment. This experiment was conducted in a glasshouse at LaTrobe University, Bundoora Victoria (37°42'S, 145°02'E) during the summer and autumn months of 2005. Average day and night temperatures, and hours of direct sunlight throughout the experimental period are presented in Figure 4.1.

Distichlis spicata (L.) Greene cv. Yensen-4a plants were grown in fine white quartz sand which had been washed and oven dried. Basal nutrients were added to the sand at the following rates (mg kg^{-1} sand): $Ca(NO_3)_2.4H_2O$ (107), $MgSO_4.7H_2O$ (70), K_2SO_4 (124), KH_2PO_4 (70), NH_4NO_3 (156), $[CH_2.N(CH_2.COO)_2]_2FeNa$ (6), $CuSO_4.5H_2O$ (0.9), $ZnSO_4.7H_2O$ (2.0) H_3BO_3 (1.3) $MnSO_4.H_2O$ (1.4), and $(NH_4)_6Mo_7O_{24}$ (0.08). Forty kilograms of sand and nutrients were placed into three plastic boxes (60 x 38 cm) with three evenly spaced 65 mm PVC tubes located vertically in the centre of the box. A constant water table of 0.5 cm from the bottom of the pot was maintained by inverting 750 ml glass bottles filled with de-ionised water in the PVC tubes. Approximately 60 bare-rooted plants were transplanted into each box in mid January 2005. The plants were allowed to establish in fresh water for the first two weeks to ensure even establishment, after which salt pre-treatments commenced. The total amount of NaCl for each box was added via solution in three one litre pulses over a two-week interval. The first two of these pulses were added via the water table, and the final pulse was added by pouring the solution onto the top of the sand. After the final salt pulse was added, white plastic beads were placed on the surface to a depth of 2 cm to prevent evaporation. After this point, the plants were watered with de-ionised water. The plants were allowed to grow for 2 weeks after the final salt pulse before the treatments commenced.

Treatment. The treatment phase of the experiment was conducted in 200 mm diameter black plastic pots lined with a plastic bag. A 65 mm PVC tube was placed in the centre of the pot, and the pot then filled with fine white quartz sand mixed with the same basal nutrients and rates as the pre-treatment phase. Sodium chloride was also added to the sand at the rates of 0, 2, 4 and 8 g NaCl kg^{-1} sand for the respective treatments. Three plants were planted into each pot and glass bottles filled with de-ionised water were again used to maintain a water table in the pots. White plastic beads were placed on the surface to a depth of 2 cm to prevent the accumulation of salt in the surface layer of sand. A composite sample of the sand was taken to determine soil electrical conductivity (EC), and Na and Cl concentrations (Table 4.1).

Table 4.1. Electrical conductivity (EC) of the soil solution, and sodium and chloride concentrations in the sand (oven-dried basis) at the final harvest.

Treatment (g NaCl kg^{-1} sand)	Soil solution EC (dS m^{-1})	Na (g kg^{-1})	Cl (g kg^{-1})
0	2.1	0.01	<0.01
2	18.8	0.76	1.07
4	33.6	1.18	2.14
8	66.2	2.85	4.55
LSD (P=0.05)	5.2	0.28	0.41

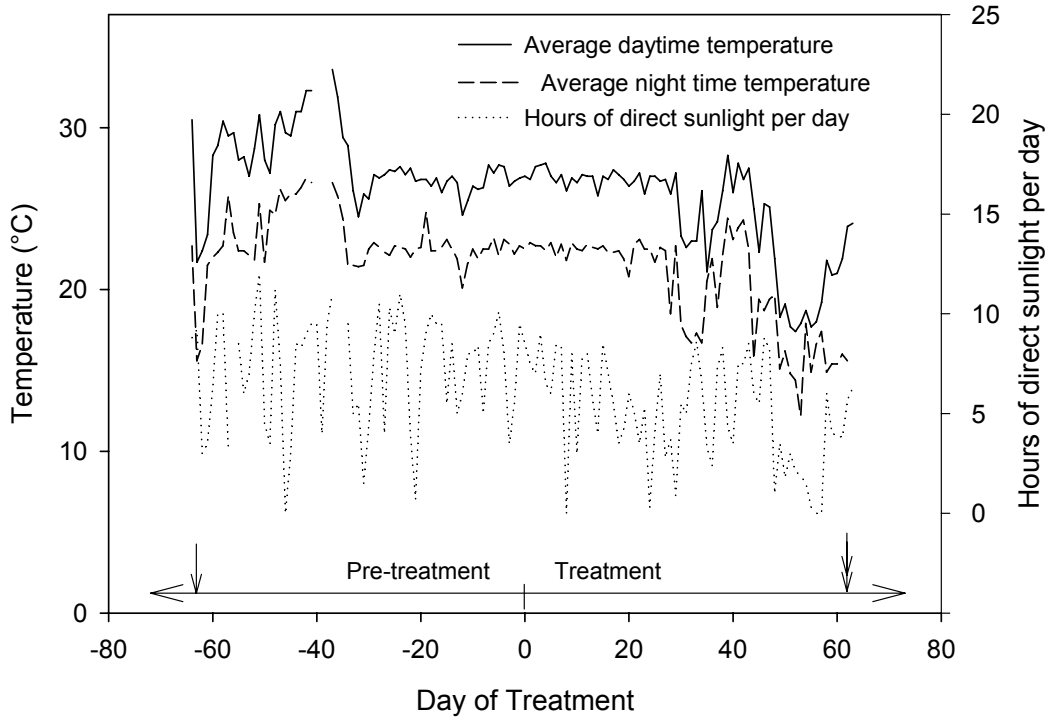


Figure 4.1. The average day time and night time temperatures and hours of direct sunlight throughout the experimental periods. The single arrow represents the commencement of the pre-treatment, and the double arrow represents the time of the final harvest at day 63.

4.2.3 Measurements

At weekly intervals throughout the experiment, plants were monitored for growth initiation (plants showing signs of growth in the form of green leaves and tillers), and the numbers of newly emerged tillers were recorded. Plants were harvested on the 8 June 2005, 63 days after transplanting. To measure the extent of salt excretion from the plants, new tillers from the 0 g NaCl kg^{-1} treatment were washed in 80 mL of de-ionised water, while the remainder of the treatments were washed in 20 mL de-ionised water. These washings were then frozen until analysis. New tiller growth was dried in an oven at 70 °C for 72 hours, weighed and then ground and stored in plastic vials. A sample of sand was also taken at harvest and stored in a sealed plastic bag and stored in a cool room for analysis.

A sample of 5.00 g of wet sand was placed into an oven at 105 °C overnight and then weighed to determine the gravimetric water content. Electrical conductivity (EC) was measured by weighing out 5.00 g of wet sand accurately and analysing a 1:2 (sand:water) solution which had been shaken for 1 hour. The EC of the sand solution was calculated based on the EC of the 1:2 extract, while allowing for the gravimetric water content of the sand.

Concentrations of Na and Cl were determined from the 1:2 sand water extracts. Chloride determination was conducted directly from these solutions using an EEL 920 Chloride Meter. Sodium determination was conducted on a Corning clinical flame photometer, with samples being diluted by a factor of 10 for the 2 and 4 g NaCl kg^{-1} treatments, and by a factor of 100 for the 8 g NaCl kg^{-1} treatments.

Approximately 0.1 g of ground oven-dried plant material was weighed out accurately into a plastic vial and 3.0 mL of de-ionised water added. Samples where less than 0.1 g of plant material was available had 1.5 mL of de-ionised water added. These vials were then placed into a shaking water bath at 60 °C for 2 hours. Concentrations of Na and Cl were determined as described above.

4.2.4 Statistical Analysis

The data were analysed with GenStat 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). A two-way ANOVA was used to test the significance of the treatment effects to the 5% confidence level.

4.3 Results

The main salt treatments had a more marked effect on *Distichlis* plants than did the pre-treatment. This can be seen from the highly significant main effects ($P<0.001$) for practically all measurements on salt treatments (Table 4.2). Salt pre-treatment effects ranged from a marginal ($P<0.1$) to a significant effect ($P<0.05$) on the number of plants that had initiated new growth, and on the number of newly emerged tillers. Highly significant interactions ($P<0.01$) occurred for new shoot dry matter, and for Na and Cl concentrations in new shoots, at day 63. Some marginal interactions at the 10% level ($P<0.1$) occurred with the number of emerged tillers following transplanting, and for the number of newly emerged tillers on days 28, 43 and 58 (Table 4.2).

Table 4.2. Significant main effects and interaction terms for the analysis of variance of different measurements undertaken on *D. spicata* plants after different periods of growth with different salinity treatments.

Measurement	Days after transplanting	Main Effects Pre-treatment (A)	Treatment (B)	Interaction A x B
New growth initiation (plants pot^{-1})	7	*	n.s.	n.s.
	12	n.s.	***	*
	15	#	***	n.s.
	28	n.s.	***	n.s.
	34	#	***	n.s.
	43	n.s.	***	n.s.
	58	n.s.	***	n.s.
Number of emerged tillers (tillers pot^{-1})	7	n.s.	**	n.s.
	12	#	***	n.s.
	15	*	***	n.s.
	28	*	***	#
	34	#	***	n.s.
	43	*	***	#
	58	n.s.	***	#
New shoot dry matter (mg pot^{-1})	63	n.s.	***	**
Na Washing	63	n.s.	***	n.s.
Cl Washing	63	n.s.	***	n.s.
% Na (shoot)	63	n.s.	***	***
% Cl (shoot)	63	*	***	**

# $p<0.1$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. not significant at $p=0.1$.

The salt treatment after transplanting had a greater effect on the initiation of new growth than did salt pre-treatment (Fig. 4.2). Increasing the post-transplanting salt level decreased the initiation of new growth, with the highest level of 8 g NaCl kg^{-1} restricting new growth to less than 1 plant per pot. A significant interaction between the pre-treatment and treatment salt levels ($P<0.05$) occurred 12 days after transplanting. This resulted from the delay in growth initiation with the 2 g compared to the 4 g NaCl pre-treatments, when plants were placed into the sand containing 4 g NaCl kg^{-1}. This situation was reversed when these plants were transplanted into sand containing 2 g NaCl kg^{-1}.

The only significant effect of the salt pre-treatment on growth initiation occurred 7 days after transplanting (Table 4.2). The pre-treatment of 4 g NaCl kg^{-1} initiated growth faster than did the nil or 2 g NaCl kg^{-1} (Fig. 4.2). However, from 12 to 42 days after transplanting, there was a trend of increasing growth initiation with the 4 g NaCl kg^{-1} pre-treatment compared to the nil salt pre-treatment, when the *Distichlis* plants were transplanted into sand containing 2 and 4 g NaCl kg^{-1} (Fig. 4.2). These differences were not displayed for plants grown at 8 g NaCl kg^{-1} where all plants grew poorly and little growth was initiated.

The salt treatment after transplanting had a greater effect on the number of newly emerged tillers throughout the experimental period, compared to pre-transplanting salt treatments. However, a marginal interaction ($P<0.1$) for tiller numbers occurred on days 28, 43 and 58 between pre-treatment and treatment salt levels (Table 4.2). The interaction resulted from a greater number of tillers in the plants that had received the pre-treatment of 4 compared to nil g NaCl kg^{-1}, when they were transplanted into sand containing 2 g NaCl kg^{-1} (Fig. 4.3). The reverse trend occurred when the plants were transplanted into sand containing no salt, where more tillers were produced by plants receiving the nil salt pre-treatment. Plants transplanted into the 8 g NaCl kg^{-1} treatment all performed poorly. However, there was a slight trend for plants that received 2 and 4 g NaCl kg^{-1} to produce more tillers than plants that received the nil NaCl pre-treatment.

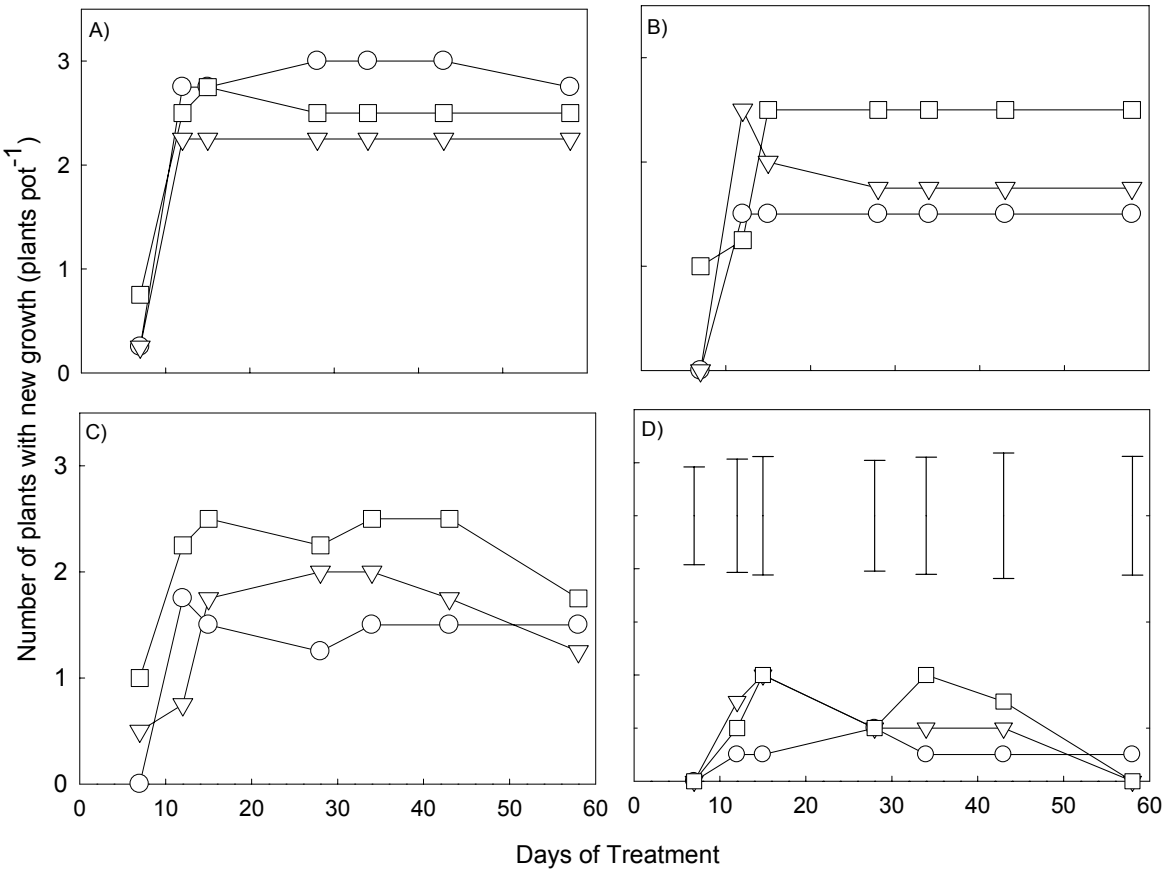


Figure 4.2. The effect of NaCl pre-treatment (○, ∇, □ for nil, 2 and 4 g NaCl kg^{-1} sand, respectively) on the number of *D. spicata* plants that had initiated new growth after being transplanted into sand containing (A) 0 g NaCl kg^{-1}, (B) 2 g NaCl kg^{-1}, (C) 4 g NaCl kg^{-1} and (D) 8 g NaCl kg^{-1} during the experiment. Error bars represent LSD (P=0.05) for the pre-treatment × main factor interaction at each observation period.

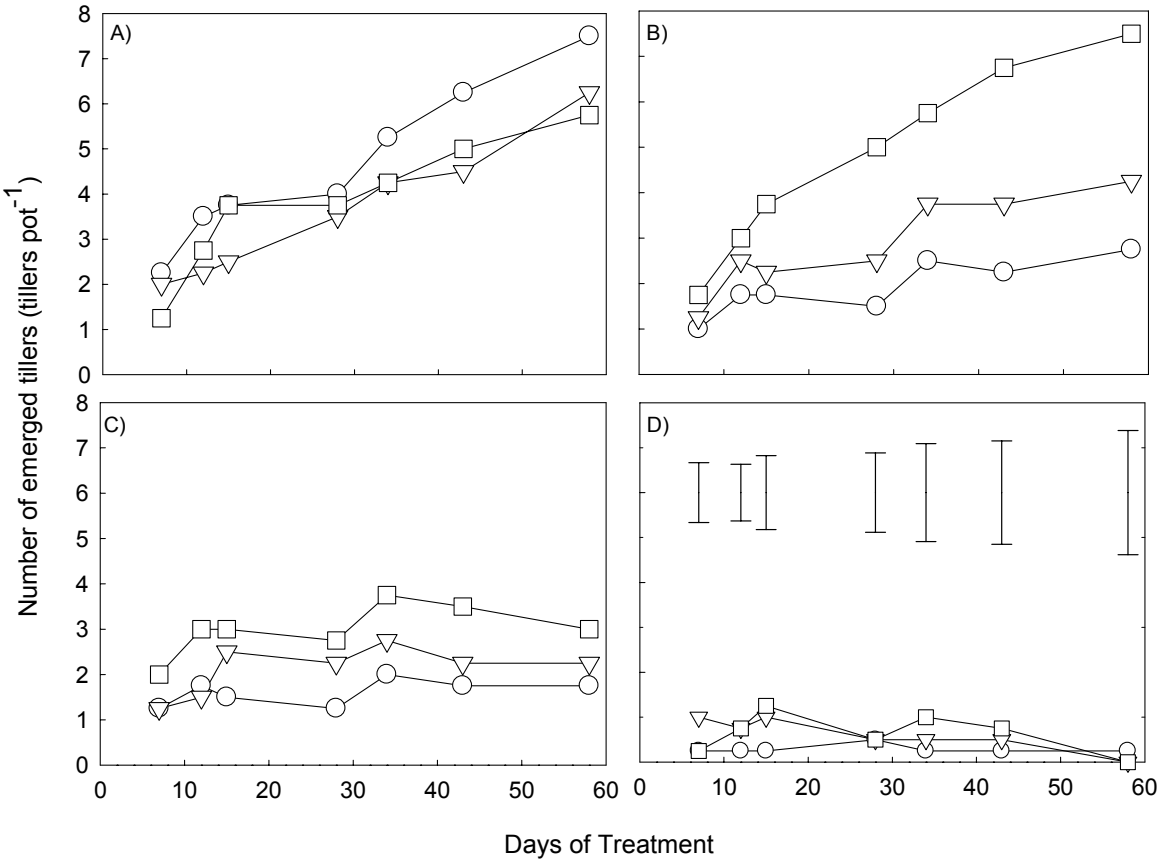


Figure 4.3. The effect of NaCl pre-treatment (○, ∇, □ for nil, 2 and 4 g NaCl kg^{-1} sand, respectively) on the number of emerged tillers of *D. spicata* plants after being transplanted into sand containing (A) 0 g NaCl kg^{-1}, (B) 2 g NaCl kg^{-1}, (C) 4 g NaCl kg^{-1} and (D) 8 g NaCl kg^{-1} during the experiment. Error bars represent LSD (P = 0.05) for the pre-treatment × main treatment interaction.

The new growth following transplanting was affected by both pre-treatment and treatment with NaCl, resulting in a highly significant interaction (P<0.01) occurring at the end of the experiment (Table 4.2, Fig. 4.4). This interaction was caused by the nearly 6-fold increase in dry matter production of the plants pre-treated with 4 g NaCl kg^{-1} and followed by the 2 g NaCl kg^{-1} treatment, compared to plants pre-treated with 0 and 2 g NaCl kg^{-1}. However, when plants were pre-treated with 4 g NaCl kg^{-1} and then transplanted into sand containing no salt, they produced approximately half the dry matter of plants that had been pre-treated with nil salt. The treatment of 8 g NaCl kg^{-1} resulted in minimal dry matter accumulation irrespective of the pre-treatment (Fig. 4.4).

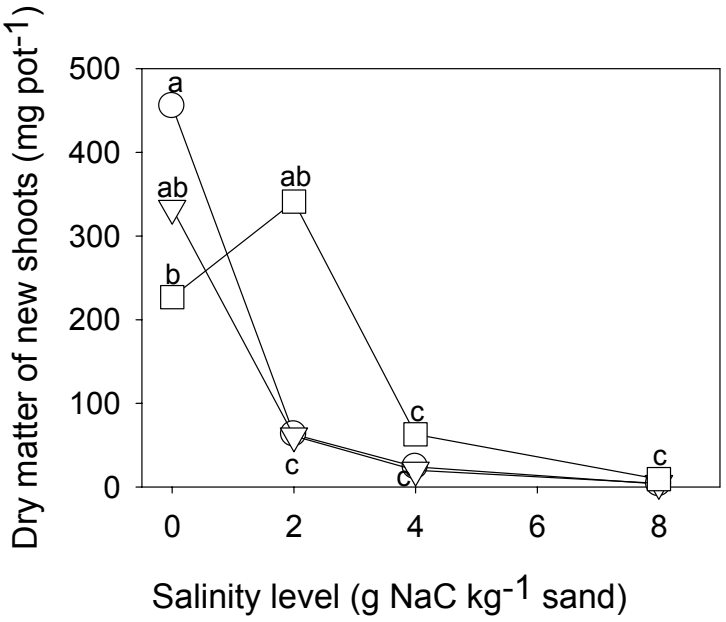


Figure 4.4. The effect of the pre-transplanting salt treatment (○, ∇, □ for nil, 2 and 4 g NaCl kg^{-1} sand respectively) on the dry matter production of new shoots of *D. spicata* plants 63 days after transplanting into sand containing increasing concentrations of g NaCl kg^{-1} sand. Means with the same letter do not differ significantly (P>0.05).

The amounts of Na and Cl that were washed from the shoot surfaces on day 63 were not affected by the pre-treatments (Fig. 4.5). The Na and Cl in washings increased with increasing salt treatment levels, up to the 4 g NaCl kg^{-1}, and then declined by around 50% at 8 g NaCl kg^{-1}. There were no interactions between pre-treatment and treatment salt levels on Na and Cl in the shoot washings (Table 4.2).

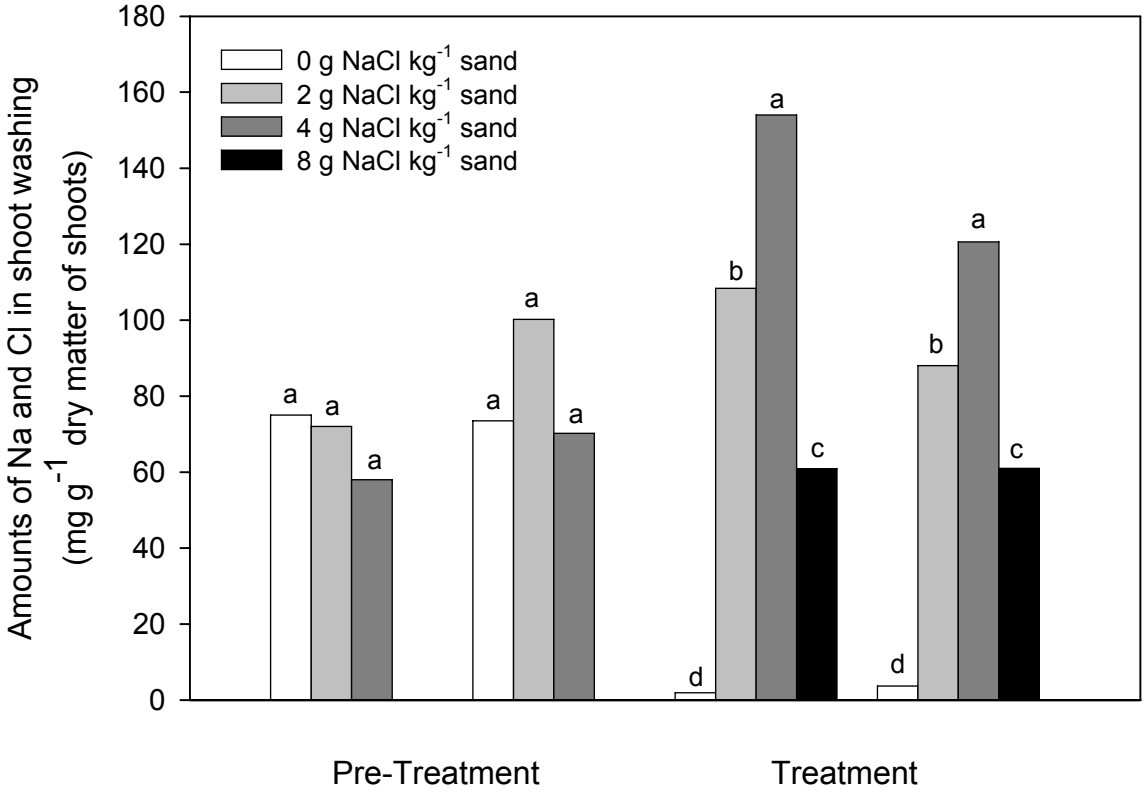


Figure 4.5. The effects of the pre-treatment and treatment of NaCl on the mass of Na and Cl in the shoot washing of *D. spicata* on day 63. Means with the same letters do not differ significantly (P>0.05).

There were significant interactions on concentrations of Na and Cl in the new shoot growth between pre-treatment and treatment salt levels (Table 4.2, Fig. 4.6). The pre-treatment did not affect concentrations of Na and Cl in plants growing in the 0 and 2 g NaCl kg^{-1} sand treatments. However, at 4 g NaCl kg^{-1}, plants receiving nil salt in pre-treatment had higher concentrations of Na and Cl than did those receiving 2 or 4 g NaCl kg^{-1} in pre-treatment. The opposite was observed with the 8 g NaCl kg^{-1} sand treatment.

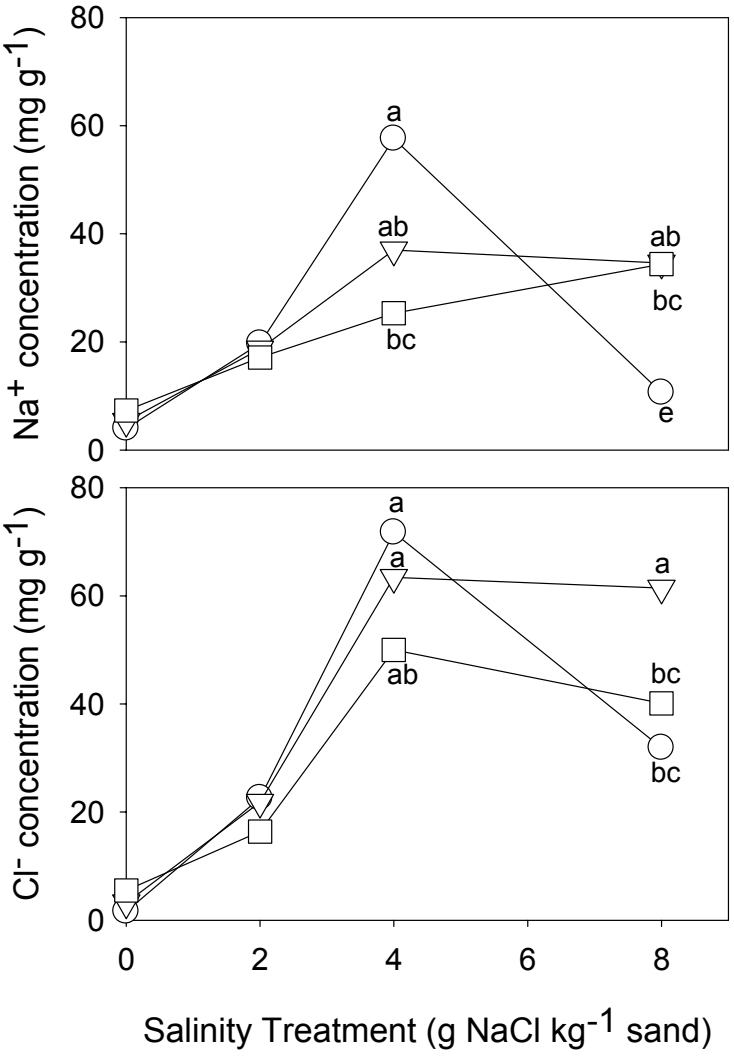


Figure 4.6. The effect of NaCl pre-treatment (○, ∇, □ for nil, 2 and 4 g NaCl kg^{-1} sand, respectively) on the concentration of Na and Cl in the new shoot growth of plants grown at various NaCl levels for 63 days. Means with the same letters do not differ significantly (P>0.05).

4.4 Discussion

This study demonstrated that salt priming of *D. spicata* plants by pre-treating them with saline conditions prior to transplanting, significantly improved establishment when transplanted into sand containing moderate salt levels (Figs. 4.2 and 4.3). A highly significant interaction ($P<0.01$) in the growth of new dry matter produced between pre- and post-transplanting salt exposure typifies the salt priming response. The effect resulted in up to 6 times more dry matter being produced from salt-primed plants (Fig. 4.4). This 6-fold increase in dry matter can be attributed to the increase in tiller numbers, along with an increase in tiller weight (Figs. 4.4 and 4.5).

Very little research into salt priming on a whole plant basis has previously been reported, with much of the research being conducted on seeds of non-halophyte species such as melons (*Cucumis melo*) (Sivritepe *et al.* 1999; 2005; 2003) and sesame (*Sesamum indicum*) (Babu and Thirumurugan 2001). An experiment conducted by Umezawa *et al.* (2000) is one such experiment where salt priming was conducted on soybean (*Glycine max*) seedlings grown in a sand culture system. In that experiment, seedlings were salt-primed with 0, 34 or 68 mM NaCl solutions which commenced 2 days after emergence and continued for 23 days. Seedlings were then exposed to salt treatments of 0, 68 and 137 mM NaCl. The plants pre-treated with 34 mM NaCl had a survival rate of 93% compared to 60% for the 0 mM pre-treatment. However, the pre-treatment effect was limited to survivability, with no increase in dry matter production as was found with *D. spicata* in this present study.

A major advantage of salt-primed *Distichlis* plants was the dramatic increase in dry matter production, when plants pre-treated with 4 g NaCl kg^{-1} were planted into sand containing 2 g NaCl kg^{-1}. This significant interaction suggests that the level of NaCl prior to transplanting is important for the performance of *Distichlis* when transplanted into saline environments. Similar results were found by Babu and Thirumurugan (2001), who showed that the salt priming of sesame seeds by soaking them in 1 M NaCl solution increased plant height, the number of leaves, seed weight and dry matter production when irrigated with a range of saline solutions, ranging from 35 to 140 mM. Salt priming of melon seeds by soaking them in 18 dS m^{-1} NaCl solution for 3 days at 20 °C also showed increased dry matter production (Sivritepe *et al.* 2005).

The mechanisms behind the success of salt priming appear to be linked to activating the salt tolerance mechanisms within *Distichlis*. This species has two main strategies for salt tolerance. These involve (i) highly active salt glands that excrete Na and Cl out of the plant tissue (Hansen *et al.* 1976; Liphschitz and Waisel 1982; Marcum 1999; Oross and Thomson 1982), and (ii)

osmoregulation capabilities to maintain the osmotic potential within the plant cell by increasing the concentration of compatible solutes (Marcum 1999). Exclusion of NaCl from the roots does not appear to be a major mechanism from this present study, as new growth tissue concentrations of Na and Cl increased at a rate greater (Fig. 4.6) than that for the increase in Na and Cl concentration in the sand (Table 4.1) from the 2 to 4 g NaCl kg^{-1} treatments (Table 4.1). Marcum (1999) found that the main osmoregulant solute in *D. spicata* is glycinebetaine which was shown to provide up to 74% of the total osmolarity in the cytoplasm when grown in 300 mM NaCl solution. The salt priming effect in this present study did not influence the amount of Na and Cl excreted from the leaves on day 63 (Fig. 4.5). However, shoot washings were only collected at the end of the experiment and so we could not determine if salt priming had any effect on the initial salt gland activity when transplanted. Nevertheless, increasing the post-transplanting salt level to 4 g NaCl kg^{-1} increased Na and Cl excretion (Fig. 4.5), which then declined with the highest post-transplant salt treatment of 8 g NaCl kg^{-1}. Liphschitz and Waisel (1982) reported that the efficiency of *Distichlis* salt glands was very high; salt excretion increased to a maximum rate when plants were grown in 200 mM NaCl, and then slowly decreased with high salinity levels. Plants in this present experiment decreased salt excretion when grown in a soil solution above 33.6 dS m^{-1} (approximately 360 mM NaCl solution), which occurred with the 8 g NaCl kg^{-1} sand treatment (Fig. 4.5). This is well above the NaCl concentration when salt excretion declined in the study described by Liphschitz and Waisel (1982). This inability of the plants grown at the 8 g NaCl kg^{-1} salt load to efficiently excrete salts out of the leaf tissue may have contributed to the poor performance and ultimate death of these plants in this study.

The death of the plants in the 8 g NaCl kg^{-1} treatment towards the end of the experiment (Fig. 4.2) was possibly related to the environmental conditions encountered during the experimental period. The experiment commenced towards the end of summer 2005 with the salt priming phase occurring during February, and the post-transplanting phase continuing into the winter months. During the experiment, the light intensity and duration of light decreased (Fig. 4.2). Salt glands of *Distichlis* are lined with many mitochondria (Oross and Thomson 1982), which generate ATP to supply energy for cellular activity from the products of photosynthesis. Kemp and Cunningham (1981) found that *Distichlis* plants grown under high light conditions (1,200 μE m^{-2} s^{-1}) were not affected by high salinity, whereas plants grown under low light (600 μE m^{-2} s^{-1}) produced significantly less dry matter under the high salinity regime. Liphschitz and Waisel (1982) also stated that ecological factors such as light affected salt gland excretion. Differences in light intensity, and consequently photosynthate supply are the likely causes of the differences in production by *D. spicata* plants under saline conditions (Leake *et al.* 2002; Sargeant 1999; Shannon *et al.* 1998). Thus the declining light regimes at the late stage of this study were possibly responsible for the poor performance of the *D. spicata* plants in the 8 g NaCl kg^{-1} treatment. The

effect of light intensity on the salt tolerance of *D. spicata* will be investigated in the following chapter.

This study also demonstrated that salt priming assisted the plants to regulate internal Na and Cl concentrations. The plants that were salt-primed with 2 and 4 g NaCl kg^{-1} were more efficient in maintaining internal concentrations of Na and Cl in new growth in both 4 g and 8 g NaCl kg^{-1} treatments (Fig. 4.6). Plants without salt priming exhibited large fluctuations in internal Na and Cl concentrations. These large fluctuations could be attributed to inefficient salt gland activity with 4 g NaCl kg^{-1}, and reduced ion uptake activity, as the plants died with the 8 g NaCl kg^{-1} treatment.

In conclusion, salt-priming is a useful practice for establishing *D. spicata* transplants in saline environments. Salt priming was shown to increase dry matter production, survival, tiller production and to assist the plants to regulate internal Na and Cl concentrations.

Chapter 5

The effect of shading on the salt tolerance of *Distichlis spicata*

5.1 Introduction

The effect of light intensity on the salt tolerance of *D. spicata* has been discussed by a number of authors (Kemp and Cunningham 1981; Liphschitz and Waisel 1982). Kemp and Cunningham (1981) found that when D. spicata was grown under high light intensity, relative growth rate was not affected by salinity, whereas at low light levels, high soil salinity did depress growth rates. Kemp and Cunningham (1981) concluded by stating that physiological processes such as salt excretion and compartmentalization may be dependant directly on light energy, and require further investigation.

Given the high density of mitochondria within the saltgland of *Distichlis* (Oross and Thomson 1982), and the observed death of plants under high salt loads and low light conditions in Chapter 4, it is reasonable to conclude that light intensity may play a role in the salt tolerance of the this species.

As *D. spicata* is traditionally planted during the cooler winter months in southern Australia, the impact of light intensity on the salt tolerance is of interest. This time of year tends to have lower light intensity along with winter rainfall, and reduced growth rates. If high light intensity does improve the salt tolerance of *D. spicata,* then the traditional planting window through the winter months will coincide with a lower salt tolerance of the grass.

The experiment documented in this chapter tests the hypothesis that high light levels increase the salt tolerance of *D. spicata* by increasing the salt secretion from the salt glands. This hypothesis was tested by growing *D. spicata* plants in a glasshouse with 1 or 3 g NaCl kg^{-1} sand, and at two light levels achieved by using shading hoods on half the plants. A number of physiological measurements were taken to determine how shading affected salt tolerance, and how the light levels affected Na^+ and Cl^- concentrations in the shoot tissue of this halophytic species.

5.2 Materials and methods

5.2.1 Experimental design

The experiment was set up in a glasshouse as a randomized block experiment with factorial combinations of 2 salinity treatments, and 2 light intensity treatments. The salinity treatments consisted of 1 and 3 g NaCl kg^{-1} sand, while the light intensity treatments consisted of direct sunlight and shading with 90% light excluding shade cloth, which was beige in colour. This gave a total of 4 treatments, which were replicated 5 times. Another six replicates were run concurrently to allow for additional measurements to be taken at the same time. The first set of replicates were used for physiological measurements, while the second set were used for dry matter measurements.

5.2.2 Growing conditions

The experiment was conducted in a glasshouse at La Trobe University in Bundoora, Victoria (37°42'S, 145°02'E). The daily maximum and minimum temperatures were recorded, along with photosynthetically active radiation (PAR) received by the plants in the different light treatments (Figure 5.1).

The *Distichlis spicata* (L.) Grenne cv. Yensen-4a plants were grown in fine white quartz sand that had been washed and dried. The following basal nutrients were added to the sand, with concentrations in mg kg^{-1} in parenthesis: $Ca(NO_3)_2.4H_2O$ (107), $MgSO_4.7H_2O$ (70), K_2SO_4 (124), KH_2PO_4 (70), NH_4NO_3 (156), $[CH_2.N(CH_2.COO)_2]_2FeNa$ (6), $CuSO4.5H_2O$ (0.9), $ZnSO_4.7H_2O$ (2.0), H_3BO_3 (1.3), $MnSO_4.H_2O$ (1.4) and $(NH_4)_6Mo_7O_{24}$ (0.08). Five bare rooted plants were established into 250-mm diameter black plastic pots without drainage holes. A 65-mm diameter PVC tube that was 30 cm in length was placed in the centre of the pot, and then the pot was filled with 5 kg of nutrient enriched sand. Upturned glass bottles filled with distilled water were placed in the vertical PVC tubes and the controlled release of water from the bottle, that occurred when air entered the bottle, maintained a 0.5 cm water-table in the bottom of the pot.

The plants were allowed to establish for 1 month prior to the salinity treatments being imposed. The salinity treatments were then imposed over a 3-stage process, where $^1/_3$ of the salt was added at each step. The first 2 steps involved adding the salt dissolved in distilled water via the upturned bottles, while the final salt was added by pouring the dissolved salt over the surface of the sand. The surface of the sand was then covered with white plastic beads to a depth of 0.02 m to prevent surface evaporation and the accumulation of salts at the surface layer of the sand. A composite

sample of sand was taken from each pot at the conclusion of the experiment to determine electrical conductivity (EC) and concentrations of Na^+, Cl^- and K^+ (Table 5.1). Soil EC was measured in a 1:5 solution by taking 5.0 g of air-dried sand and shaking in 25 mL of distilled water for 1 h, after which the EC of the solution was measured. Concentrations of Na and Cl were also measured from the 1:5 sand:water extract. Chloride was determined directly from this solution using an EEL 920 Chloride Meter, while Na^+ was determined using a Corning clinical flame photometer, with samples being diluted by a factor of 10 for all treatments.

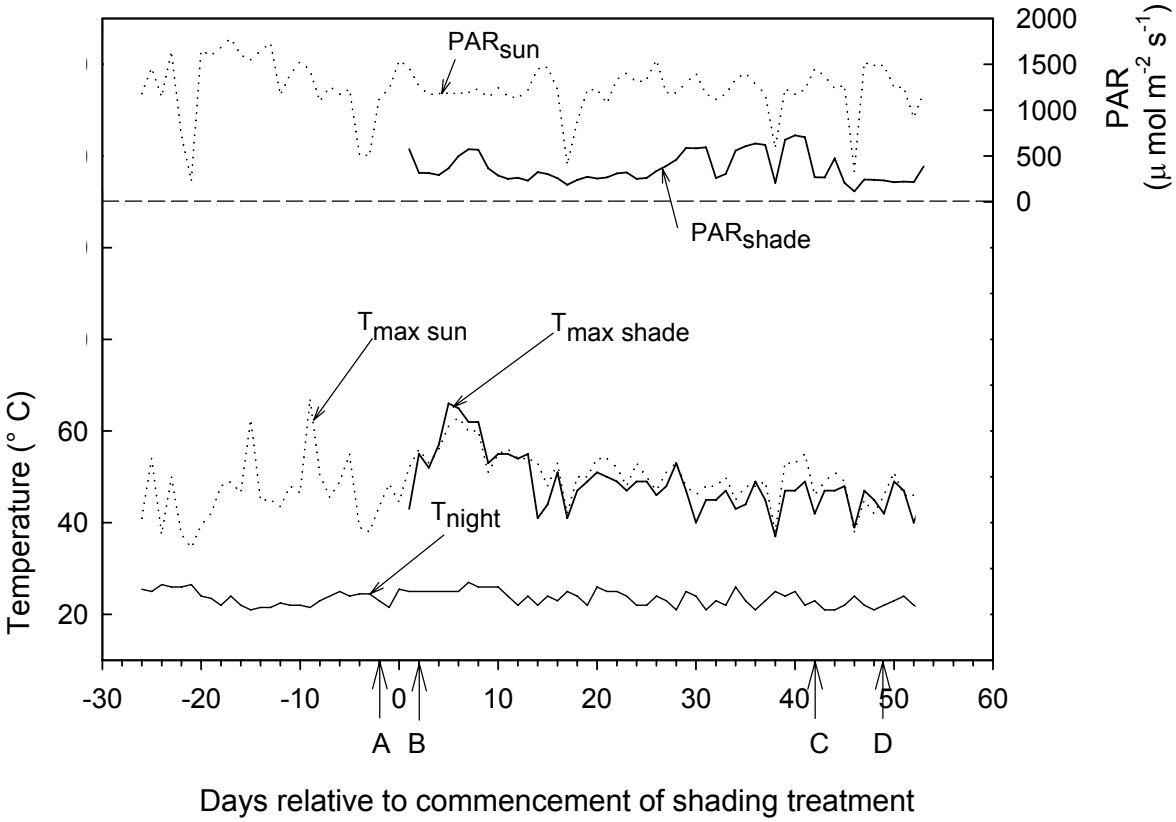


Figure 5.1. The environmental conditions (temperatures and light levels) for the full sun and shaded treatments throughout the experimental period. T_{max} sun, T_{max} shade and T_{night} indicate the daily maximum temperature for the full sun, shaded and night-time temperature respectively. PAR_{sun} and PAR_{shade} indicate the daily light levels for the respective light treatments. Letters indicate where different measurements were taken. A) represents the first transpiration measurements, B) the first osmolarity measurements and first ion excretion measurements, C) second transpiration measurements, and D) final transpiration and ion excretion measurements, after which the final harvest was completed.

Table 5.1. Electrical conductivity (EC) and concentrations of sodium, chloride and potassium ions in the soil solution at the final harvest. Values in parenthesis represent the relative proportion of each ion relative to the other ions measured.

Treatment	Sand EC ($dS\ m^{-1}$)	Sodium ($g\ kg^{-1}$)	Chloride ($g\ kg^{-1}$)	Potassium ($g\ kg^{-1}$)
Low salt	11.3	35 (32%)	33 (31%)	40 (37%)
High salt	38.6	130 (36%)	177 (50%)	49 (14%)
LSD ($P = 0.05$)	2.5	0.7	22.9	6.8

5.2.3 Measurements

There were a number of physiological measurements taken both pre and post-shading during the experiment. Leaf osmolarity was determined on two occasions (day 2 and 49). On day 2, four leaves (fourth youngest fully-expanded leaves) were harveseted from each pot, and immediately stored on ice and frozen. On day 49, one tiller from each pot was harvested, stored on ice and frozen; the four youngest fully-expanded leaves were subsequently removed from the tiller and used for leaf osmolarity determination. The leaves were placed in a cool room at 4 °C overnight and leaf juices (leaf sap and cell contents) extracted with a hydraulic press the following morning. Twenty μL of leaf juice was collected and made up to 150 μL with distilled water, which was then used for osmolarity determination in a freezing point Helbmiko osmometer.

Salt excretion from the leaves was measured at 24 h intervals for 3 days prior to imposing the shading treatments, then at 12 h intervals for 3 days after shading was imposed, and again on day 49 over a 24-h period. Two leaves (the 4th fully expanded leaf) were tagged from each pot and washed with distilled water. The same leaf was then washed either 12 or 24 h later with distilled water which was collected by placing the leaf into a funnel and washing the leaf with 1 mL of distilled water from a wash bottle. The wash solution was collected in a vial through a plastic tube connected to the funnel. This leaf washing was then frozen until analysis. The 4th fully expanded leaf was selected at day 49 to ensure that the leaf used was the same physiological age as the earlier leaves. The area of each leaf was also measured to calculate salt excretion per unit area. Concentrations of Na^+ and K^+ were determined on a Corning clinical flame photometer, with samples being diluted by a factor of 10 for the Na^+ analysis. Chloride determination was conducted directly from the leaf washing solution on an EEL 920 Chloride meter.

Transpiration was measured at days -3, +42 and +49 on a LCi portable photosynthesis system. This was carried out on the same leaf that was used for salt excretion. Measurements were taken between 8 and 10 am to ensure that the temperature within the leaf chamber did not exceed 50 °C. Transpiration values were recorded two minutes after the leaf chamber was closed, with the leaf orientated 90° to the sun at the time of measurement. The associated data that were collected from the LCi portable photosynthesis system were also used to calculate the stomatal conductance (g_s) of water vapour from the following equation.

$$g_s = \frac{(\frac{e_s}{p}) - (\frac{e_{an}}{p})}{E} - r_b$$

Where:

e_s = saturated vapour pressure at leaf surface temperature (mbar)

e_{an} = vapour pressure of water out of the chamber (mbar)

p = atmospheric pressure (mbar)

E = transpiration rate (mol m^{-2} s^{-1})

r_b = boundary resistance to H_2O (chamber constant = 0.32 m^2 s mol^{-1})

On day 49, the plants were harvested by cutting the tillers at the base of the plastic beads and dried in an oven at 70 °C until a constant weight was achieved. Samples were then weighed for total dry matter production, and then ground using a coffee grinder. Approximately 0.1 g of finely ground, oven-dried tiller material (leaves plus stems) was then weighed into a plastic vial and 3.0 mL of deionised water added. These vials were then placed into a shaking water bath at 60 °C for 2 h. Concentrations of Na, Cl and K in the extract were determined as described above.

5.2.4 Statistical analysis

The data were analysed with GenStat 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). A 2-way ANOVA was used to test the significance of the treatment effects to the 5% confidence level. Dry matter data were $\log_{10}$ transformed to ensure the normality of the residuals.

5.3 Results

5.3.1 Shoot growth

Shading surprisingly had no effect on the total shoot growth of *Distichlis spicata* over the 7 week regrowth period, under the conditions of this experiment (Fig. 5.2). This occured despite a reduced light regime that averaged 400 μmol m^{-2} s^{-1} (PAR) during the day-time, which was around $^1/_3$ of that of the full sun treatment (Fig. 5.1). In contrast, the high salt regime reduced shoot growth to around $^1/_5$ of the growth with low salt. There was no interaction between the salt levels and light treatment. However, despite the lack of any effect on total dry matter production over the experimental period, the net photosynthetic rate at day 49, was reduced by about 50% under the shading treatment, compared to the plants grown in direct sunlight.

5.3.2 Concentration of osmotica in shoots

The leaf osmolarity two days after the shading treatments were imposed was significantly affected by the salinity treatments, but not by the shading treatments (Fig. 5.3). Leaf osmolarity increased approximately 40% under the high salt load treatment, while the main effect leaf osmolarity means for the full sun and shaded treatments were similar.

Long-term shading for 49 days resulted in a significant shading × salt interaction ($P<0.05$) in leaf osmolarity (Fig. 5.4). The basis for this interaction was the high leaf osmolarity in full sun, high salt treatment; the osmolarity in this treatment was 140 millosmol kg^{-1}, which was 50-60% higher than the other treatments.

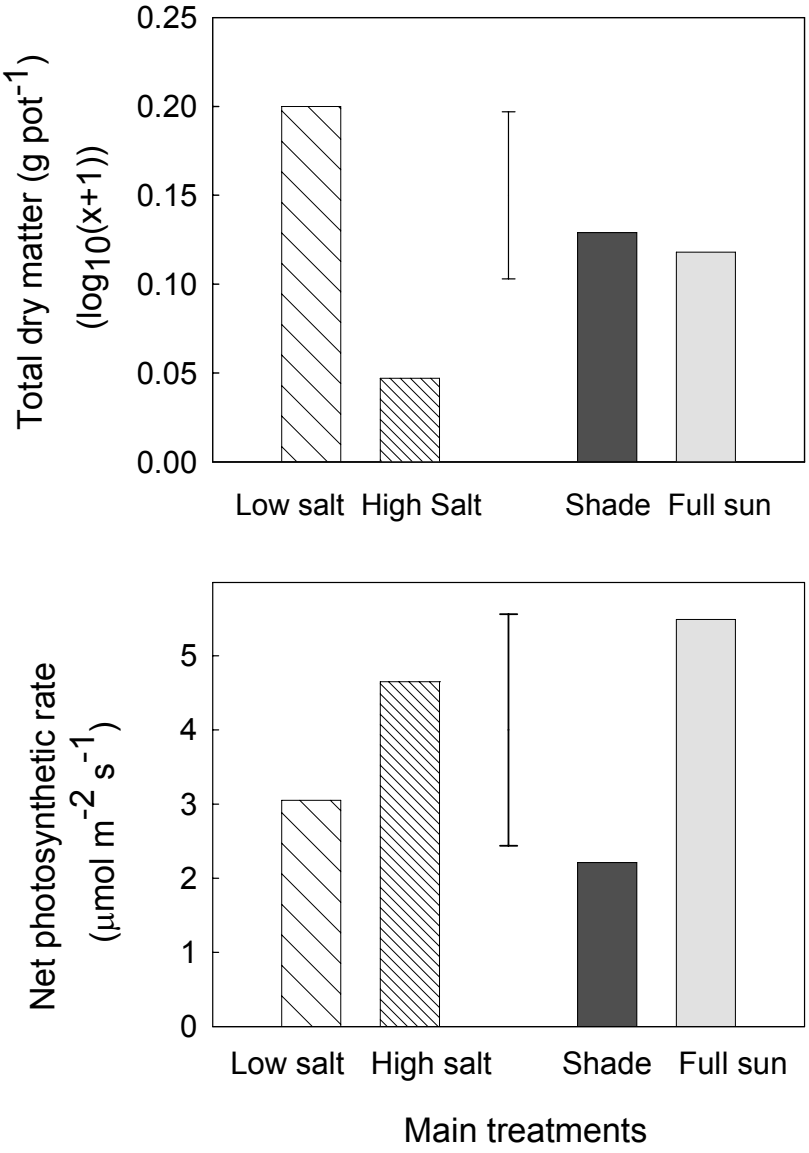


Figure 5.2. Main effect means for salt and shading treatments on the shoot regrowth of *D. spicata* over 49 days along with the net photosynthetic rate at day 49. Error bars represent LSD ($P = 0.05$) for the salt and shade main effects.

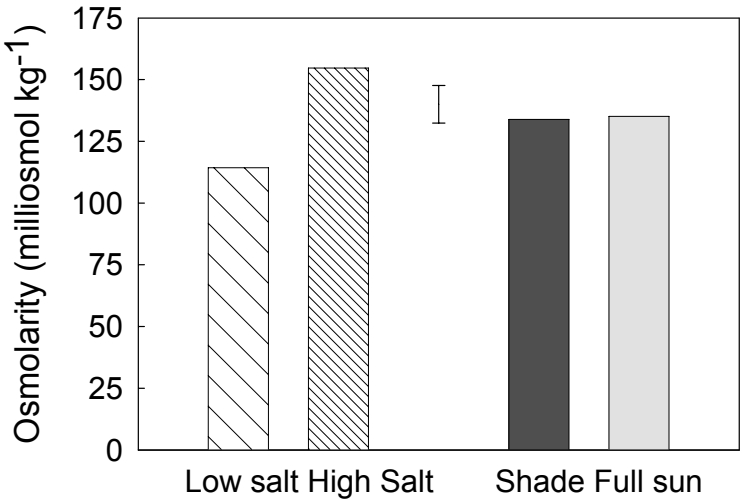


Figure 5.3. Main effect means for salt and shading treatments on leaf osmolarity two days after shading treatments were imposed. Error bars represent LSD (P = 0.05).

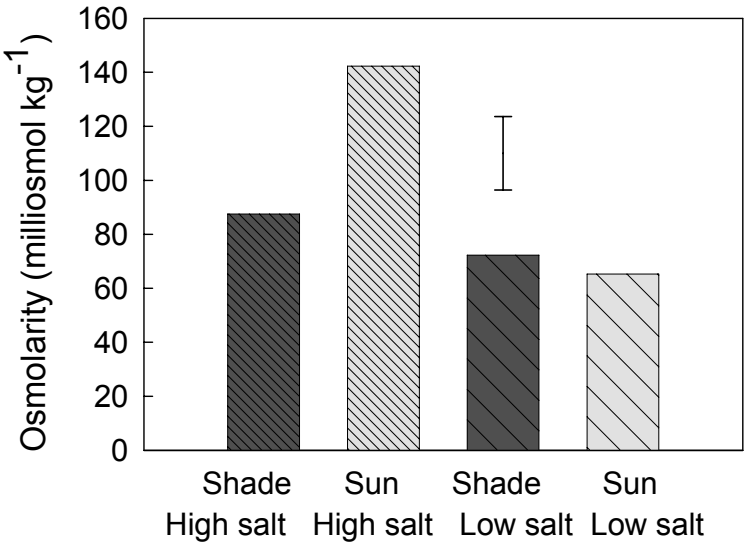


Figure 5.4. Leaf osmolarity at 49 days after the shading treatments were imposed. Error bar represents LSD (P = 0.05) for the shade × salt interaction.

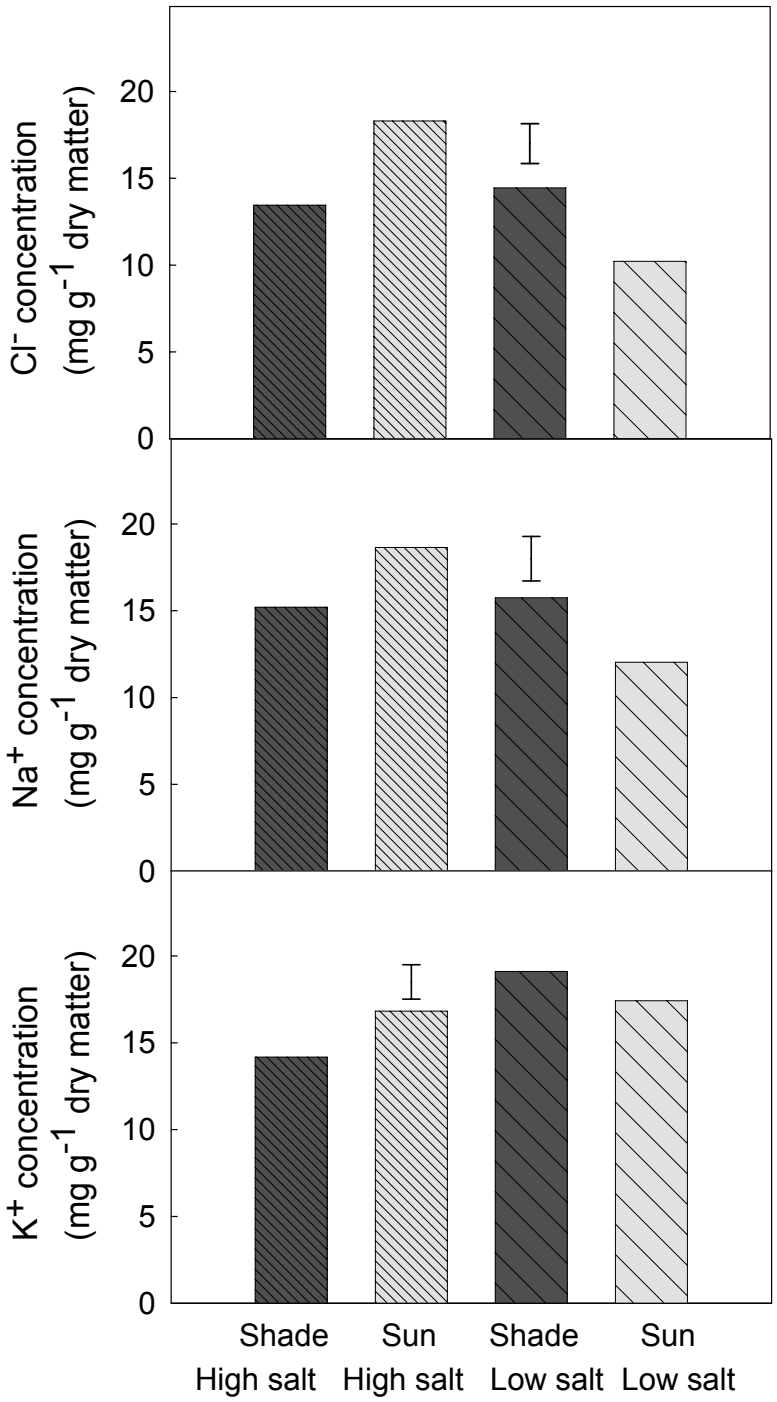


Figure 5.5. Chloride, sodium and potassium concentrations in shoot tissue 49 days after the shading treatments were imposed. Error bars represent LSD (P = 0.05) for the shade × salt interaction.

The concentrations of Na^+, Cl^- and K^+ in dried shoot (leaf and stem) tissue, 49 days after shading, ranged between 12 and 18 mg g^{-1} (Fig. 5.5). All concentrations were subject to significant (P<0.05) shade × salt interactions. The basis for the interactions for the Na^+ and Cl^- concentrations was the increased ion concentrations in shoots with the high salt – full sun treatment, compared to the high salt – shaded treatment. Furthermore, there were reduced ion concentrations in the low salt – full sun, compared to the low salt – shaded treatments. There were no differences in ion concentration between the high or low salt shaded treatments. The basis behind the K^+ interaction was the lower concentration in the shaded treatment with high salt, while K^+ concentrations in shaded and full sun treatments with the low salt were similar.

5.3.3 Ion excretion by salt glands

There appeared to be a short lag phase, before shading affected the Na^+ and Cl^- excretion by salt glands in the leaves of *D. spicata* (Table 5.2). This lag-phase period occurred as the significant salt × shading interaction first occurred for Na^+ and Cl^- excretion during the night-time hours on the second day after shading was imposed. This interaction continued over the following day, but not for the 3rd night-time period. Significant main effects for salt occurred for Na^+ and Cl^- excretion during the second day after shading. There was no significant effect of either salt or shade treatments on salt excretion over the 24-h period at day 49 post-shading. Potassium excretion was not affected by shading treatments in the short or long-term. There were significant main effects for salt on the second day and night after shading, and on day 49 post-shading.

A diurnal pattern of salt excretion occurred from the *D. spicata* leaves two days after shading was imposed. Here the majority of the excretion occurred during the day time period. During both the day and night periods, there were significant shading × salt interactions for both the Cl^- and Na^+ excretion (Table 5.2, Fig. 5.6). The day-time interactions were caused by marked increases in the excretion rate for both ions with the high salinity – full sunlight treatment, compared to other treatments These high salt – full sunlight plants excreted about 25% more Cl^- than Na^+ during this day-time period. The night-time interactions resulted from significantly less Na^+ and Cl^- excretion in the low salinity – shaded treatment, compared to the other treatment combinations.

Table 5.2. Significant main effects and interaction terms for salt and shading treatments when chloride, sodium and potassium salt gland excretion data were subject to an analysis of variance.

Measurement	AOV term	Days following imposition of shading								
		-3 days	-2 days	-1 day	+0.5 day	+1.0 day	+1.5 days	+2.0 days	+2.5 days	+49 days
		Period which excretion occurred: [#]								
		24	24	24	12N	12D	12N	12D	12N	24
Cl^-	Salt	-	*	*	*	*	-	***	***	-
	Shade	-	-	-	-	-	-	*	*	-
	Salt×Shade	-	-	*	-	-	*	*	-	-
Na^+	Salt	-	*	*	***	*	*	***	***	-
	Shade	-	-	*	-	-	-	*	-	-
	Salt×Shade	-	-	-	-	-	*	*	-	-
K^+	Salt	-	-	-	-	-	*	*	-	*
	Shade	-	-	-	-	-	-	-	-	-
	Salt×Shade	-	-	-	-	-	-	-	-	-

*p<0.05, *** p<0.001, - = not significant at $P = 0.05$
[#] 24 (excretion over a 24 h period), 12N (12 h of night-time excretion), 12D (12 h of day time excretion)

Potassium excretion from the leaves during the second day and night-time periods, after the shading was imposed was significantly higher from the high than from the low salt treatments, but was not affected by shading (Fig. 5.7). There were no salt × light interactions in K^+ excretion during the day or night. Interestingly, the levels of K^+ excretion from the leaves for all treatments were generally similar during the night and the day, for each of the treatment main effects.

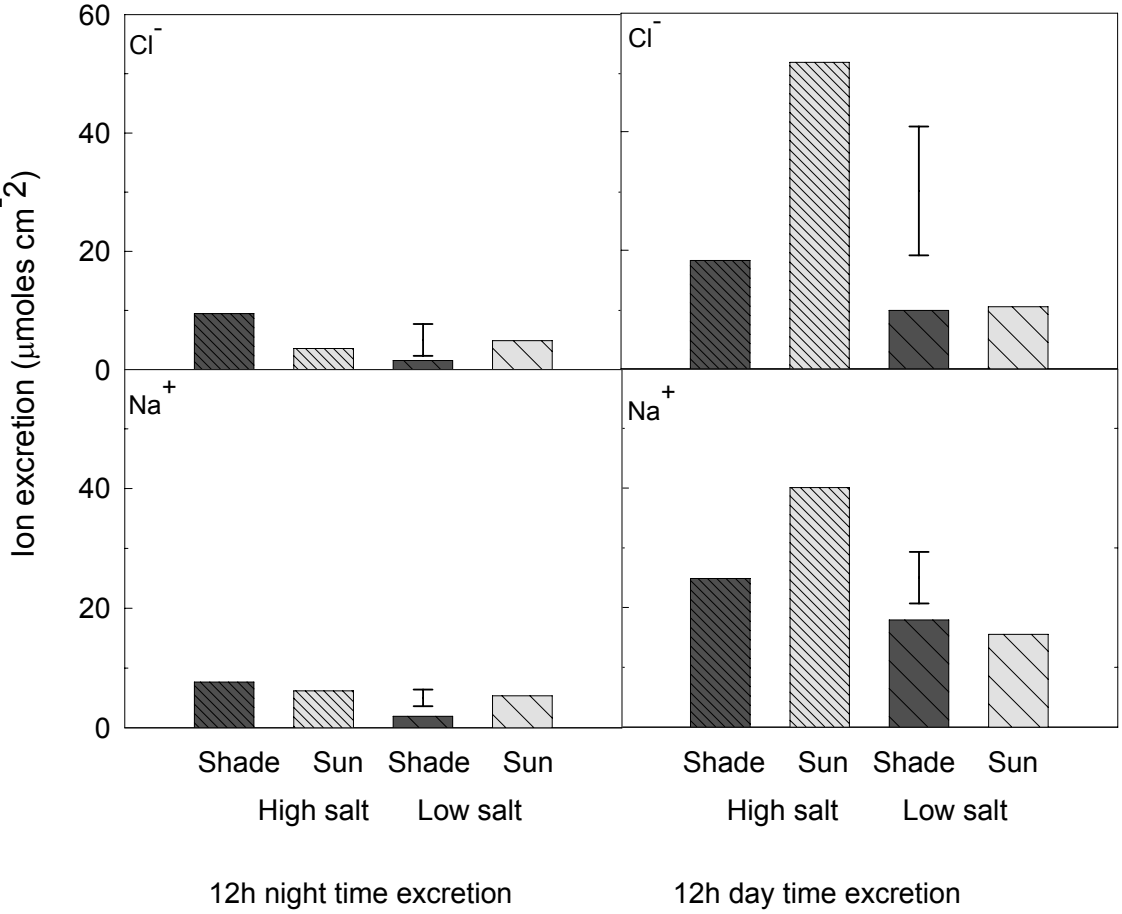


Figure 5.6. Diurnal chloride and sodium excretion from the leaf surface over a 24-h period 2 days after the shading treatments were imposed. Error bars represent LSD (P = 0.05) for the salt × shading interaction.

The amount of Cl^- and Na^+ excreted from the *D. spicata* leaves 49 days after shading was imposed, was not statistically affected by the salt load, or light intensity (Fig. 5.8). Similarly, there was no salt × shading interaction for the measurement 49 days after the shading was imposed.

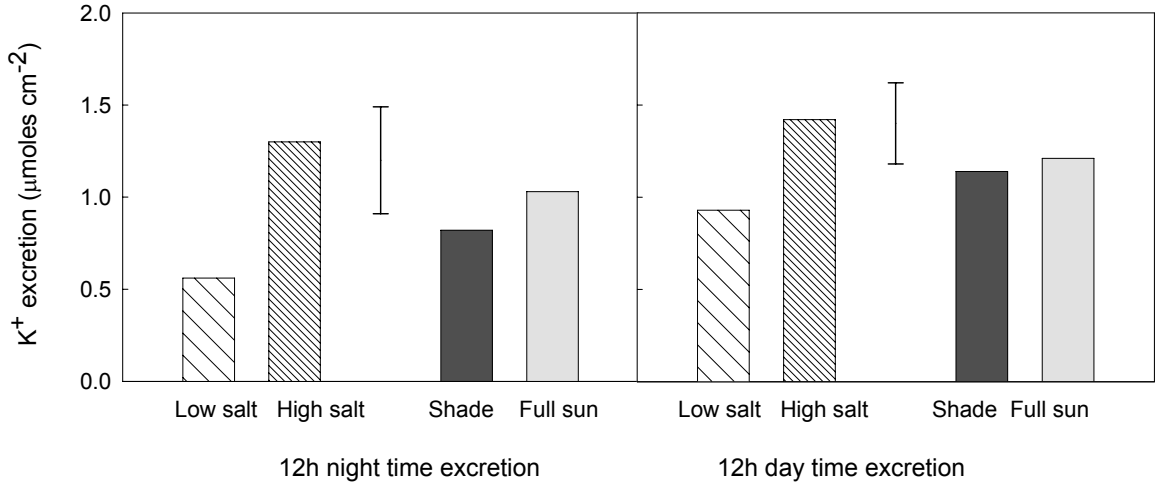


Figure 5.7. Diurnal potassium excretion from the leaf surface over a 24 h period for the main treatments (low and high salt, along with shaded and full sun) 2 days after the shading treatments were imposed. Error bars represent LSD ($P = 0.05$) for the salt and shading main effects.

The amount of K^+ excreted 49 days after the shading treatments were imposed, was significantly affected by salt load, but not light treatment (Fig. 5.9). Again, there was more K^+ excreted from plants under the high salt load compared to those under a low salt load. Quantities of K^+ excreted over the 24 h period 49 days after shading were about $^1/_3$ of that excreted 2 days after shading (Fig. 5.7 and 5.9). There was no significant interaction between salt × shading treatments 49 days after shading treatments were imposed.

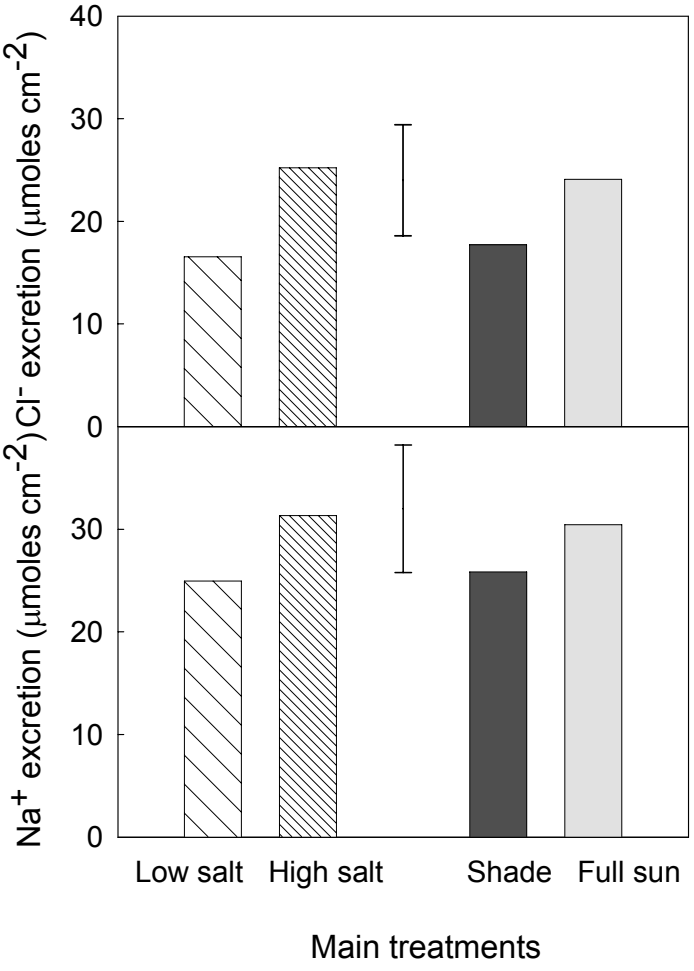


Figure 5.8. The effect of salt level, and shading treatments on the chloride and sodium excretion rates from leaves over the 24 h period, 49 days after the shading treatments were imposed. Error bars represent LSD (P = 0.05) of the salt and shading main effects.

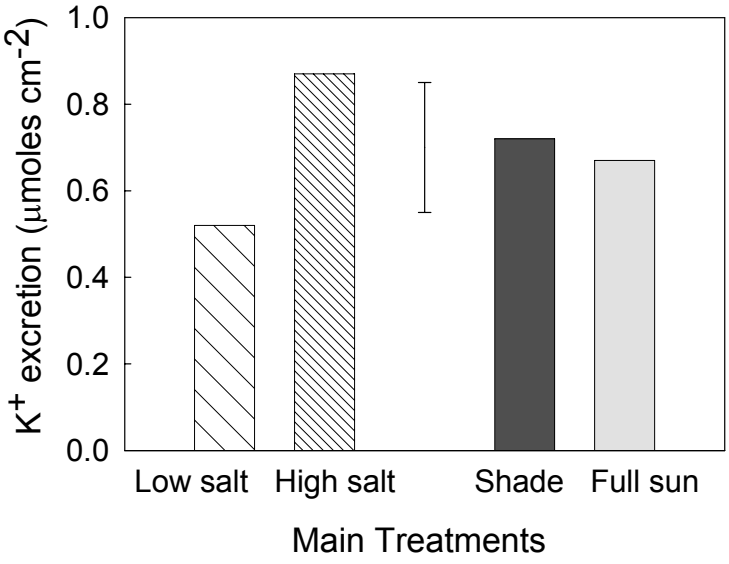


Figure 5.9. The effect of salt and shading treatments on the potassium excretion from leaves over the 24 h period, 49 days after the shading treatments were imposed. Error bars represent LSD (P = 0.05) for the salt and shading main effects.

5.3.4 Transpiration

The transpiration and stomatal conductance of water vapor by *D. spicata* leaves were significantly affected by both the salt load and light levels at different times during the experiment (Fig. 5.10). High salt levels reduced stomatal conductance 3 days before shading was imposed. Then on day 49 after shading, high salt reduced both the transpiration rate, and the stomatal conductance of the *D. spicata* leaves. Transpiration and stomatal conductance was nearly halved when the *Distichlis* plants were shaded, at both day 42 and 49 after the shading treatments commenced.

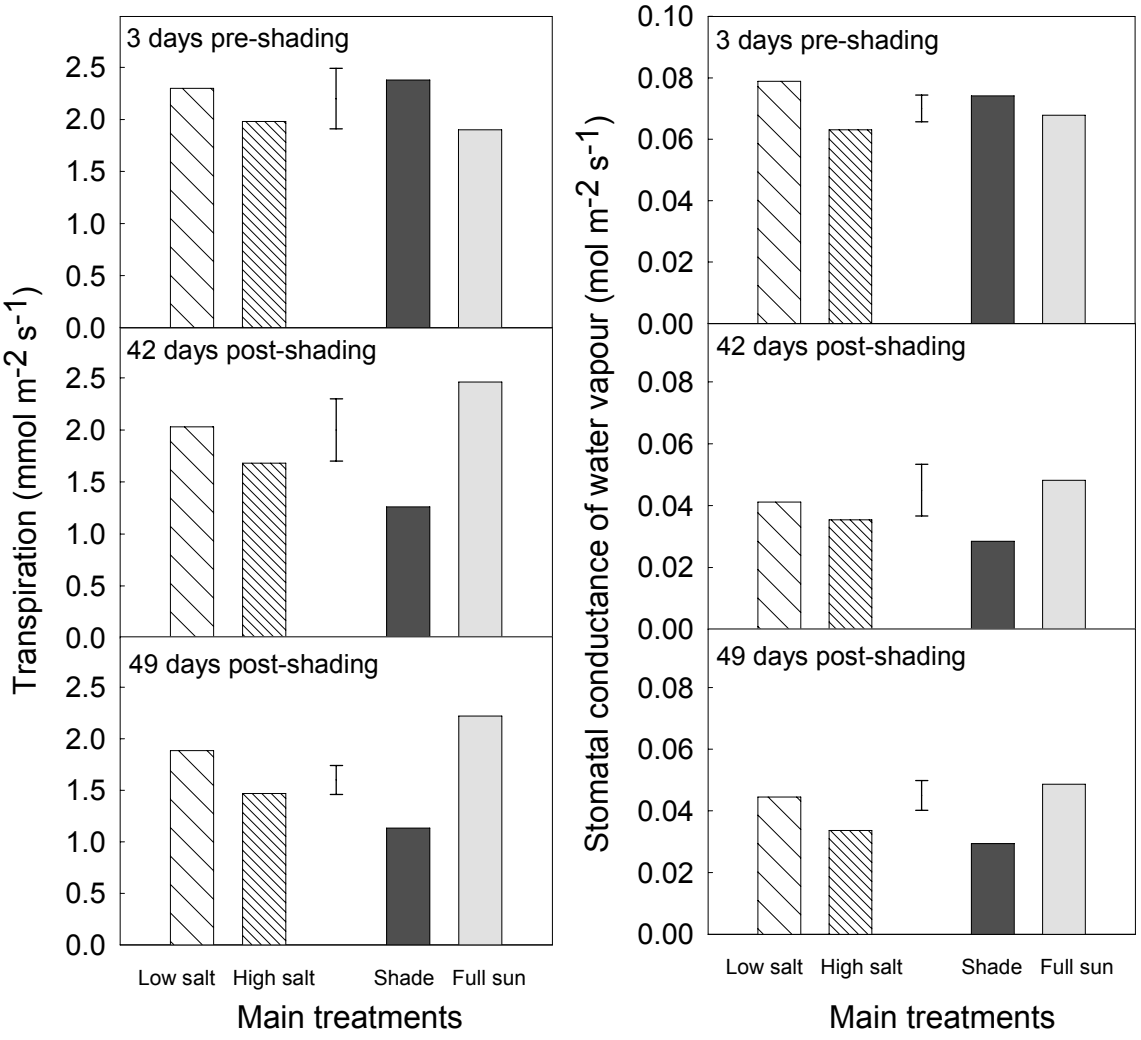


Figure 5.10. Effect of salt and shading treatments on transpiration and stomatal conductance of water vapour at day -3, +42 and +49 days relative to the commencement of the shading treatments. Error bars represent LSD (P = 0.05) for the salt and shading main effects.

5.4 Discussion

The initial hypothesis put forward was that the salt tolerance of *D. spicata* is directly linked to its ability to excrete salts through salt glands in the epidermis of the leaf. It was further hypothesized that salt excretion is a highly energy-dependant process, and therefore, plants grown under high light intensities would be able to excrete greater quantities of salts, and therefore tolerate high salt concentrations. This hypothesis was supported by Kemp and Cunningham (1981) who concluded that physiological processes such as salt excretion, compartmentalization and manufacture of natural osmotica may be dependant directly on light energy. Fahn (1988) and Liphschitz and Waisel (1982) both point out that salt gland cells contain high numbers of mitochondria, further suggesting that salt excretion is a highly energy-dependent process. The proposition was further supported by visual observations during a prior glasshouse experiment where plants exposed to high salt levels died following a period of overcast weather, while plants exposed to low levels of salinity survived (Chapter 4). The results presented in this chapter do not support this hypothesis. Shading did not affect *D. spicata* growth over a 49 day period under the conditions of this study (Fig. 5.2).

If the hypothesis that salt excretion is directly linked to light intensity is correct, then one would expect that shaded plants would excrete significantly less Na^+ and Cl^- than plants growing in direct sunlight. This is only seen in the short term, 2 days after shading treatments were imposed (Fig. 5.6). In the longer term, 49 days after shading treatments were imposed, there was no significant difference between Na^+ or Cl^- excretion between plants growing under shade or in direct sunlight. A further expectation would be that leaf osmolarity would be higher in the shaded plants, as they would be unable to excrete salts as effectively as the plants grown in direct sunlight. Again, the osmolarity over the short term did not differ between plants grown in shade or direct sunlight. In the longer term, after 49 days, the highest leaf osmolarity occurred in plants grown in full sunlight and with high salt levels (Fig. 5.4). It would also be expected that plant growth would be significantly less with the possibility of some plant death in the shaded plants, as their salt tolerance would be decreased. However, plants that were shaded produced similar shoot yields as plants in the direct sunlight, despite the shaded plants having a much reduced net photosynthetic rate at day 49. This is surprising as the shaded plants grew for 7 weeks in low light that was around $^1/_3$ of the full sunlight. A possible explanation for this result is that the shaded plants might have been mobilizing and utilizing energy reserves from the rhizomes. It is possible that carbohydrates stored within the rhizomes prior to the commencement of shading were able to supply energy substrates to supplement any shortfall in the current photosynthetic rate in the shaded plants during the course of this experiment.

Findings from this study point to the high possibility of ion exclusion at the root level being an important salt tolerant mechanism for *D. spicata*,, although this was not shown to be an important mechanism in Chapter 4. This assumption is based on the concentration of salts within the sand medium compared to that in the shoots of *D. spicata.* However, as the roots were not analysed, it is possible that there may have been some accumulation within the root tissue. Assuming that there was no accumulation within the root tissue, then the concentration of these ions in the plant tissue, along with those excreted would reflect the ratio in the sand medium, where the concentration of Na^+ and Cl^- was about 300% higher than in the high salt treatment (Table 5.1). However, the concentration of Na^+ and Cl^- in the shoot tissue was only 20-30% higher in the high salt treatment, rather than the 300% that would be expected (Fig. 5.5). Further to this, the salt excretion two days after shading was around 100 and 180% higher for Na^+ and Cl^-, respectively, for the high salt treatment (Fig. 5.6). Over the longer term, this difference declined, such that by day 49 after shading was imposed, the higher excretion rates were around 25 and 50%, respectively, for Na^+ and Cl^- in the high salt treatment, compared to the low salt treatment (Fig. 5.8). Again, we do not see a 300% increase in salt excretion as would be expected, if ion exclusion at the root level was not occurring. Saline ion exclusion at the root level has previously been identified as a major mechanism of salt tolerance in the halophyte grass *Spartina alterniflora,* which also occupies the same physiological niche as *D. spicata,* preventing up to 85% of the saline ions that should have passed through the plant if there was no exclusion at the root level (Bradley and Morris 1991).

Although it appears that salt excretion is not directly linked to light, there was a diurnal pattern of excretion, with the majority of salt excretion occurring during the day-time (Fig. 5.6). This finding is contrary to the findings of Hansen *et al.* (1976) who found that salt excretion in *D. spicata* primarily occurred during the night. The conditions of the experiment of Hansen *et al.* (1976) were quite different to this study, as it was a field experiment conducted in a desert playa. These contrary findings may be attributed to the different growing conditions, where differences between the experiments occured in light intensity, water and nutrient availability. Different patterns of salt excretion in response to different enviornmental conditions have been previously noted by Waisel (1972). The salt excretion from the *D. spicata* leaves also shows a significant specificity towards Na^+ and Cl^- ions, which can be seen from the relative amounts of these ions excreted, compared to K^+. The concentration of Na^+, Cl^- and K^+ within the plant tissue, are relatively similar, however significantly more Na^+ and Cl^- was excreted, compared to K^+. A high specificity towards Na^+ and Cl^- has previously been reported in the literature (Liphschitz and Waisel 1982). Another interesting observation is the reduction in excretion of ions over the period of the experiment. Sodium and Cl^- excretion declined by 20-40% in the high salt

treatments from the commencement of shading to the completion of the experiment. Potassium excretion also decreased over this period. However, it is likely that much of the decrease in ion excretion by the salt glands could be attributed to an equivalent decline in transpiration from 3 days of pre-shading, to 49 days of post-shading. Lower transpiration rates would mean that less salt would be transported to the leaf tissue, reducing the requirement to excrete salt from the salt glands.

The decline in transpiration that occurred on days 42 and 49 after shading was imposed, appears to be due to a partial closure of the stomata (Fig. 5.10). On days 42 and 49, the transpiration rate and stomatal conductance of water vapour by shaded plants was approximately half that of plants in direct sun light. This partial closure of the stomata would reduce the transpiration rate of the shaded plants, and hence the delivery of salt to the shoots in the xylem sap. Thus the closure of stomata would have assisted the shaded plants to tolerate high salt levels in the sand medium. Robinson *et al.* (1997) also proposed that stomatal closure was an important mechanism of salt tolerance in the halophyte *Aster tripolium*, where stomatal opening was inhibited by the presence of excess Na^+ ions. The authors concluded that the regulation of transpiration is a major salt-tolerance mechanism in some species. In this study, it appeared that the reduction in transpiration assisted the shaded *D. spicata* plants to tolerate the high salt levels. However the cause of the partial stomatal closure is likely to be different to that reported in the experiment by Robinson *et al.* (1997). The most likely cause for the partial closure in the *D. spicata* plants was the reduced light levels of the shaded plants, where the PAR was about 25% of full sunlight treatments on day 42 and 49. It was also observed on day 49 after shading, that there was a significant reduction in transpiration and stomatal conductance in the high salt treatment. There are two possible explanations for this. These include a) a lower osmotic potential within the sand solution, thus causing partial stomatal closure, and b) a mechanism described by Robinson *et al.* (1997) where excess Na^+ ions are involved in the closure of stomata.

Another salt tolerance mechanism in *D. spicata* is its ability to accumulate glycinebetaine as a compatible solute within its tissues to achieve osmotic adjustment within the cytoplasm (Marcum 1999). Leaf osmolarity at day 49 shows that plants growing in the high salinity and direct sunlight treatments had higher tissue osmolarity than other treatments (Fig. 5.4). This was also the pattern for shoot Na^+ and Cl^- concentrations (Fig. 5.5). However, the total osmoregulation as indicated by the osmolarity measurements, can not be explained by Na^+ and Cl^- concentrations alone as osmolarity increased 150% between the low and high salt treatments, yet concentrations of Na^+ and Cl^- only increased around 25% in plants exposed to the high salt treatment. This suggests that in the shoots other osmoregulants such as glycinebetaine played an important role in this osmotic adjustment by the high salt – full sun plants.

The concentration of Na^+ and Cl^- of *D. spicata* shoots was also quite low compared to other halophyte species commonly found in saline areas throughout Australia. The highest concentration of Na^+ and Cl^- found within the *D. spicata* shoots was in the high salt – direct sunlight treatments, where concentrations reached around 2% (Fig. 5.5). This treatment would be the closest treatment to a saline discharge zone within the mixed farming region, and the resulting salt concentration of 2% is substantially lower than that for other halophyte species commonly found within southern Australia; their concentrations of Na^+ and Cl^- are generally between 5-10% (Norman *et al.* 2002). However, at low salinity, the shaded treatment had a higher concentration of Na^+ and Cl^- within the shoots than the full sun treatment, the reverse of that found at high salinity (Fig. 5.5). The reason for this reversal is unknown. However at low salinity, concentrations range from 1-1.5%, which is within the range of a number of legumes and herbs found on saline sites (Norman *et al.* 2002). These values are significantly lower than those reported for other halophytic pasture species, such as old man saltbush (*Atriplex nummularia*) and bluebush (*Maireana brevifolia*) which have concentrations of Na^+ ranging from 6.1 to 7.7% and 7.2-8.4%, respectively. Likewise, the concentrations of Cl^- in these species ranged from 7.8-10.0 and 5.8-7.8%, respectively (Norman *et al.* 2002). Animals that consume plants containing high concentrations of salt need to expend energy for mineral metabolism and for maintaining electrolyte homeostasis, along with greater supplies of good quality drinking water (Arieli *et al.* 1989; Barrett-Lennard 2003b; Potter 1961).

While the findings from this experiment enable the hypothesis relating salt tolerance to light intensity to be rejected, there were a number of limitations in this study. Firstly, a number of measurements, such as transpiration rates immediately following the imposition of shading treatments were not able to be made. It would also have been very useful to have recorded photosynthetic rates both prior to and post shading. These measurements would have indicated whether there were short-term reductions in stomatal closure and photosynthesis rates. The transpiration and photosynthetic measurements were not able to be carried out because of equipment failure. A second limitation was that the restricted number of plants used prevented measurements of xylem salt concentration and the flow rates of xylem sap, in different treatments. This would have enabled a more quantitative assessment of ion exclusion mechanism in *D. spicata*.

This glasshouse experiment provides the data that point to a variety of salt tolerant mechanisms in *D. spicata* that enable *D. spicata to* grow in highly saline soil. These include saline ion exclusion at the root level, accumulation of compatible solutes to achieve osmotic adjustment within the plant tissue and salt excretion onto the leaf surface. In addition to these, there was also

the indirect mechanism of transpiration control by partial stomatal closure under shading conditions which may assist establishment during the cooler more overcast winter months of southern Australia. These mechanisms all play an important role in the ability of *D. spicata* to tolerate high salt concentrations in the root zone. No evidence could be found to support the hypothesis that salt excretion by salt glands, and associated salt tolerance, was directly related to high light intensities.

Chapter 6

Field establishment of *Distichlis spicata* at saline discharge sites

6.1 Introduction

Distichlis spicata cv. yensen-4a is a halophytic pasture grass that has been selected for forage production on saline soils. It appears to be suited to saline areas on mixed farms in southern Australia. However, the establishment of *D. spicata* in saline discharge soils has had mixed results since it was introduced into Australia in the mid 1990s. Plantings in Western Australia and Victoria have shown that vegetative establishment into saline soils has been patchy (Leake *et al.* 2002). These patchy establishments have been attributed to the large variation in soil properties of saline soils (Semple and Koen 2004).

The establishment of *D. spicata* cv. yensen-4a has been traditionally conducted with bare rooted tillers that have been planted through a vegetable planter in early autumn and late winter. Vegetative establishment is the only option for establishment, as this particular selection is a dioecious male plant. Being a C_4 grass, the majority of growth occurs throughout the warmer summer months, with limited growth occurring during the winter period. However, the majority of the rainfall in southern Australia occurs during winter and spring, when moisture regimes are favorable for establishment, but temperatures are not ideal for active *D. spicata* growth. Establishment into saline soils when moisture is limiting may pose considerable stresses on plants, as the salinity will exacerbate the moisture deficit stress. The question arises as to when and how best to establish *D. spicata* plants into these saline environments to maximise plant survival.

The experiments outlined in this chapter aim to address the problems and difficulties of establishing *D. spicata* in saline discharge sites within the Wimmera and Mallee regions of north western Victoria. These areas are typically low rainfall, which limits the opportunities for favorable conditions for establishment. A number of establishment experiments were conducted at saline discharge sites at Manangatang and Donald over a 2-year period. Different approaches were used to improve establishment, particularly at the dry saline site at Manangatang in the Victorian Mallee.

6.2 Experiment 1

This experiment was initially set up as part of a larger field experiment. The study was to investigate the impact of *D. spicata* on the local environment, particularly on the levels of the watertable at the discharge site. It was hypothesized that *D. spicata* would establish across the site but its growth would be restricted in the high salinity areas of the site.

6.2.1 Materials and methods

Site description

The site was located on the east side of a saline discharge zone (35°02'S, 143°05'E) in the Mallee region of north western Victoria, near the township of Manangatang. The saline discharge site was surrounded by sandy rises, which were cropped (Plate 6.1). The upper eastern edge of the discharge site had been planted with trees and saltbush (*Atriplex* spp.), and a road passed through the centre of the site (Plates 6.1 and 6.2). Established samphire plants (*Halosarcia* spp.) were growing in the lower region of the transect. There was also a dam and dead trees located in the discharge site, on the west side of the road, indicating that the site had not been saline at an earlier time. The soil type in the discharge site was a sandy clay loam over a clay subsoil.

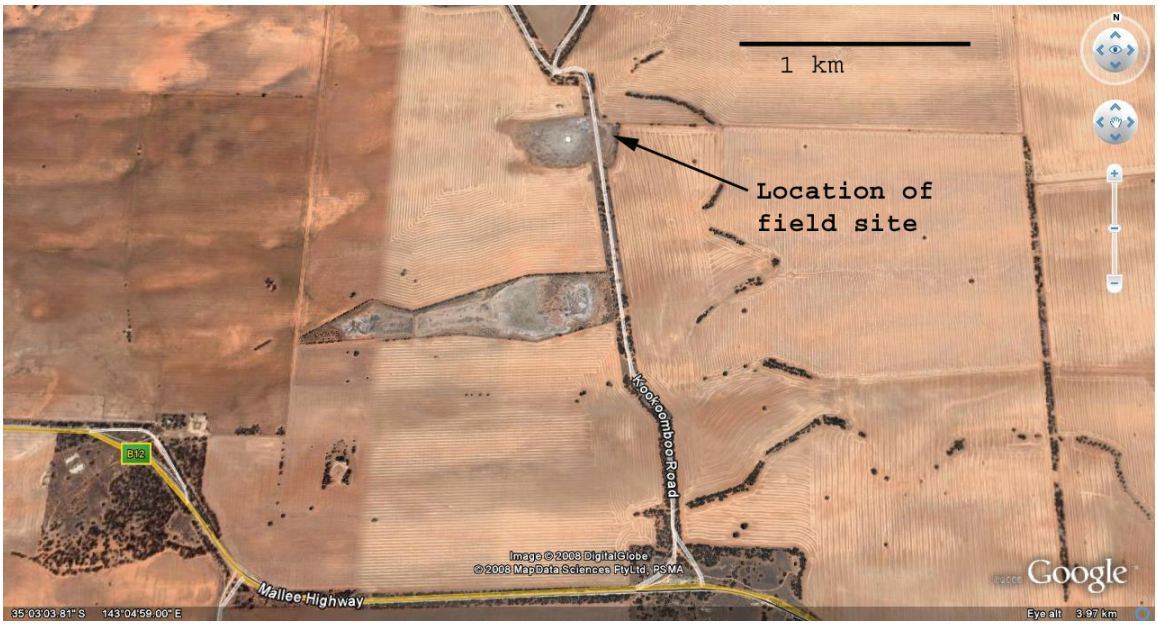


Plate 6.1. Satellite image of the Manangatang field site.

Plate 6.2. Looking over the Manangatang field site. The white poles are located along the three transects.

Experimental design and treatments

Three transects, 10 m in length, and 3 m wide were set out running from the upper edge of the discharge site, where saltbush, annual grasses and legumes were growing (Plates 6.2 and 6.3). The focus of the experiment changed when the *D. spicata* failed to establish at the lower end of each transect. Each plant became an independent measure of establishment success. The experiment became a regression analysis relating establishment success (survival) to adjacent soil properties.

Plate 6.3. Looking across the three transects. The top of the transects were located higher up the landscape, and ran down into the more saline area.

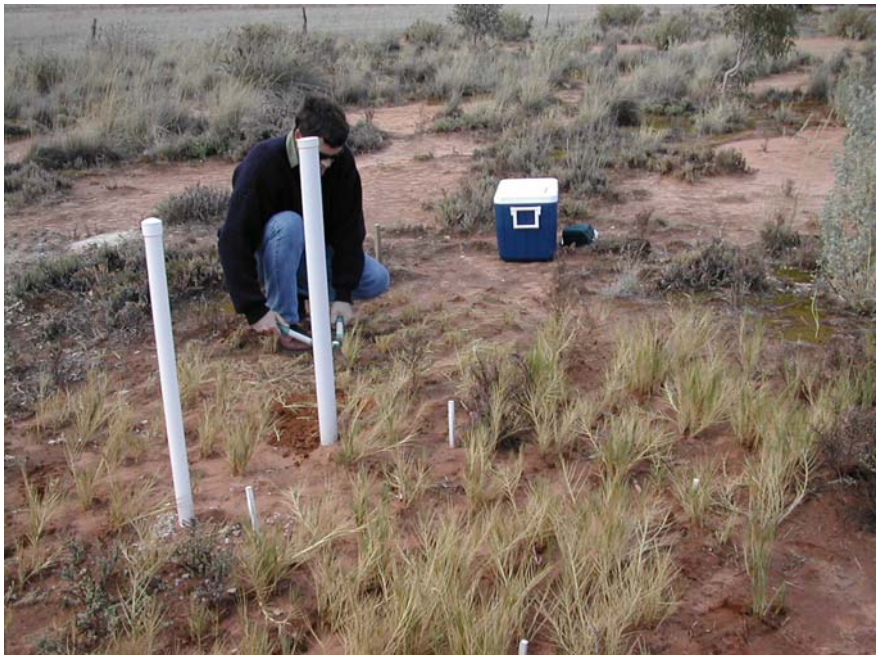


Plate 6.4. The top end of the first transect in mid June 2005. This area achieved the highest survival rate.

Growing conditions

All three transects were treated identically and were rotary hoed to a depth of 10 cm. Glasshouse-raised rooted tillers of *D. spicata* from La Trobe University, that had been trimmed to a height of 10 cm, were planted 20 cm apart along the transects. Planting was conducted on 17 September 2004 and survival assessed 2 months later on 18 November 2004 (Plate 6.4).

Approximately 20 mm of rain was received at the site in the 10 days prior to planting (Fig. 6.1). However, only 7.8 mm of rain was received in 6 small rainfall events in the month following planting. All of the November rain fell in the first 2 weeks of the month, with 56 mm received during these 2 weeks. The daily maximum temperatures fluctuated between 18 and 40 °C during the 2 months following planting.

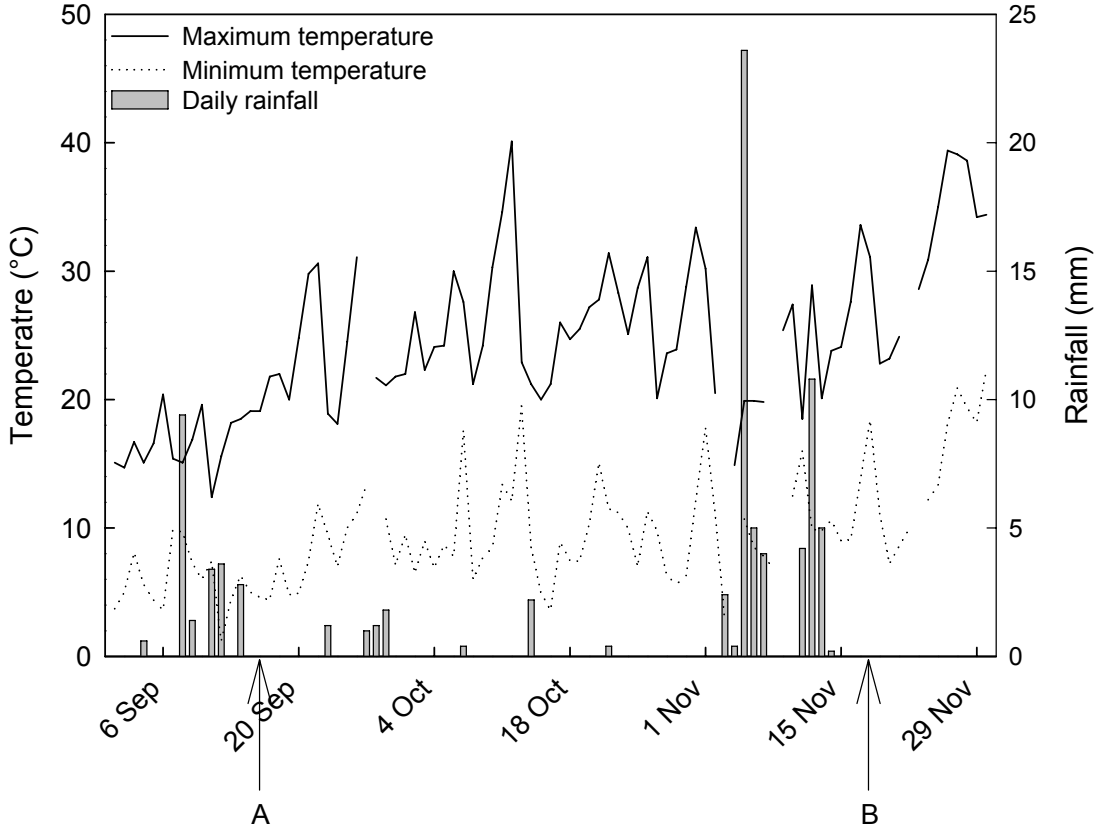


Figure 6.1. The daily maximum and minimum temperatures, along with daily rainfall from September to December 2004. The planting date was on 20 September (A), while survival was assessed on 18 November (B). Temperature and rainfall data sourced from closest Bureau of Meteorology Station at Manangatang (18 km west for rainfall data) and Ouyen (70 km west for temperature data).

Measurements

One hundred plants were randomly selected across the three transects. These plants represented the range from complete mortality, to plant survival with active growth. The shoots were removed at a height of 10 cm above the soil surface; these shoots therefore represented the re-growth after planting. A soil sample was taken to a depth of 10 cm beside each harvested plant. The plant material was dried in an over at 100 °C until a constant weight was achieved. The soil samples were air dried, and then analysed for electrical conductivity (EC) and pH in a soil:water, 1:5 suspension that was shaken for 1 h. Plant growth data were then plotted against soil EC and soil pH to determine the relationship between survival and soil properties.

6.2.2 Results of Experiment 1

Improved survival occurred at the top of the transects, where approximately 80% of the plants survived (Plate 6.4). This was in stark contrast to the bottom of the transects where all plants died. Plant survival appeared to follow the pattern of vegetation cover at the site prior to establishment, with plants surviving where the natural vegetation of saltbush, annual grasses and legumes occurred on the slope.

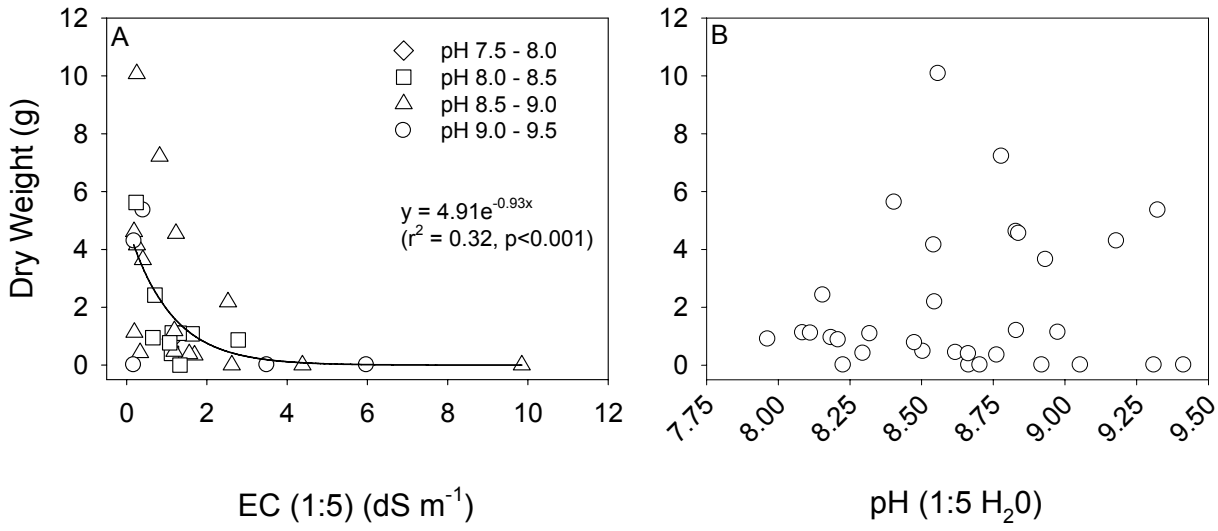


Figure 6.2. The relationship between dry matter production and the soil electrical conductivity and pH (Experiment 1).

Plant survival was closely related to soil EC, with increasing shoot regrowth occurring at low soil EC (Fig. 6.2). Survival declined exponentially with increasing soil EC, with virtually no plants surviving where soil EC was 3 dS m^{-1} or greater (Fig. 6.2A). There was no relationship between survival and soil pH (Fig. 6.2B).

6.3 Experiment 2

The low survival of plants in the more saline regions of the transects of Experiment 1, and the improved survival of plants that had been salt primed in Chapter 4, led to a new approach to establishing *D. spicata* in the field. The major factor that appeared to limit establishment in Experiment 1 was the high soil EC. It was hypothesized that a two-pronged approach would increase establishment success: these were to (i) establish the *D. spicata* plants on raised beds that had been leached by winter rain to reduce the soil salinity levels, and (ii) to improve the salinity tolerance of the *D. spicata* plants by pre-exposing them to saline conditions, using the process termed 'salt-priming' that was evaluated in Chapter 4.

6.3.1 Material and methods

Experimental design and treatments

The experiment was set up at the Manangatang site as a randomized split-split plot design with four replicates. There were 3 main plots representing location on the transect (upper, middle and lower). The 2 split-plot treatments were the raised beds and the adjacent flat area. There were 5 split-split plot treatments, which represented duplicate plants of *D. spicata* from different sources with different treatment histories (Table 6.1). Survival data were acrsin transformed to ensure the normality of the residuals, and the statistical analysis was conducted on the transformed data.

Table 6.1. The source and treatment history of *D. spicata* plants used in the field experiment.

Plant type	Plant history
Wickepin	Plants sourced from a saline discharge zone at Wickepin, Western Australia.
Manangatang	Plants were sourced immediately prior to planting from plants that survived experiment 1, at the same site.
Salt-primed Manangatang	Plants sourced from experiment 1, 6 weeks before planting, and were then grown in a glasshouse and irrigated with saline water (10 dS m^{-1}) daily, and a commercial soluble nutrient solution weekly.
Glasshouse	Plants were sourced from the nursery stock of plants grown in a glasshouse at La Trobe University. These plants had not been previously exposed to salt.
Salt-primed Glasshouse	Plants were sourced from the nursery stock of plants at La Trobe University, however plants were irrigated with saline water (10 dS m^{-1}) daily, and a commercial soluble nutrient solution weekly.

Growing conditions

Linear shaped raised beds, with a length of 1 m and a width of 0.3 m were formed from top soil to a height of 0.15 m at 3 locations along the transect on 16 June 2005. After the raised beds were formed, a total of 74 mm of rainfall was received between 16 June and 31 August (Fig. 6.3). Above average rainfall was received during the winter months, with a total of 115 mm received between 1 June and 31 August 2005. This rainfall exceeded the long-term average of 89 mm.

The site was planted on 31 August. Two bare-rooted tillers of each plant type were planted beside each other on the raised beds, and on adjacent flat area beside the bed. Rainfall in the month of September was 33 mm, which was 10% above the average for the month. However, there was only one rainfall event greater than 2 mm in the first 3 weeks following planting. Daily maximum temperatures also increased slowly following planting ranging from 10 to 27 °C (Fig. 6.3).

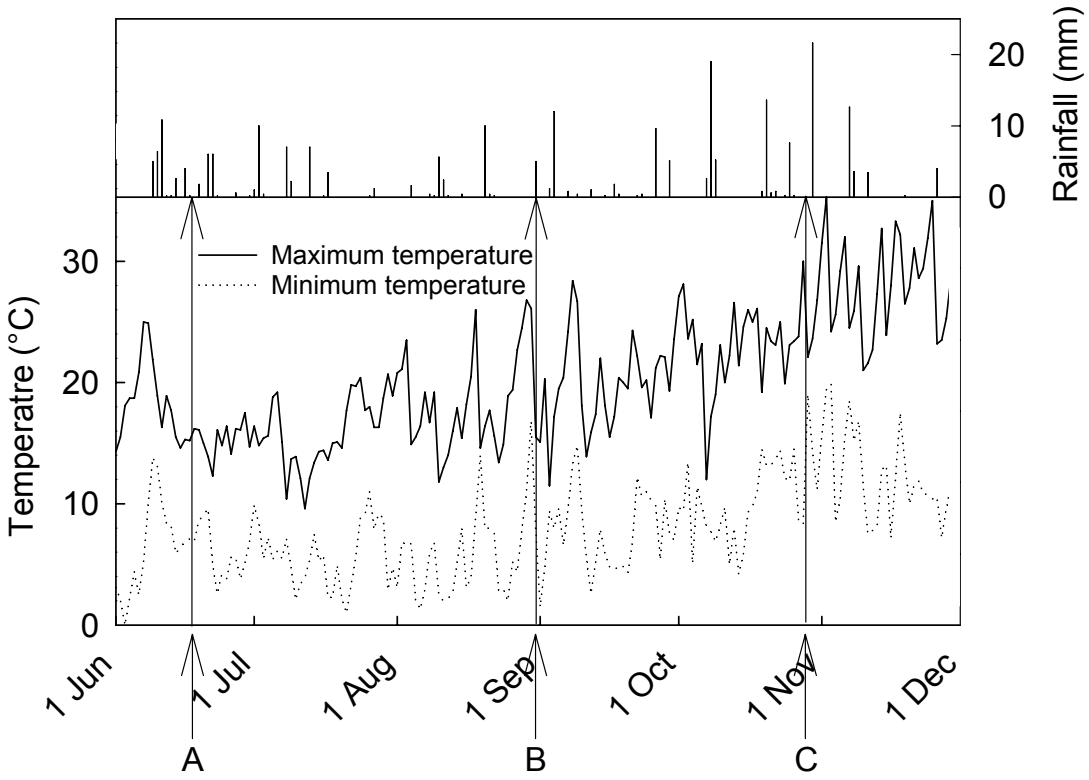


Figure 6.3. The daily maximum and minimum temperatures, along with daily rainfall from June to December 2005. The raised beds were formed on 16 June (A), *D. spicata* tillers were planted on 31 August (B) and assessed for survival on 28 October (C). Temperature and rainfall data sourced from closest Bureau of Meteorology Station at Manangatang (18 km west for rainfall data) and Ouyen (70 km west for temperature data).

Measurement

The survival of *D. spicata* plants were assessed by the presence of at least one green leaf on the plant on 28 October 2005. Soil samples were taken when the raised beds were formed, and when they were planted. Sub-samples were then taken for gravimetric water content for soils sampled at the time of planting. All remaining samples were air dried, and analysed for EC and pH in a 1:5, soil:water suspension that was shaken for 1 h.

6.3.2 Results from Experiment 2

Plant survival in this experiment was quite low. Survival was affected by raised beds ($P<0.1$) and location on the transect ($P<0.05$), but not affected by the plant type (Table 6.2). Raised beds decreased plant survival, with only 7% of plants surviving, compared to 12.5% survival on the adjacent flat area. Both the upper and middle areas on the transect had a similar survival of around 15%, whereas no plants survived in the lower portion of the transect. There were no differences in the survival, between the 5 plant types. Importantly, there was no difference between salt-priming and no salt-priming for plants from Manangatang or from the glasshouse collection.

Table 6.2. Plant survival for the raised bed, location on the transect and plant type treatments (Experiment 2).

Treatment		Survival (%)
Raised beds		
	Raised beds	7.0
	Flat	12.5
	Significance	#
Location on transect		
	Upper transect	14.8
	Middle transect	14.5
	Lower transect	0
	Significance	*
Plant type		
	Wickepin	9.2
	Manangatang	16.7
	Salt-primed Manangatang	4.2
	Glasshouse	8.3
	Salt-primed glasshouse	10.4
	Significance	n.s.

# P<0.1, * P<0.05, n.s. not significant at P=0.1.

The soil EC was significantly increased by the creation of the raised beds, when the beds were initially formed on 16 June, and measured later on 31 August. Soil EC was also 2.5-3 times higher at the lower location on the transect, compared to the upper and middle regions, with the soil being quite saline, with a EC of 5 dS m^{-1} in the lower transect at the time of planting. The moisture content was also higher at the lower location on the transect, with a gravimetric water content of 26%, compared to 12-19% in the upper and middle regions at planting. Soil pH was not affected by raised beds or location on the transect.

Table 6.3. Soil electrical conductivity (EC), pH and gravimetric water content (ω%) of the different treatments when the raised beds were formed on 16 June (initial) and when planting occurred on 31 August.

Main Treatment		EC (dS m^{-1})		pH (H_2O)		ω
		16 June	31 Aug	16 June	31 Aug	(%)
Raised beds						
	Raised beds	2.8	3.4	8.5	8.4	18
	Flat	2.1	2.3	8.5	8.5	20
	LSD (*P*=0.05)	0.7	0.7	n.s.	n.s.	n.s.
Location						
	Upper transect	1.3	1.3	8.5	8.7	12
	Middle transect	1.7	1.9	8.4	8.4	19
	Lower transect	4.3	5.4	8.5	8.3	26
	LSD (*P*=0.05)	1.8	1.6	n.s.	n.s.	4

n.s. not significant

6.4 Experiment 3

In response to the establishment failures in Experiment 2, a different approach to establishing plants in the field was taken. This approach involved using established plants with a functioning root system growing in an undisturbed soil core. The plants were planted in the field in the early autumn in 2006, when temperatures were high enough to enable *D. spicata* to grow rapidly. The aim was to minimize the transplanting shock, by planting with undisturbed root systems, after plants had undergone salt priming. This was achieved by growing the plants during the summer months from November to March in black plastic pots 7 cm in diameter, and watering with a solution containing nutrients and a high concentration of salt.

A second attempt was made to establish these plants, using the same preparation procedures in the late winter after the March planting had failed at Manangatang. This second planting time was trialed to determine whether the cooler and wetter conditions in late winter would improve *D. spicata* establishment.

6.4.1 Material and methods

Site description

This experiment was conducted at the Manangatang site used in experiment 1 and 2, and also at a site at Donald (36°16'S, 143°02'E) in the Wimmera region of north western Victoria. The Donald site was at the edge of a saline discharge area, where water collected throughout the year, forming a small lake (Plate 6.5). At this site the plants were planted along a transect, extending from dry land on the edge of the ponded area, down into the very wet soil with ponded water about 1 m away from the bottom of the transect (Plate 6.6). The upper region of the transect supported a mixture of spiny rush (*Juncus acutus*) and samphire (*Halosarcia* spp.), with the lower region supporting a lower density of mature samphire plants.

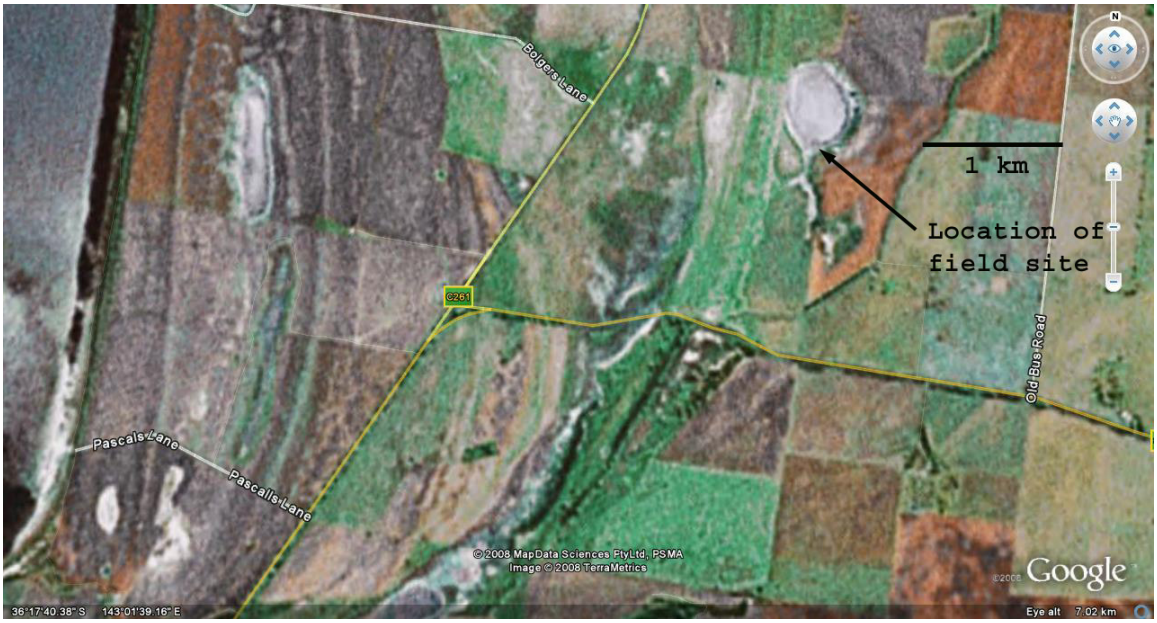


Plate 6.5. View of the Donald site from 7,000 m (Source: Google Earth)

Plate 6.6. The establishment area at the Donald site.

Experimental design and treatments

Plants were planted in a transect that ran down the slope into the discharge zone at both sites. The transect was 10 m long, and plants were planted every meter along the transect. At each location along the transect, 3 intact salt-primed plants were planted alongside 3 bare rooted tillers harvested from a pre-existing stand of *D. spicata* adjacent to the discharge zone at the Donald site. The paired plantings along the transect enabled plant survival to be analyzed using a paired t-test.

Growing conditions

The intact salt-primed plants were grown in 7 cm diameter pots that were free draining, and were allowed to establish over a period of 30 days with a nutrient solution applied automatically through a dripper system on to the soil surface of each pot. After this period, the salinity of the nutrient solution was increased by adding NaCl to the solution over a 2-week period to 45 dS m^{-1} and the pots irrigated daily with approximately 100 mL of the saline nutrient solution been applied to each pot. The nutrients contained in the solution were N (3.2 mM), K (1.2 mM), Ca (0.8 mM), P (0.4 mM), S (0.3 mM), Mg (0.3 mM), B (5 μM), Mn (2 μM), Zn (2 μM), Cu (0.5 μM), Mo (0.5 μM) and Fe (20 μM). The plants were grown for 2 months on this saline nutrient solution prior to planting at the field site.

Tillers were trimmed to a height of 15 cm 2 days before planting, to minimize moisture loss via transpiration. Plants were established into the moist soil at the discharge sites at Donald and Manangatang on the 4 and 5 March 2006, respectively, and again at the Manangatang site on 24 August 2006.

There was very little rainfall during the March period at both Manangatang and Donald (Figure 6.4). Maximum temperatures were generally high at Managatang and Donald and fluctuated between 20 and 40 °C during March, with higher temperatures during the first half of March.

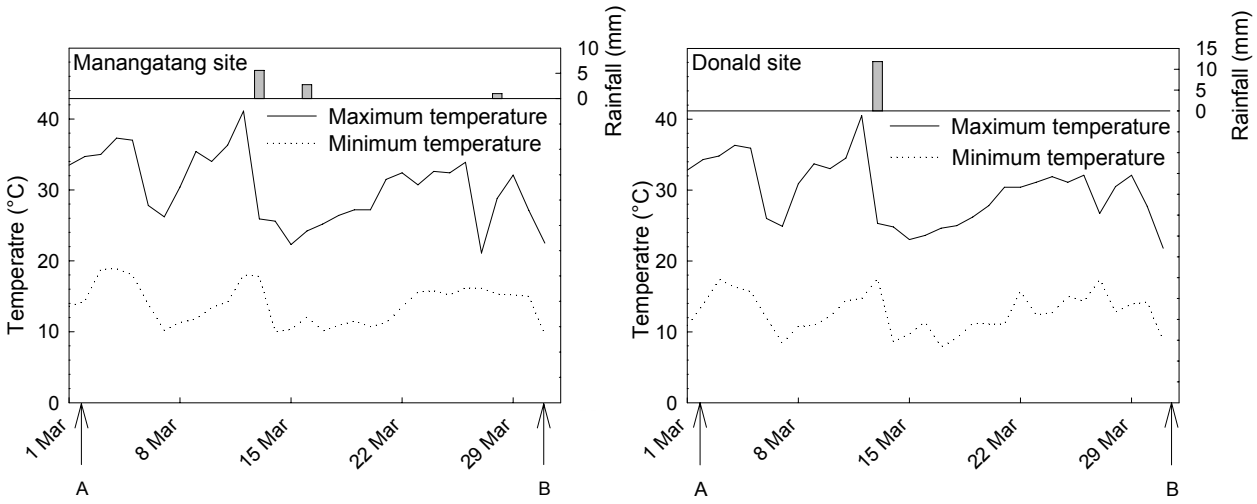


Figure 6.4. The daily maximum and minimum temperatures, along with daily rainfall at Manangatang and Donald for March 2006. The plants were planted on 4 and 5 March (A), and plant survival assessed on 31 March. The temperature and rainfall data were sourced from closest Bureau of Meteorology Station. The data for the Manangatang site was collected from Manangatang (18 km west for rainfall data) and Ouyen (70 km west for temperature data), while the Donald data was collected from Donald (10 km south for rainfall data) and Charlton (28 km east for temperature data).

A follow-up planting at Manangatang occurred on 24 August using *D. spicata* plants prepared in the same way, with salt-priming occurring in the glasshouse at La Trobe University, during the winter months. These plants were planted out in the same transect as the March planting, with bare rooted tillers being planted between the salt-primed plants with roots in an undisturbed mass.

August rainfall at Manangatang was very low with only 2 mm received for the month, which was far below the average of 30 mm for the month of August (Fig. 6.5). The September rainfall was about half of the average of 29 mm. Maximum daily temperatures averaged about 20 °C, while minimum temperatures ranged from 1-10 °C.

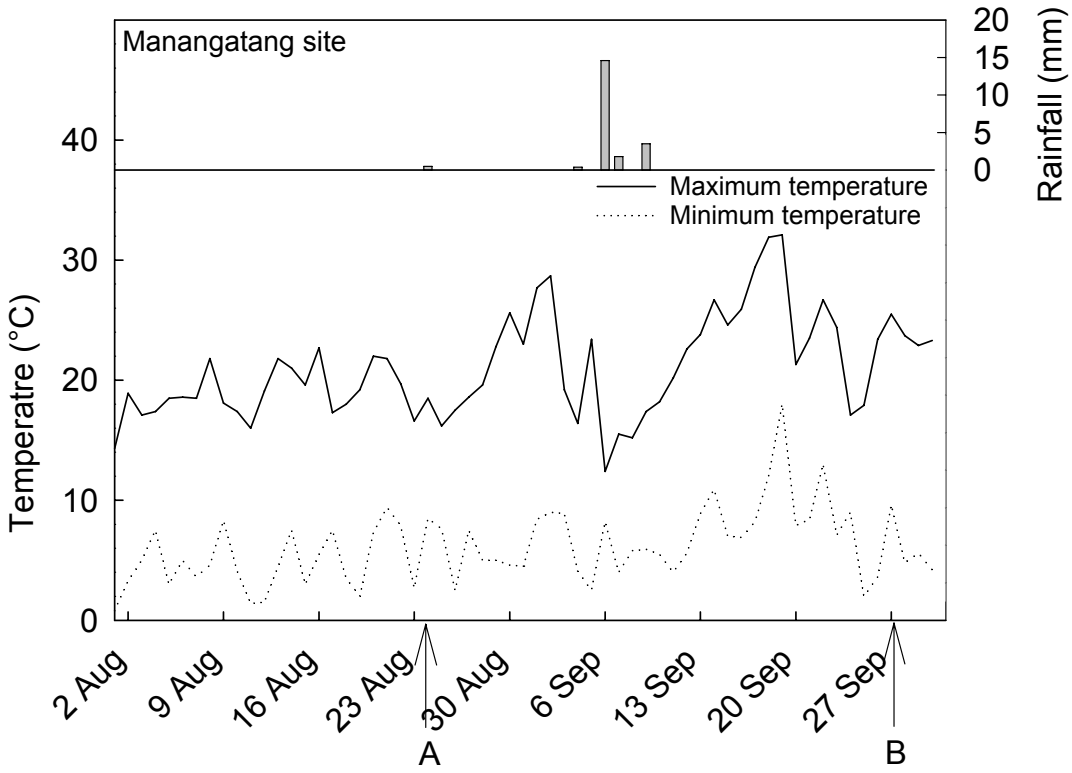


Figure 6.5. The daily maximum and minimum temperatures, along with daily rainfall at Manangatang for August 2006. The plants were planted on 24 August (A), and plant survival assessed on 30 September (B). Rainfall and temperature data sourced from closest Bureau of Meteorology Station at Manangatang (18 km west for rainfall data) and Ouyen (70 km west for temperature data).

Measurements

Plant survival was assessed by the presence of at least one green leaf on 31 March and 30 September. Soil samples were taken when the transects were planted to a depth of 10 cm. A sub-sample was then used to determine the gravimetric water content, and the remaining sample was air dried for soil EC and pH determination. This was done using a 1:5, soil:water suspension that was shaken for 1 h.

6.4.2 Results from Experiment 3

Plant survival at the Donald site was increased significantly by planting *D. spicata* with undisturbed roots, growing in an intact soil mass, compared to the bare-rooted tillers ($P<0.001$). Excellent establishment success, with 100% of plants surviving, occurred with the salt-primed, intact root system plants. This was a 5-fold increase in survival, compared to the survival of the bare-rooted tillers.

Both the intact root system and bare rooted plants failed to establish at the Manangatang site in March and August (Table 6.4). Soil EC and pH were similar at both sites. However, the gravimetric water content of the soil at the Donald site was almost 4 times higher than the drier Manangatang site (Table 6.5). The gravimetric water content at the Manangatang site measured in August 2006 was not recorded. However, it would be likely to be similar to the water content in March, as practically no rain fell during the April-August period in 2006, due to the extreme drought conditions that prevailed in the Victorian Mallee in the winter of 2006.

Table 6.4. Survival of intact and bare-rooted plants at Donald and Managatang (± SE mean) (Experiment 3).

Planting time / Site		Survival of intact plants (%)	Survival of bare rooted plants (%)
March			
	Donald	100 ± 0	20 ± 6.7
	Manangatang	0 ± 0	0 ± 0
August			
	Manangatang	0 ± 0	0 ± 0

Table 6.5. Soil electrical conductivity, pH and gravimetric water content (ω%) at the Donald and Manangatang sites at planting in March (± SE mean) (Experiment 3).

Site	Soil EC ($dS\ m^{-1}$)	Soil pH	ω (%)
Donald	8.5 ± 0.5	8.8 ± 0.01	35 ± 0.7
Manangatang	8.7 ± 0.3	8.7 ± 0.05	9 ± 0.4

6.5 Discussion

The results from the field establishment experiments demonstrate that *D. spicata* can be successfully established into hostile saline discharge sites under certain conditions. However, a delicate balance exists between soil moisture and soil salinity and this balance will determine whether successful establishment will be achieved. Establishment was successful at the Donald site despite the high soil EC of 8.5 dS m^{-1}. In contrast, the attempts to establish equivalent *D. spicata* plants in the lower transect at Manangatang failed completely (Table 6.4) even though this site had a similar EC of 8.4 dS m^{-1} (Table 6.5). The major differences at these two sites were the soil texture and moisture content of the soil. The Donald site had a gravimetric water content of 35%, compared to the much drier Manangatang site with 9% (Table 6.5). At the Donald site, water availability was greater and helped to alleviate the osmotic drought created where high concentrations of salts are present. Plants respond in a similar manner to both drought stresses and salinity (Munns 2002). Hence, dry soil conditions at the Manangatang site, exacerbated the osmotic drought created by the high concentrations of salts, and none of the *D. spicata* plants survived.

A number of approaches were trialed with the experiments to combat the salinity-moisture balance, including efforts to reduce soil salinity by leaching the raised beds, and altering the planting time for a more suitable moisture status. None of these efforts proved successful for establishing *D. spicata* in the saline lower transect areas at Manangatang (Table 6.2). In fact, the construction of the raised beds impeded establishment, as salts were not leached out of the raised beds during the winter months. This was despite above-average rainfall received during the winter months. Compared to the flat, the EC of the raised beds was higher, which was most likely due to more saline soil being used to construct the beds, as they were more saline immediately following construction. It is likely then that average rainfall for one winter period was not sufficient to leach salts out of the raised beds in this soil type, so this approach is unlikely to result in establishment in dry saline discharge soils.

Under the extremely saline conditions found at the Manangatang site, salt-priming was also found to have no beneficial effect on plant establishment. Salt-priming had previously been shown to improve establishment and subsequent growth under the high salt and high moisture regimes in the glasshouse in Chapter 4. Salt-priming of seeds has also been shown to improve the establishment of many glycophyte species (Babu and Thirumurugan 2001; Sivritepe *et al.* 1999; Sivritepe *et al.* 2005; Sivritepe *et al.* 2003; Umezawa *et al.* 2000). However, it needs to be considered that there was only one rainfall event greater than 2 mm in the 3 weeks following planting, while temperatures continued to rise. Although salt-priming in this extreme situation did not improve *D. spicata* survival, it may be a useful practice to improve establishment success in discharge areas where the water status is higher.

The use of salt-primed, undisturbed root systems to minimize transplanting shock was successful at the Donald site compared to the use of bare-rooted tillers. Close *et al.* (2005) defines transplanting shock as the negative effects on plant growth after transplanting and needs to be minimised if the fastest possible establishment is to occur. Many factors are involved, including the nutritional status of the plant and environmental stresses (Close *et al.* 2005), as well as the mass, or volume of the root system being planted (Close *et al.* 2003). Close *et al.* (2003) also found that seedling growth after transplanting was correlated to the plug depth (depth of the root and soil mass), rather than the plug volume. The deeper undisturbed rooting medium presumably improves root architecture in the establishing plant and increases the initiation of primary roots (Nelson 1996). The transplanting shock in Experiment 3 was minimised by using plants with salt-primed, undisturbed root systems in a plug that was 10-15 cm deep which would have resulted in good root architecture. Plants were also irrigated daily with a nutrient-rich solution prior to planting, ensuring that the nutritional status of the plant was high. Trimming of the plant tillers, reduced the potential loss of moisture form the plant, by restricting transpiration. These factors all assisted in minimizing the transplanting shock for the *D. spicata* plants and 100% survival resulted. In contrast only 20% of the bare-rooted plants used in Experiment 3 survived. The results show that efforts to minimize transplanting shock were successful.

The results from Experiment 3 suggest that it may be possible to establish *D. spicata* plants in the lower end of the transect at Manangatang. The most likely way to achieve this would be to use salt-primed plants with undisturbed roots during late winter, in years where average winter rainfall is received to ensure maximum levels of soil moisture. The chances of success would increase in particularly wet winters when free water accumulates and ponds on the surface of the discharge area. This approach is supported by the presence of mature samphire plants and lack of juvenile plants low in the landscape, suggesting that opportunistic establishment of the samphire

species occurs when conditions are favorable. The conditions of Experiment 2 in 2005 would be an example of a suitable year, as above average rainfall was received during the winter months, and the gravimetric water content was 26% in the lower transect soil with a soil EC 5.4 dS m^{-1} (Table 6.3) in late August. These conditions were closer to the soil conditions encountered at Donald that resulted in a successful establishment (Table 6.5). It is likely that the run of dry years, with 4 out of the last 6 years having below average rainfall would have lowered the water-table at the discharge site at Manangatang, and reduced moisture levels in the soil. If establishment of salt-primed undisturbed root system plants could be achieved during years of average winter rainfall, then it is likely that the roots of *D. spicata* would be able to extend down into the water-table during the spring which would enable the plant to survive in the dry summer months. Further field trials are required to test this proposition, and to refine establishment procedures.

In conclusion, the results from these field experiments have demonstrated that successful field establishment of *D. spicata* is reliant upon a favorable salinity-moisture balance in the soil. Successful establishment can occur in highly saline environments provided that there is adequate soil water present. Further field research is required to determine whether salt-priming and the use of plants with undisturbed root plugs are necessary to successfully establish *D. spicata* at saline discharge sites in late winter, if average winter rainfall is received. The following chapter will investigate the use of rhizome fragments as an alternative means of establishment to see if they offer benefits for establishment in saline environments.

Chapter 7

Rhizome establishment

7.1 Introduction

Conventional planting techniques for *D. spicata* have focused on planting bare-rooted tillers. The previous chapter (Chapter 6) investigated a number of techniques that aimed to improve the success of this technique. It was found that field establishment was quite variable between sites, with moisture and salinity having a major impact on this variability, along with variable planting material. Minimal research has been carried out to investigate the suitability of rhizome fragments for establishing *D. spicata*, either for the re-vegetation of salt marshes or for agricultural use, despite rhizome and sprig fragments being used to vegetatively establish some grasses (Fernandez 2003; Satorre *et al.* 1996). The only published research on *D. spicata* rhizomes concluded that rhizome fragments were able to tolerate a wide range of osmotic stresses as well as cold storage prior to planting, and were capable of sprouting in any season and had optimum growth when soil temperatures were warmer (Pavlicek *et al.* 1977).

Rhizomes have two primary functions. The first is they act as a storage organ for carbohydrate reserves, while the second is that they provide a means for the plant to spread vegetatively by elongating through the soil. These rhizomes then allow the production of tillers from nodes along the length of the rhizome. In *Distichlis*, starch is the major non-structural carbohydrate, with concentrations of up to 18.8% being recorded by Smith (1968). This reserve of starch within the rhizomes is remobilised during times of demand, and used to initiate and sustain growth over the short term until the photosynthetic supply is restored. Therefore, this reserve presumably gives the rhizome the ability to sprout and establish when suitable conditions occur.

The experiments outlined in this chapter investigate the hypothesis that rhizome fragments can initiate growth under a range of moisture and salt regimes. This hypothesis was tested by planting rhizome fragments of varying age in sand under a range of moisture and salt regimes. The relative age of the rhizome was also investigated along with the starch concentration in the

rhizome fragment, and whether starch concentrations impact on the success or failure of the fragment to initiate tiller growth.

7.2 Materials and Methods

7.2.1 Experimental design

Experiments were conducted in a constant temperature room set at 25 °C at La Trobe University, Bundoora, Victoria. The experiments involved measuring the tiller emergence from rhizome fragments placed in sand in small containers. Experiment 1 involved a completely randomized design with 3 salinity treatments (0.3, 1.0 and 3.0 g NaCl kg^{-1}) and 2 gravimetric moisture contents (2 and 10 % (w/w)) replicated 4 times. Each replicate contained 4 rhizome fragments. Experiment 2 involved a completely randomized design with 2 rhizome ages, where 3 node fragments were taken from the younger distal or older proximal end of a 12 node rhizome, and 3 salinity treatments of 0.3, 1.0 and 3.0 g NaCl kg^{-1}, replicated 4 times. Each replicate contained 7 rhizome fragments of both ages.

7.2.2 Growing conditions

Rhizome pieces were harvested from field-grown *Distichlis spicata* cv. yensen-4a (NyPa Forage) plants at Wickepin, Western Australia. These plants were growing in a sandy saline discharge site, and were harvested in October 2006. The rhizome pieces were stored in a cool room at 4 °C until the pieces were sorted and allocated to the 2 experiments.

7.2.2.1 Experiment 1. Rhizome pieces were then cut into fragments containing 6 nodes. These 6 node fragments were then sorted into 3 groups based on the number of tillers that were attached to the rhizome (either 3, 4 or 5 tillers attached to each fragment). These 3 categories were then evenly allocated across the treatments. The fragments were then cut down to a 4-node length by removing 1 node from each end of the fragment. The internodes that were removed from each end were then dried in an oven at 70 °C until a constant weight was achieved.

Replicates consisted of plastic containers (17 × 12 cm) with 500 g of moist saline sand. Rhizome fragments were placed on the sand surface, and then a further 200 g of moist saline sand was placed on top of the fragments, covering them with 1 cm of sand. Plastic lids were then placed on the containers, and weighed. The containers were then placed in a larger plastic box containing an open vessel of water to maintain a high humidity in the box. Each container was weighed every 2 days to determine the loss in moisture. This loss was replaced by applying a fine mist to the surface of the sand.

7.2.2.2 Experiment 2. Intact rhizome lengths were cut into an older (proximal end) and younger (distal end) rhizome fragments with each fragment consisting of 7 nodes per fragment. One internode length was then cut off each end of the younger and older fragments and dried in an oven at 70 °C for starch analysis. The older and younger fragments from the same rhizome were kept together, so they could be planted into the same sand container. They were then randomly allocated to containers for each treatment and covered with sand as per experiment 1. In this experiment there were 7 older, and 7 younger rhizome lengths placed in each of the 4 replicated containers for each treatment. The gravimetric water content of the sand used was 10% (w/w).

7.2.3 Measurements

The plastic containers containing the rhizomes were checked for new growth every 2 days and were allowed to grow for 36 days from planting. New tillers that had emerged from the sand were tagged and their height measured. At each subsequent inspection, the tillers were again measured. At the completion of the experiment, the rhizome fragments were harvested, washed and nodes that had burst, but had not produced a tiller, were recorded. The percentage of rhizomes to initiate growth included those in which a bud had burst, and those that had emerged tillers. The term 'rhizome sprouting' is used if a node along the rhizome had burst to initiate tiller growth. A selection of rhizome fragments from experiment 2 were chosen for starch analysis from the nodes removed prior to planting.

The starch analysis was conducted by the Western Australian Chemistry Centre by the rapid enzymic test procedure (McLeary *et al.* 1994) and is a specific analysis for total starch determination. Amylase and amyloglucosidase are used to effectively hydrolyse the Alpha 1-4 and 1-6 linkages between glucose molecules. Glucose concentration was then determined colourimetrically (AACC 1995; AOAC 1995)

7.2.4 Statistical analysis

A 2-way analysis of variance was carried out on the rhizome data, using GenStat 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). The rhizome data for the initiation of tiller growth and for emerged tillers, expressed as a percentage of tillers, were arcsin transformed prior to analysis. Significant differences between treatments were identified at the 5% level.

7.3 Results

The ability of rhizomes in experiment 1 to initiate tiller growth was significantly affected by both moisture and salt content of the sand medium in which they were planted (Table 7.1). In addition, there was a significant moisture × salt interaction ($P<0.05$) for the initiation of tiller growth. The ability of the rhizomes to produce emerged tillers, and tiller height were only affected by the moisture content of the sand.

In experiment 2, there were significant effects of salt on the initiation of tiller growth ($P<0.05$), and the emergence of tillers ($P<0.1$), and on tiller height ($P<0.01$) (Table 7.1). Rhizome age significantly affected both the initiation and emergence of tillers ($P<0.1$), but had no effect on tiller height. There were no salt × rhizome age interactions on *D. spicata* rhizomes. The starch concentration of the rhizome fragments was significantly affected by the age of the rhizome, and was also related to the ability of the fragments to initiate tiller growth.

7.3.1 Experiment 1

The significant salt × moisture interaction ($P<0.05$) affecting the initiation of tiller growth resulted from the high moisture content of the sand (10% w/w) overcoming the effect of increasing salt concentration which reduced the initiation of tiller growth (Fig 7.1). Fragments that were planted in the low moisture sand (2% w/w) had a strike rate of approximately 60% in the 0.3 g NaCl kg^{-1} sand, which declined rapidly with increasing salt concentration, down to about 10% of fragments initiating growth at 3 g NaCl kg^{-1} sand. However, where the moisture content was maintained at 10% (w/w), the percentage of rhizome fragments to initiate tiller growth remained relatively constant between 60 and 85% over the range of salt concentrations from 0.3 to 3 g NaCl kg^{-1} sand.

Table 7.1. Significant main effects and interaction terms for moisture, salt and rhizome age treatments for experiments 1 and 2 when the analysis of variance is performed on data for the percentage of rhizomes to initiate growth, to produce emerged tillers, and on emerged tiller heights and starch concentrations in the rhizome.

Treatment	Rhizomes %		Emerged tiller height	Starch concentration of the rhizome
	Initiated tiller growth	Produced emerged tillers		
Experiment 1				
Moisture (ϖ%)	**	***	***	
Salt	*	n.s.	n.s.	
Moisture × Salt	*	n.s.	n.s.	
Experiment 2				
Salt	*	#	**	
Rhizome age	#	#	n.s.	
Salt × Rhizome age	n.s.	n.s.	n.s.	
Starch				
Rhizome age				***
Sprouted				***
Sprouted × Rhizome age				*
Distance from the growing tip				*

# $P<0.1$; * $P<0.05$; **$P<0.005$; *** $P<0.001$; n.s. not significant at $P=0.1$.

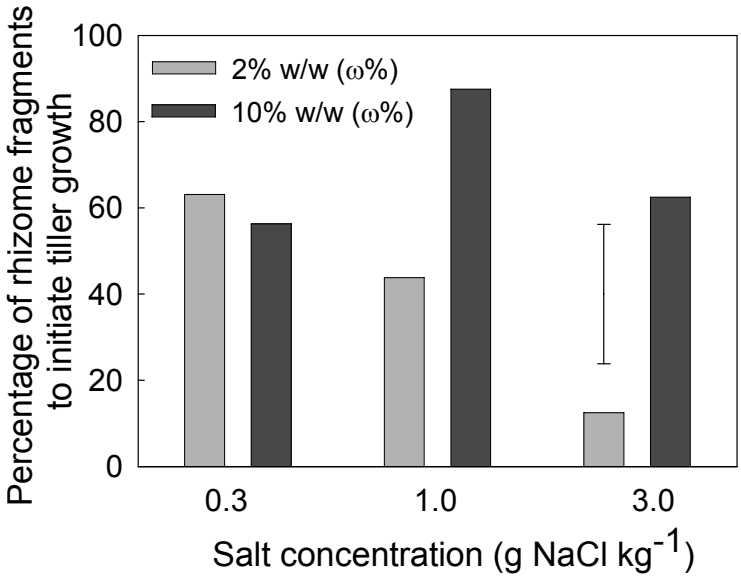


Figure 7.1. The effect of gravimetric water content (ϖ%) and salt concentration in the sand on the percentage of rhizome fragments to initiate tiller growth. Error bar represents the LSD (*P*=0.05) for the moisture × salt interaction.

The percentage of rhizome fragments to produce emerged tillers and the subsequent tiller height were both affected by the moisture content of the sand. For both measurements, the higher water content of 10% (w/w), produced a higher percentage of rhizome fragments producing tillers (Fig. 7.2A), as well as taller tillers (Fig. 7.2B). Around 45% of rhizome fragments planted into the sand maintained at 10% (w/w) produced tillers, as compared to 10% (w/w) when planted into sand at 2% (w/w) gravimetric water content. A similar difference in tiller height was also observed, with tillers in the 10% (w/w) water content treatment being about 4 times higher, than those in the drier 2% (w/w) treatment.

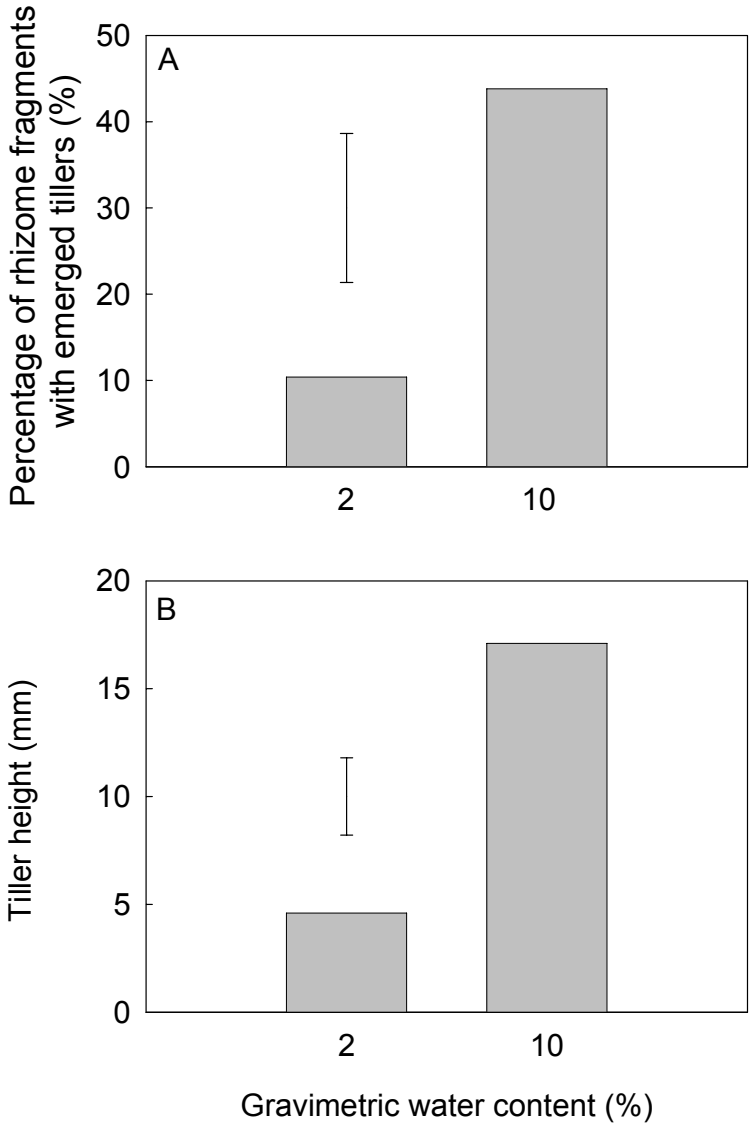


Figure 7.2. The effect of gravimetric water content (ϖ%) of the sand on the percentage of rhizome fragments with emerged tillers (A), and the emerged tiller height (B). Error bars represent the LSD (P=0.05).

7.3.2 Experiment 2

The initiation of tiller growth from rhizome fragments, the number of emerged tillers and the emerged tiller height were all significantly reduced by increasing salt concentrations in the sand. The initiation of tiller growth from the rhizome fragments was reduced (P<0.05) to about 9% with the higher salt treatment of 3 g NaCl kg^{-1}, compared to 27% for the lower 0.3 g NaCl kg^{-1} treatment (Fig. 7.3A). A similar pattern, significant at (P<0.1), occurred for the percentage of rhizome fragments that produced emerged tillers (Fig. 7.3B). The height of the emerged tillers also declined from a height of 4.8 mm with the low salt treatment of 0.3 g NaCl kg^{-1} to a height of only 0.5 mm, with the highest salt concentration of 3 g NaCl kg^{-1} (Fig. 7.3C).

Younger, more distal fragments of *D. spicata* rhizomes initiated more tillers (Fig. 7.4A), and produced more emerged tillers (Fig. 7.4B), than the older more proximal portion of the rhizome. These effects were significant at the 10% level (Table 7.1). However the strike rate for these rhizome fragments was not particularly high as only around ¼ of the younger rhizome fragments initiated tiller growth and produced emerged tillers compared to about ⅛ of the older fragments, which initiated tiller growth and produce emerged tillers. Rhizome age did not have any affect on the emerged tiller height (Fig. 7.4C).

The starch concentration in rhizome fragments was affected by a significant interaction (P<0.05) between the age of the rhizome (distance from the rhizome tip) and whether or not the rhizome had sprouted. Three times more starch was found in older rhizomes fragments that had sprouted, compared to older fragments that did not sprout. A similar trend was also observed in younger fragments, but the differences were not significant (Fig. 7.5). The starch concentration also increased significantly with increasing distance from the growing tip (Table 7.1 and Fig 7.6). The starch concentration remained relatively constant until the 8^{th} internodes from the growing tip, but then the concentration of starch doubled between 8^{th} and 11^{th} internodes to about 4% starch.

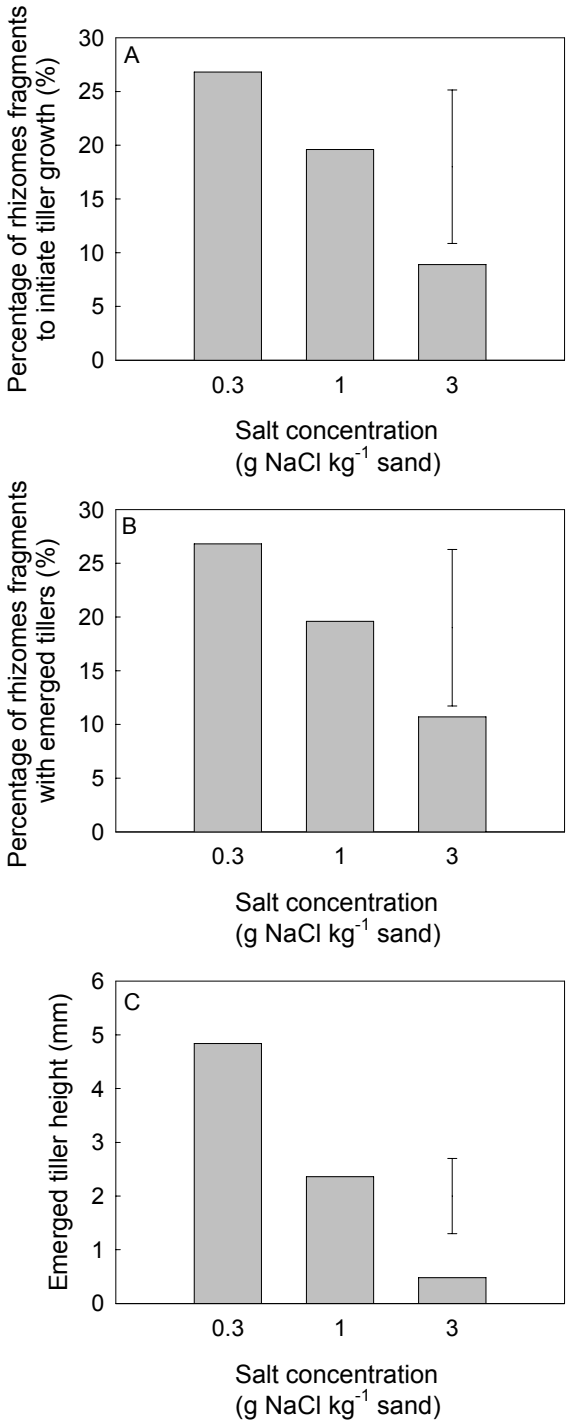


Figure 7.3. The effect of salt concentration in the sand on the percentage rhizome fragments to initiate tiller growth (A), produce emerged tillers (B) and the emerged tiller height (C). Error bars represent the LSD (P=0.05).

Figure 7.4. The effect of rhizome age on the percentage of rhizome fragments to initiate tiller growth (A), produce emerged tillers (B), and the emerged tiller height (C). Error bars represent the LSD (*P*=0.05).

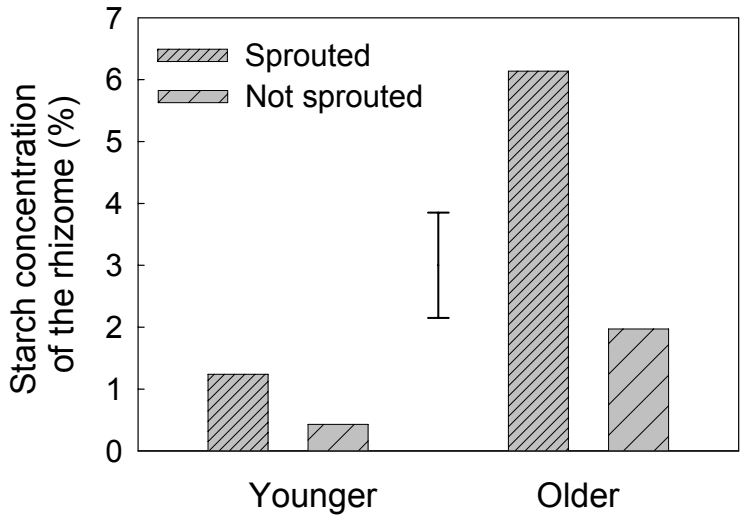


Figure 7.5. The starch concentration of the rhizome fragments for the different age rhizomes relative to their ability to sprout. Error bar represent the LSD (*P*=0.05).

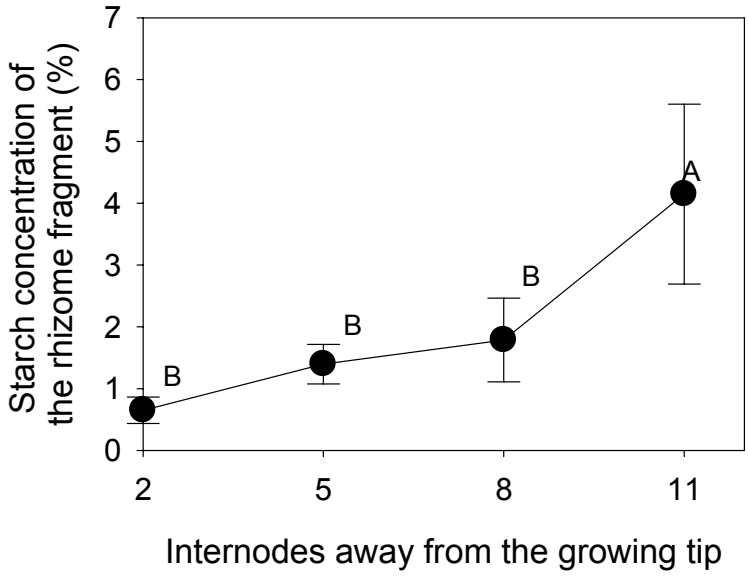


Figure 7.6. The starch concentration of the rhizome relative to the growing tip of the rhizome. Error bars represent the (±) SE mean. Means with the same letters do not differ significantly (*P*>0.05).

7.4 Discussion

The results of the two rhizome experiments documented in this chapter demonstrate that rhizome fragments are capable of producing tillers when planted in moist sand under optimal conditions. Again, it appears that both moisture and salt concentrations have a large impact on the success of rhizome fragments to initiate tiller growth, with high moisture levels having the capacity to mitigate the effects of high salt concentrations (Fig. 7.1). This is similar to the findings from Chapter 6, where *D. spicata* plants at the moist Donald site were able to establish successfully compared to the much drier Manangatang site, when both sites had similar soil EC values.

In both experiments, high salt concentrations in the sand were found to impede tiller initiation, whereas high moisture levels were found to improve tiller initiation in the high salt treatment. Again, as was found with the establishment of rooted tillers in the field (Chapter 6), a delicate balance exists between soil salinity and moisture levels. This delicate balance is exacerbated under highly saline conditions, due to the decreased water availability of saline soils (Munns 2002). Pavlicek *et al.* (1977) also came to a similar finding, with 66% of rhizome fragments sprouting under high salinity and high moisture when bathed in a 0.5M NaCl solution compared to 93% survival at 0.05M NaCl. If the use of rhizome fragments as a means of establishing rhizomes in the field was to be successful, then the timing of establishment will remain a critical factor in the success of establishment to ensure that sufficient moisture was present when the planting occurred. One possible advantage of establishing rhizomes is that they are not transpiring when planted as there are no leaves attached to the rhizomes. In comparison, rooted tillers are transpiring as soon as they are planted, and consequently require an active root system and sufficient available moisture within the planted rooting zone. The rhizomes that are not yet transpiring could be planted deeper into the soil, where the soil temperature would be higher during late winter/early autumn, along with the higher moisture regime in the deeper soil layers.

The age of the rhizome fragment was also found to affect tiller emergence, with the younger fragments being nearly twice as successful at producing emerged tillers. It was observed that the older fragments appeared to be more lignified and scaly compared to the younger fragments. It is not known whether these factors had any influence on the emergence of tillers. However, a random selection of rhizome fragments from the experiment, found that the starch concentration was higher in fragments that sprouted compared to fragments that did not sprout within each age group. Starch is the major carbohydrate stored within *Distichlis* rhizomes (Smith 1968), and consequently would be used as an energy reserve to initiate and sustain growth until photosynthesis can occur from leaves on the new tillers (White 1973). Hence, the fragments with a higher starch concentration would have a greater ability to sprout and sustain tiller growth over

the observed period. Despite this finding, it was also found that the starch concentration decreased towards the younger end of the rhizome (Fig. 7.6). This appears to demonstrate that while higher starch concentrations improve the ability for a fragment to initiate a tiller, there are some other factors that influence the ability of the rhizomes to sprout.

One explanation for this reduction in rhizome activity with increasing age could be linked to increasing bud dormancy with age. This has previously been found in *Agropyron repens*, where bud dormancy increased in the nodes most distant from the rhizome apex (Leakey *et al.* 1977a; McIntyre 1970). It was also noted by Leakey *et al.*(1977b*)* that bud dormancy was related to the time of year when rhizomes were harvested, with dormancy occurring in spring-harvested rhizomes, whereas rhizomes harvested during autumn did not exhibit bud dormancy. This dormancy was attributed to the total nitrogen concentration in the fragments, with nitrogen in older rhizomes being mobilized for tiller growth in the spring, and then being replenished in the autumn following nitrogen accumulation in rhizomes during the summer growing period (Leakey *et al.* 1977b). Further, Leakey *et al.* (1977a) reported that when rhizome fragments were bathed in a nitrogen solution, bud dormancy could be broken in *A. repens*. Where fragments were bathed in a KNO_3 solution of 210 ppm N, 100% of the buds produced tillers, compared to only 11% of buds when bathed in the distilled water control. This effect was not limited to KNO_3, with similar results occurring for other nitrogen solutions. Exposing these fragments to low temperatures also reduced bud dormancy, but the low temperature treatment was not as effective as bathing them in a nitrogen solution. The authors suggested that dormancy resulted from an impairment in sugar metabolism resulting from the low nitrogen status of the plant (Leakey *et al.* 1977a). It is possible that a similar bud dormancy mechanism exists in *D. spicata*. Farmers have observed how applications of nitrogen fertilizer to *D. spicata* swards resulted in a vigorous growth of new tillers (R Matthews, personal communication). It is possible that some bud dormancy in these situations could have been broken by the nitrogen applications, resulting in the flush of new tillers in the field. Clearly, this is an area for further research with the aim of improving *D. spicata* establishment. The effect of nutrient supply on *D. spicata* growth will be investigated in the following chapter.

The results from this experiment demonstrate that rhizome fragments of *D. spicata* are able to produce tillers, and can therefore result in plant establishment. However as with other planting techniques, moisture and salt levels are critical for successful tiller emergence. The use of rhizome fragments for planting *D. spicata* is attractive because it has the potential to keep establishment costs to a minimum. Further research is required to determine whether rhizomes may be more successful than rooted tillers in some environments as they have the potential to be planted deeper in moist soil at saline discharge sites. Research also needs to be conducted to confirm the presence of seasonal bud dormancy in *D. spicata*, and to determine if nitrogen applications are a suitable way of breaking this bud dormancy.

Part III

Managing *Distichlis spicata* swards on saline land

Chapter 8

Nutrient requirements for *Distichlis spicata* – glasshouse trial

8.1 Introduction

Although *D. spicata* has been grown for a number of years within Australia, very little is known about managing this species to maximise the feed quality and dry matter production. Farmers have observed visual yield responses to nitrogenous fertilizer applications under grazing and that sheep grazed nitrogen-fertilized strips of *D. spicata* more heavily than adjacent unfertilized areas (R. Matthews, pers comm.). These observations are supported by the considerable amount of research that has come out of the dairy industry, where nitrogen is used widely to increase dry matter production, and increase pasture quality (Davies 1977; Davison *et al.* 1985; Jacobs *et al.* 1999; Jacobs *et al.* 2006; McKenzie *et al.* 2002). Similarly, research by Fenton *et al.* (2004) with puccinellia (*Puccinellia ciliata*), a salt-tolerant pasture grass growing on saline land, showed that the live weight gain of sheep was greater with nitrogen and phosphorus fertilizer applications, compared with unfertilized puccinellia. Thus, preliminary evidence suggests that the yield and quality of halophytic grasses such as *D. spicata* will improve with increasing nitrogen and phosphorus supply.

The experiment documented in this chapter tests the hypothesis that high rates of nitrogen and phosphorus will increase feed quality by altering the plant morphology of *D. spicata*. This hypothesis was tested by growing *D. spicata* plants and irrigating them in a free draining medium with twelve different nutrient solutions, containing three levels of nitrogen, two levels of phosphorus and two salinity levels in a factorial combination. A range of plant morphological and chemical analysis were conducted to assess the effect of these treatments on forage quality and shoot growth.

8.2 Materials and Methods

8.2.1 Experimental design

The experiment was setup as a randomized block design with factorial combinations of 2 phosphorus nutrient solutions, 3 nitrogen nutrient solutions and 2 salinity treatments. The phosphorus treatments consisted of 0.04 and 1.0 mM P, the nitrogen 0.4, 3.2 and 16 mM N while the salt treatments were 10 and 33 dS m^{-1}. This gave a total of 12 treatments, each of which was replicated 5 times.

8.2.2 Growing conditions

The experiment was conducted over 2 phases. The first phase was conducted in a glasshouse at La Trobe University, Bundoora, Victoria (37°42'S, 145°02'E) from August to October 2007, while the second phase was conducted outside in direct sunlight from November 2007 to February 2008. The daily maximum and minimum temperatures, along with rainfall, are presented in Figure 8.1.

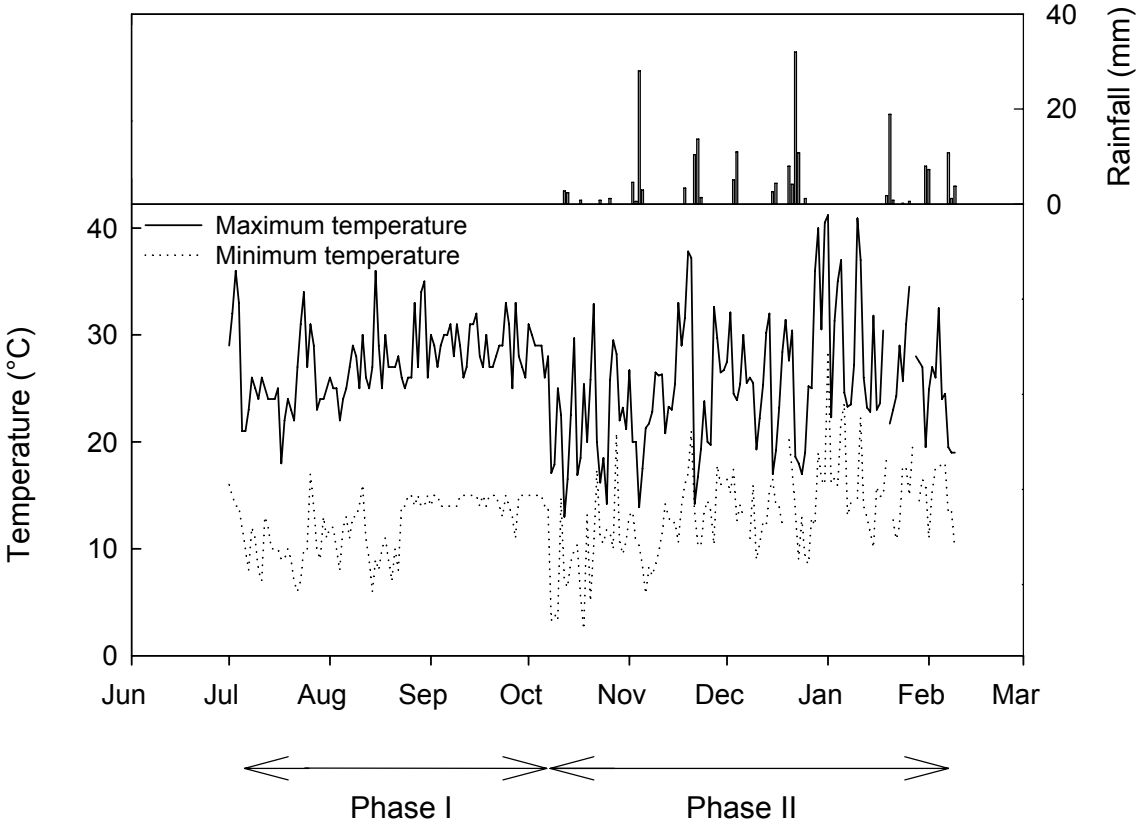


Figure 8.1. The average maximum and minimum temperatures during phase I (inside glasshouse) and phase II (outside glasshouse), together with the daily rainfall during phase II.

Bare rooted *Distichlis spicata* (L.) Grenne cv. yensen-4a plants were harvested from Donald in the Wimmera region of Victoria. These were planted into black plastic pots, 7 cm in diameter, that were filled with soil collected from a discharge zone at Manangatang in the Mallee region of northen Victoria. The soil was mixed with a fine white quartz sand to provide a loamy sand texture (87% sand, 10% clay and 3% silt) and to ensure adequate drainage. Each pot contained four plants. The surface of the soil was covered with white plastic beads to prevent evaporation and the accumulation of salt in the surface layer. The pots were flushed daily with water for 4 weeks prior to regular flushing with the nutrient solutions.

The nutrient solution used was based on Hoagland solutions with variations in the nitrogen and phosphorus levels, which were achieved by varying the concentrations of NH_4NO_3 and $NH_4H_2PO_4$ to provide combinations of 0.4, 3.2 and 16 mM N, and 0.04 and 1.0 mM P. The electrical conductivity (EC) of the solutions were also varied by the addition of NaCl to achieve EC's of 10 and 33 dS m^{-1}. Other basal nutrients were added to the flushing solution in the following composition: K_2SO_4 (0.6 mM), $CaCl_2$ (0.8 mM), $MgSO_4$ (0.2 mM), H_3BO_3 (5 μM), $MnSO_4.H_2O$ (2 μM), $ZnSO_4.7H_2$) (2 μM), $CuSO_4.5H_2O$ (0.5 μM), Na_2MoO_4).$2H_2O$) (0.5 μM) and $[CH_2.N(CH_2.COO)_2]_2FeNa$ (128 μM).

During both phases of the experiment, the plants were randomized weekly. Treatments were imposed by applying 100 mL of the nutrient solution every second day, which was sufficient to cause excess solution to leach from the bottom of the pots and prevent salt and nutrient accumulation within the soil.

The plants were allowed to grow for their allotted time, and harvested at the completion of each phase. At the end of phase I, the tillers were cut at the surface of the white beads, and then placed outside in direct sunlight to commence phase II. At the end of phase II, the tillers were again harvested at the surface of the white beads. At each harvest, three average sized tillers from each pot had their leaves removed and were scanned with a Win Rhizo root scanner to measure leaf area. All harvested tillers were then dried in an oven at 70 °C until a constant weight was achieved. Leaves and stems were then weighed separately to determine the leaf to stem ratios.

Samples were then ground and analysed for *in-vitro* dry matter digestibility (IVDMD) (phase I) and acid-detergent fibre (ADF) (phase II) using wet chemistry by FeedTest Laboratories, Hamilton (Victorian Department of Primary Industries). There was insufficient sample to allow IVDMD analysis on both leaf and stem portions from phase I, so these were combined to give an overall IVDMD value for each treatment. The IVDMD analysis used was a pepsin-cellulase

technique over four days as outlined by the Australian Fodder Industry Association (2005). Acid-detergent fibre was also determined according to the Australian Fodder Industry Association (2005), using the Ankom technique. Total nitrogen was analysed by dry combustion using a CNHS auto-analyzer (Elementar, Varios EL). Total nitrogen was then multiplied by 6.25 to estimated crude protein concentration of the plant material.

The data were analysed with GenStat 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). A analysis of variance was used to test the significance of the treatment effects to the 5% confidence level.

8.3 Results

Nitrogen treatments significantly affected all measurements ($P<0.05$ and $P<0.001$) of feed quality in both phases of the experiment (Table 8.1). Phosphorus did not have such a wide effect, but did significantly affect dry matter production ($P<0.001$) during phase I and II, leaf area in phase I and II ($P<0.005$ and $P<0.001$), leaf to stem ratio in phase II ($P<0.001$), IVDMD in phase I ($P<0.001$) and ADF in phase II ($P<0.001$). Salt treatment also had a significant effect on many of the plant measurements including dry matter production in phase II, leaf to stem ratio in phase II, crude protein in phase I and II and ADF in phase II. Crude protein and ADF were also significantly affected by plant part in phase II of the experiment ($P<0.001$).

There were however, a number of interactions that occurred between the nitrogen and phosphorus, nitrogen and EC, phosphorus and EC, and nitrogen, phosphorus and EC (Table 8.1). These interactions generally occurred in phase II of the experiment, with the exception of crude protein and IVDMD measurements, which had a small number of interactions in phase I. There were also a number of significant second-order interactions ($P<0.05$; $P<0.001$) involving the nitrogen, phosphorus, EC and plant parts for growth and quality measurements during the experiment.

Table 8.1. Main effects and interaction terms for the analysis of variance of leaf size, leaf to stem ratio, crude protein, in-vitro dry matter digestibility (IVDMD), acid detergent fibre (ADF) and shoot dry matter re-growth data, measured at the end of phase I and II of the experiment for the nitrogen (N), phosphorus (P), salt (EC) treatments and for different plant parts.

	Dry matter		Average leaf area		Leaf to stem ratio		Crude protein[B]		IVDMD[A]	ADF[A,B]
	(g)		(cm^2)				(%)		(%)	(%)
	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
N	***	***	***	***	*	***	***	***	***	***
P	***	***	**	***	n.s.	***	n.s.	n.s.	***	***
Salt (EC)	n.s.	***	n.s.	n.s.	n.s.	***	**	*	n.s.	***
Plant part (pp)								***		***
N×P	#	***	n.s.	***	n.s.	***	***	*	***	***
N×EC	n.s.	***	n.s.	**	n.s.	**	n.s.	*	n.s.	n.s.
P×EC	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*
N×P×EC	n.s.	*	n.s.	***	n.s.	***	*	n.s.	n.s.	n.s.
P×pp								n.s.		***
EC×pp								n.s.		***
N×EC×pp								n.s.		#

[A] Measurement for IVDMD and ADF were only conducted for phase I and II respectively.

[B] Measurement for crude protein and ADF were the only measurements conducted on plant part, and were only conducted in phase II.

*** p<0.001. ** p<0.005, * p<0.05, # p<0.1, n.s. not significant.

8.3.1 Growth and morphology of D. spicata

There was substantially more re-growth of *D. spicata* shoots during phase II, compared to phase I (Fig. 8.2); the re-growth was 2 to 4 times higher under the full sunlight from the established plants growing during phase II. Given that phase II provided light and temperatures typical of field conditions in summer, the focus of the results will be on phase II results.

Distichlis spicata re-growth in phase II increased with increasing nitrogen supply. The significant main effect for nitrogen (Table 8.1) resulted from a more than doubling of the re-growth from 2.39 g pot^{-1} with 0.4 mM N, to 5.23 g pot^{-1} with the 3.2 mM N solution, and then a slight decline to 5.02 g pot^{-1} with 16 mM nitrogen. However the level of phosphorus supply, and the salt regime modified the response to nitrogen, as significant N×P and N×EC and interactions occurred (Table 8.1). These interactions resulted from larger responses in shoot re-growth to nitrogen supply with

the high phosphorus or low salt regimes, respectively. In addition, there was a significant P×EC interaction where the response to phosphorus supply was greatest at the low salt level (data not presented).

The effect of P supply and EC regimes on the shoot re-growth of *D. spicata* in phase II to increasing nitrogen supply, further played out with a significant N×P×EC second order interaction (Fig. 8.2). This resulted from a substantially larger shoot re-growth response to 3.2 and 16 mM nitrogen, when high phosphorus (1 mM P) and low salt (10 dS m^{-1}) concentrations were present in the nutrient solution. Shoot re-growth with these high rates of nitrogen, together with high phosphorus and low salt, had more than twice the re-growth of other treatments.

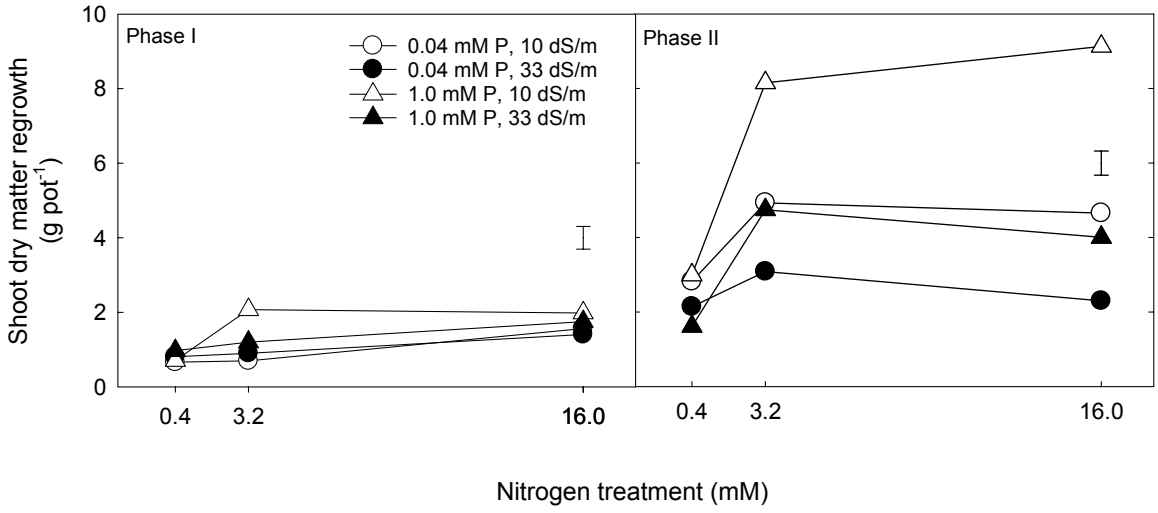


Figure 8.2. Dry matter of shoot regrowth from the *D. spicata* when irrigated with solutions containing different concentrations of nitrogen, phosphorus and salt levels in phase I and II. There was a N×P×EC interaction in phase II, but not in phase I. Error bars represent LSD (P = 0.05) for the N×P×EC interaction means.

The area of individual leaves of *D. spicata* was increased by high concentrations of nitrogen and phosphorus in the nutrient solution in phase I and II (Table 8.1, Fig. 8.3). Unlike dry matter production, the leaf area continued to increase from 4.4 to 6.0 cm^2 with each increment in nitrogen supply from 0.4 to 16 mM during phase II. Higher phosphorus supply also increased individual leaf size from 4.9 to 5.6 cm^2. However, significant N×EC (P<0.01) and N×P (P<0.001) interactions in leaf area occurred in phase II. The former interaction resulted from larger leaf sizes with the low salt (10 dS m^{-1}) when nitrogen supply was increased to 3.2 and 16

mM in the nutrient solution, whereas salt levels had no effect at low nitrogen (0.4 mM). The N×P interaction, on the other hand, occurred with high N supply where leaf area was 30% larger with high phosphorus concentration (1 mM) in the nutrient solution (data not presented).

The response in individual leaf area was similar to that for shoot dry matter, in that a strong N×P×EC interaction (P<0.001) occurred again. This resulted from significantly higher leaf areas in excess of 7 cm^2, for plants receiving the low salt, high phosphorus concentration, as compared to plants, with low phosphorus and high nitrogen (16 mM) concentrations in the solution. There were no differences in leaf area between treatments at the two lower nitrogen concentrations.

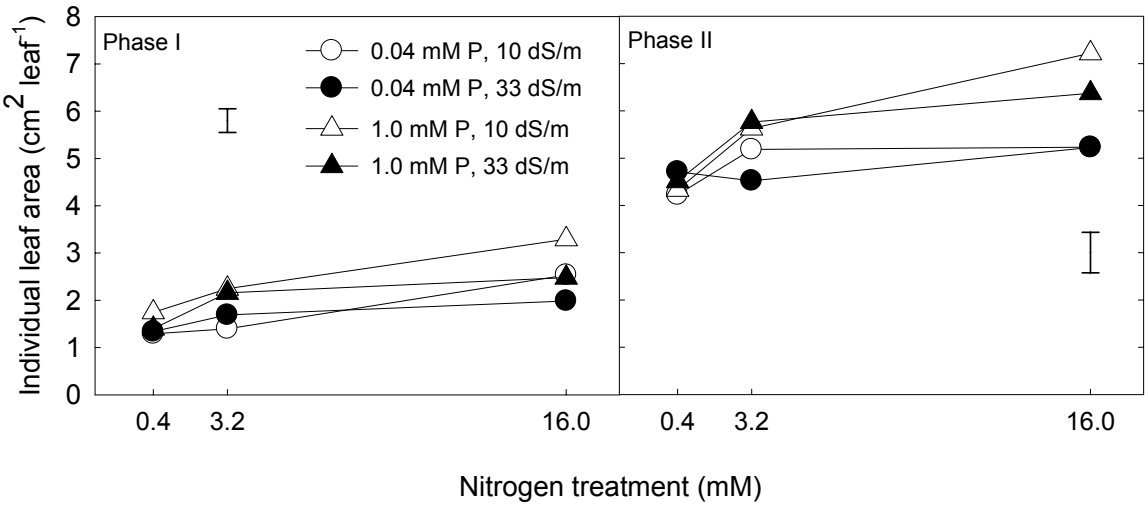


Figure 8.3. The individual leaf area of *D. spicata* when irrigated with solutions containing different nitrogen, phosphorus concentrations and salt levels in phase I and II. There was a N×P×EC interaction in phase II, but not in phase I. Error bars represent LSD (P = 0.05) for the N×P×EC interaction means.

Significant differences in the 'leafiness' of *D. spicata* shoots, measured as the leaf to stem ratio, occurred among nitrogen, phosphorus and salt main effect means (Table 8.1). Here, the leaf-to-stem ratio of *D. spicata* shoots was increased by the highest rate of nitrogen supply (16 mM) in phase I and II, and by high phosphorus supply in phase II (Table 8.1). These increases were caused by a decrease in stem weights by the higher nitrogen concentrations, and an increase in leaf weights with higher phosphorus concentration.

The high-salt treatment increased the leaf-to-stem ratio by 20% from 0.74 to 0.89 in phase II. This was caused by less suppression of leaf growth (40%), as compared to the suppression of stem weights (50%) (data not presented). There were also N×P and N×EC interactions in phase II. The N×P interaction followed a similar trend to shoot dry matter, in that the ratio increased with increasing nitrogen supply, but only with high phosphorus supply (1 mM). The N×EC interaction in phase II was different from the response for shoot dry matter and individual leaf area, as the higher salt (33 dS m^{-1}) concentration resulted in a higher leaf-to-stem ratios at all nitrogen concentrations, with a large increase occurring as nitrogen supply increased from 0.4 to 3.2 mM with the high salt solution (data not presented).

As with shoot dry matter and leaf area measurements, there was a significant (P<0.001) N×P×EC interaction for leaf-to-stem ratio, but the cause of the interaction was different. It resulted from two contrasting patterns in the response to increasing nitrogen concentrations, from 0.4 to 16 mM in the nutrient solutions (Fig. 8.4). With high phosphorus and high salt levels, there was a linear increase in the ratio from 0.8 to 1.15 from the low to high nitrogen regimes. However, with low phosphorus and low salt concentrations in the solution, there was no change in the ratio, with the ratio remaining constant at around 0.6. The response to nitrogen with other phosphorus and salt combinations were intermediate between these two straight line responses.

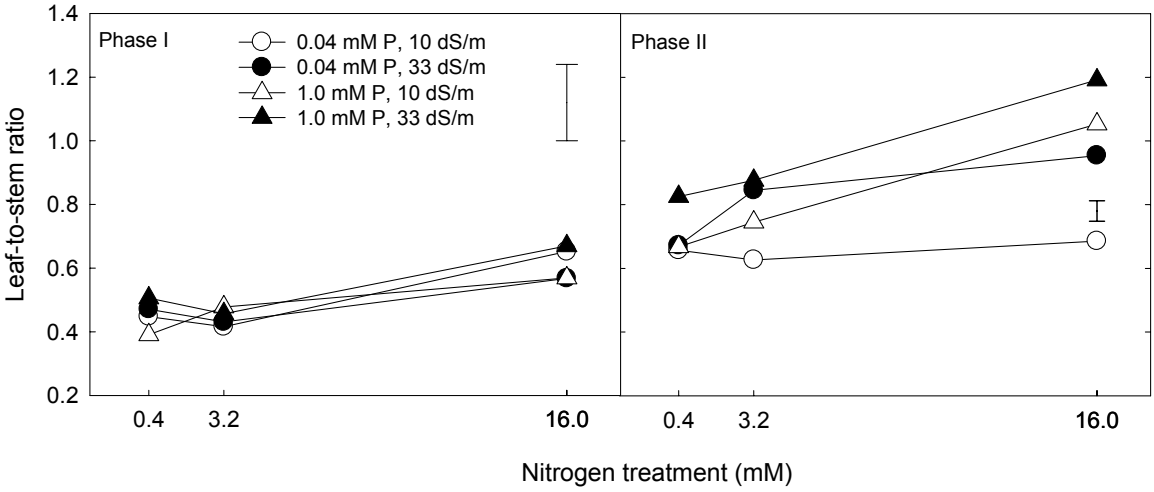


Figure 8.4. The leaf-to-stem ratio of *D. spicata* when irrigated with solutions containing different nitrogen, phosphorus concentrations and salt levels in phase I and II. There was a N×P×EC interaction in phase II, but not in phase I. Error bars represent LSD (P = 0.05) for the N×P×EC interaction means.

The main-effect means for crude protein concentrations in *D. spicata* shoots increased significantly with increasing rates of nitrogen supply and EC levels in both phases of the experiment (Table 8.1). Concentrations of crude protein in the shoots doubled when nitrogen supply was increased from the low (0.4 mM) to high (16 mM) nitrogen supply in both phases (Figs. 8.5 and 8.6), while increasing the EC of the nutrient solution from 10 to 33 dS m^{-1} increased the concentration of crude protein from 11.2 to 12.3% and 10.6 to 11.6% in phase I and II, respectively. There was also a significantly higher concentration of crude protein (P<0.001) in the leaves compared to the stems, with leaves having 14.9%, which was almost double that of the 7.6% concentration in the stems (Fig. 8.5A).

8.3.2 Nutritive value of D. spicata shoots

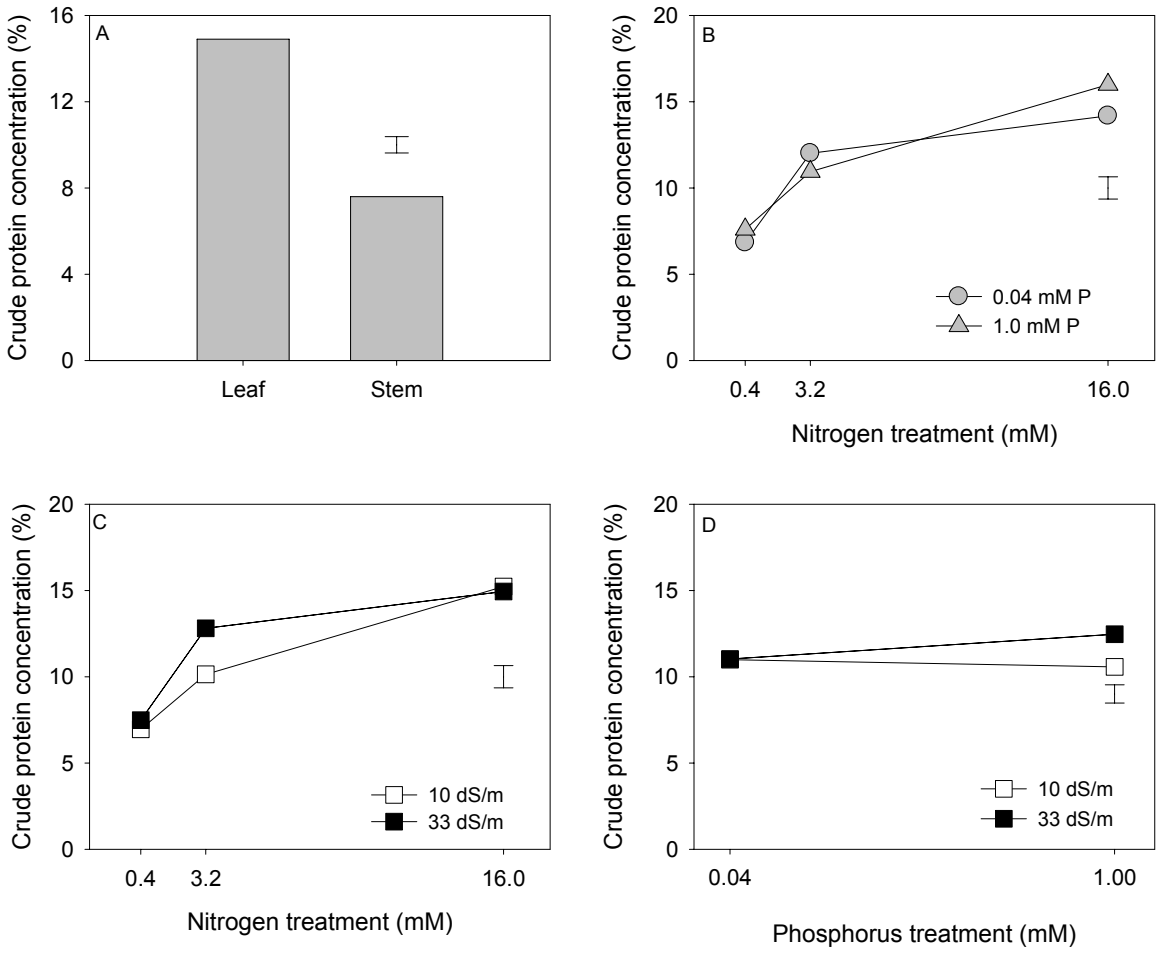


Figure 8.5. The concentration of crude protein in leaves and stems of *D. spicata* during phase II of the experiment, showing (A) plant part main effect, (B) N×P, (C) N×EC and (D) P×EC interactions. Error bars represent LSD ($P = 0.05$) for the main effect or interaction means.

There were also a number of first-order interactions that occurred in phase I and II of the experiment, including N×P, N×EC and P×EC (Table 8.1). All interactions with nitrogen involved increasing concentration of crude protein with increasing nitrogen supply. In both phases of the experiment, the N×P interaction was caused by the differences in response to the highest nitrogen level: with high phosphorus, the crude protein increased significantly (P<0.05) between the moderate (3.2 mM) and high (16 mM) nitrogen treatment. There was no increase at low phosphorus between these two nitrogen treatments (Fig. 8.5B).

The N×EC interaction in phase II resulted from the effect of high salt modifying the linear increase in crude protein concentration in response to increasing nitrogen supply that occurred with the low salt treatment (Fig. 8.5C). At high salt, the response was asymptotic with a rapid initial increase with moderate nitrogen supply (3.2 mM), but no further increase in concentration of crude protein at high nitrogen (16 mM). There was also a further P×EC interaction in phase II, which resulted from an increase in the concentration of crude protein at high phosphorus supply (1 mM) with the high salt treatment (33 dS m^{-1}), whereas there was no effect of salt at low phosphorus supply (0.04 mM).

During phase I, a significant second order interaction (P<0.001) N×P×EC also occured. This interaction resulted from the high salt concentration modifying the effects of the N×P interaction described above. High salt displaced upwards the linear response in shoot crude protein concentration to nitrogen with high phosphorus supply, compared to this response under low salt concentration (Fig. 8.6). In contrast, there were no differences between salt treatments at low phosphorus, when the response to nitrogen was more asymptotic, with no difference in crude protein concentration between the moderate and high nitrogen treatments.

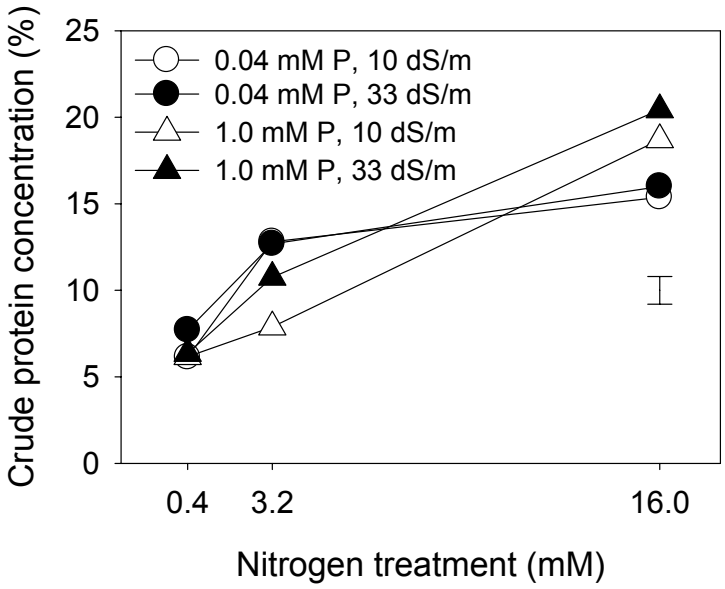


Figure 8.6. The concentration of crude protein in the shoots of *D. spicata* during phase I of the experiment, showing the N×P×EC interaction. Error bar represent LSD (P = 0.05) for the N×P×EC interaction means.

The *in-vitro* digestibility of shoot dry matter measured at the end of phase I was significantly affected by the main effects of nitrogen and phosphorus (Table 8.1). Both nutrients affected the IVDMD to a similar extent, as increasing nitrogen from 0.4 to 16 mM increased IVDMD from 46 to 51%, while increasing phosphorus from 0.04 to 1.0 mM increased digestibility from 46 to 50% (Fig. 8.7). However, a highly significant N×P interaction (P<0.001) occurred where increasing nitrogen supply had no effect on IVDMD at low phosphorus (0.04 mM), but high phosphorus (1 mM) resulted in a linear increase in IVDMD from 45 to 55% as N concentration increased.

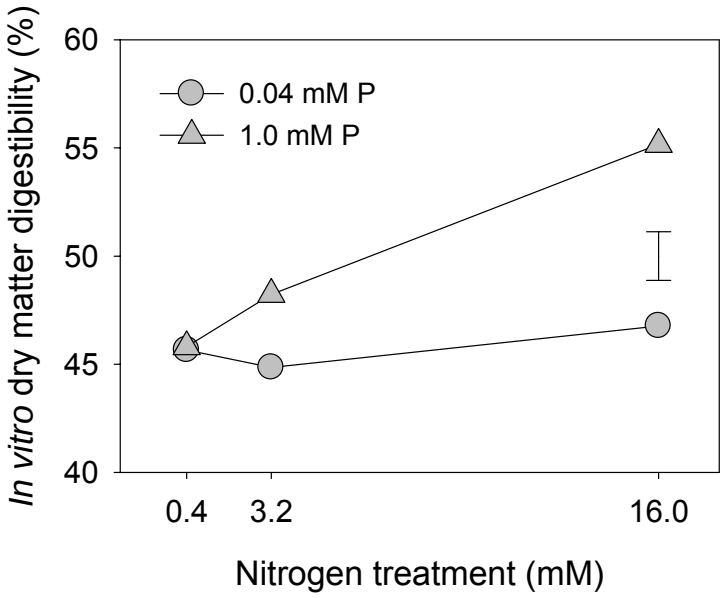


Figure 8.7. The effect of nitrogen and phosphorus supply on the in-vitro digestibility of shoot dry matter of *D. spicata* at the completion of phase I. Error bar represents the LSD (P = 0.05) for the N×P interaction means.

The acid detergent fibre (ADF) concentration in shoots of *D. spicata* during phase II was affected by the main effect of nitrogen, phosphorus, salt and plant part (Table 8.1), with the fibre decreasing with increasing nitrogen, phosphorus and salt concentrations in the nutrient solution (data not presented). Leaves had significantly lower ADF concentration than stems with means varying from 37 to 28% for stem and leaves respectively.

As with all previous growth and quality measurements, there was again a range of interactions that occurred between different treatments (Table 8.1). The N×P interaction was caused by a reduction in ADF concentration where nitrogen supply was increased from 3.2 to 16 mM, with the high phosphorus treatment. No such reduction occurred at low phosphorus (Fig. 8.8A). The P×EC interaction resulted from a larger decrease in ADF concentration with low salt (10 dS m^{-1}) compared to high salt (33 dS m^{-1}) where the phosphorus concentration was increased from 0.04 to 1 mM in the nutrient solution (Fig. 8.8B). Similarly, the P×Plant part interaction resulted from a large decline in fibre concentrations in stems, where phosphorus supply increased from 0.04 to 1.0 mM, whereas the ADF concentration in the leaves remained constant (Fig. 8.7C). The second-order N×EC×Plant part interaction in ADF concentration resulted from the greater fibre reductions in stems than in leaves with high salt (33 dS m^{-1}), in particular at low nitrogen (0.4

mM) concentrations (Fig. 8.8D). In fact, there was no difference in the fibre concentration in leaves at low nitrogen when the salt concentration of the solution was increased from 10 to 33 dS m^{-1}(Fig. 8.8D).

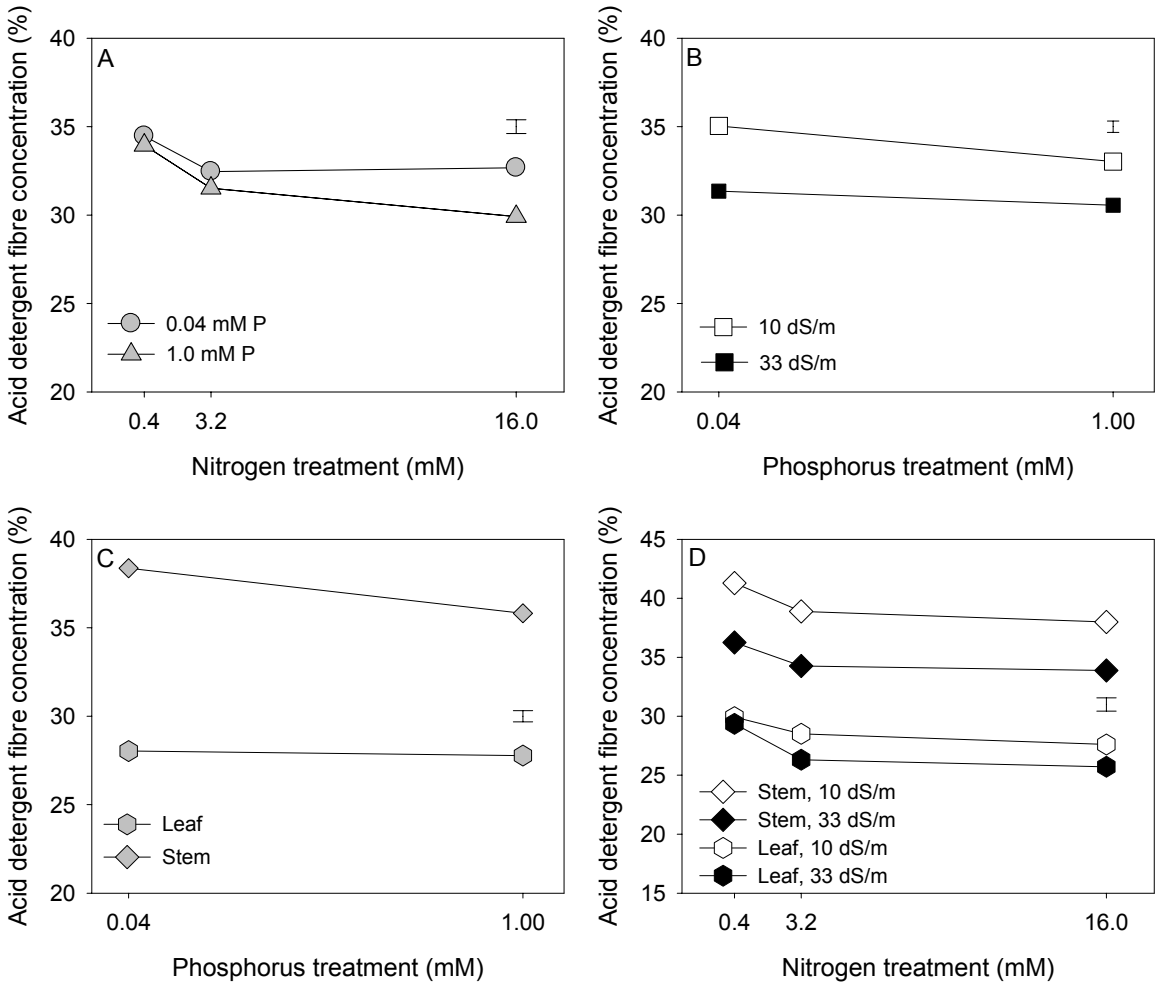


Figure 8.8. The percentage of acid detergent fibre of *D. spicata* during phase II of the experiment, showing the (A) N×P, (B) P×EC, (C) P×Plant part and (D) N×EC×Plant part interactions. Error bar represents the LSD ($P = 0.05$) for the different interaction means.

The *in-vitro* digestibility values of dry shoots were also positively related to the phosphorus and nitrogen concentrations of *D. spicata* shoots at the end of phase I (Fig. 8.9). In both cases, the linear regression was highly significant (P<0.001) with half of the variation in digestibility being explained by the variation in both nitrogen and phosphorus concentrations.

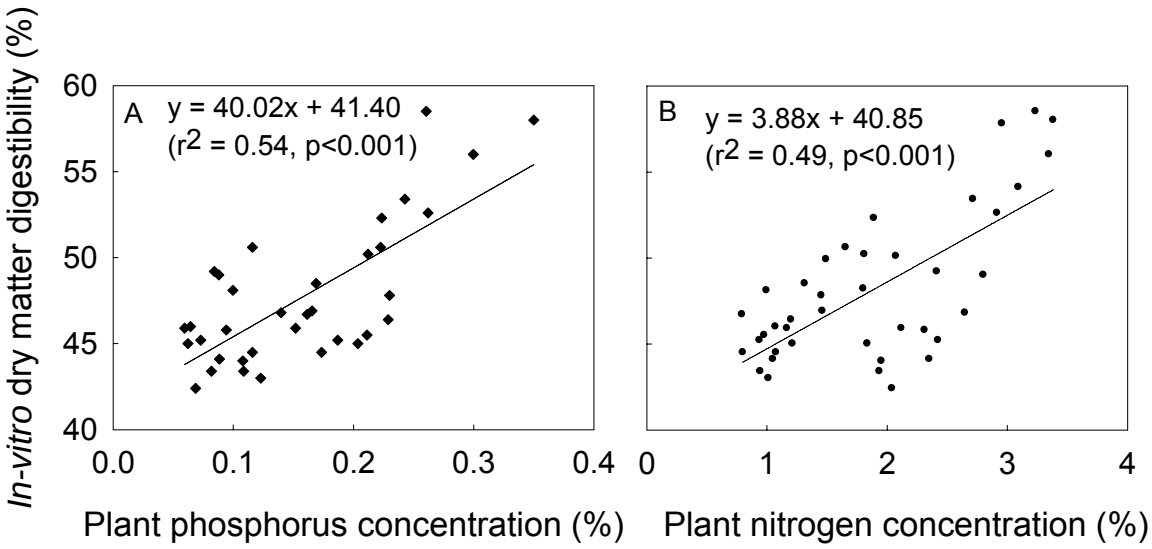


Figure 8.9. The relationship between in-vitro dry matter digestibility and concentrations of phosphorus (A) and nitrogen (B) in the shoot tissue (leaves and stems combined) of *D. spicata* at the completion of phase I of the experiment.

The ADF concentrations in the stems and leaves were negatively related to the nitrogen concentration of these tissues (Fig. 8.10B). There was a similar negative relationship between the ADF concentration in the stems of *D. spicata* and the stem phosphorus concentration (Fig. 8.10A). However the phosphorus concentration had no effect on the fibre concentration in the leaves of *D. spicata*.

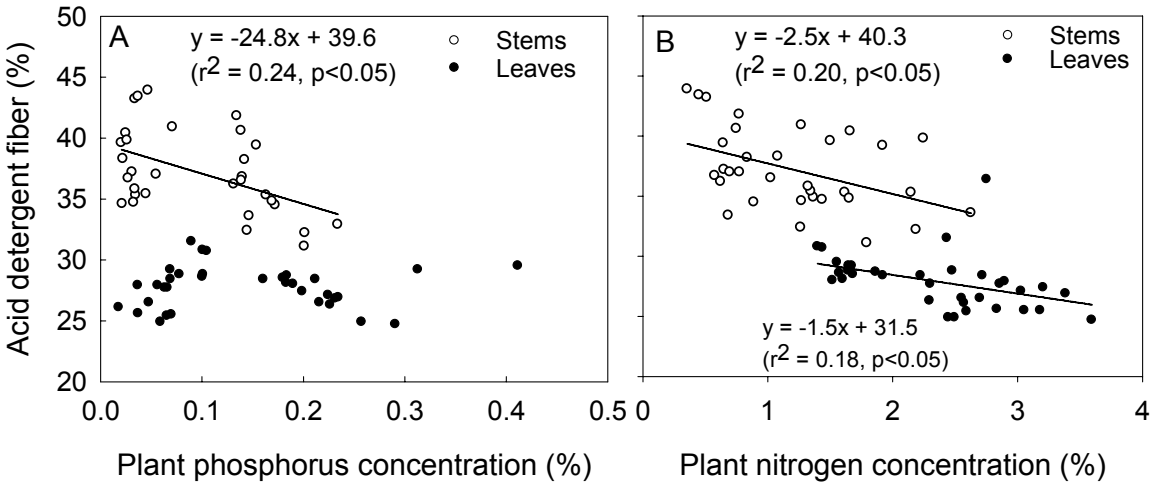


Figure 8.10. The relationship between acid-detergent fibre and concentrations of phosphorus (A) and nitrogen (B) in the plant tissue of *D. spicata* in phase II of the experiment.

8.4 Discussion

Halophytic pastures have previously been reported as having low-quality forage (Brizuela *et al.* 1990; Ludwig and McGinnes 1978). The results in this chapter demonstrate that the morphology and chemical composition of *D. spicata* shoots can be manipulated to improve the feed quality by varying nitrogen and phosphorus supply over a range of salinities. This was achieved by increasing the leaf area, leaf-to-stem ratios and the composition of the shoots with increases in crude protein, *in-vitro* dry matter digestibility and decreases in concentration of acid detergent fibre. Overall, plants receiving high concentrations of phosphorus and nitrogen were able to produce more dry matter, as well as produce higher quality forage.

Plant morphology, in terms of "leafiness" of the shoots, with more leaves and less stems, can be improved by high rates of nitrogen and phosphorus supply. Increased leafiness is a desirable trait, as leaves are less fibrous than stems, and are more digestible (Terry and Tilley 1964). During both phases of this present study, the higher phosphorus treatment produced larger leaves with increases in areas of individual leaves. Larger leaf sizes have been found where high rates of phosphorus were applied for a range of species, including cotton (*Gossypium hirsutum*), white clover (*Trifolium repens*) and wheat (*Tricicum aestivum*) (Radin and Eidenbock 1984; Rodriguez *et al.* 1998; Singh and Sale 1997a). Rodriguez *et al.* (1998) concluded that low phosphorus supply limited the number of cells per leaf, along with cell expansion rate. Radin and Eidenbock (1984) and Singh and Sale (2000) observed a reduction in root hydraulic conductivity under low phosphorus supply, which coincided with a similar decrease in leaf area. Further work carried out by Singh (1998) also demonstrated that cell wall elasticity was higher in plants that had a higher phosphorus supply, meaning that the cell extension would not be as restricted. This combined with the greater hydraulic conductance, and delivery of water to the shoots, may be an important part of the story of larger leaves under high phosphorus supply to *D. spicata*.

The leaf-to-stem ratios during the experiment were shown to increase where adequate nutrients were applied (Fig. 8.4). Possibly, the most unexpected result was the increase in leaf-to-stem ratio with the higher salt treatment. The higher salt treatment depressed both leaf and stem growth, but depressed stem growth more than leaf growth, resulting in a higher leaf-to-stem ratio. Leaf growth was depressed by 40%, whereas the stem growth was depressed by 50% under the higher salinity treatment. One explanation for the increased leaf-to-stem ratio under the high salinity treatment could be the reduction in the internode length of the shoots (N. Yensen, pers. comm.). This response has also been observed in *Atriplex* spp., where the internode length was reduced by up to 85% when exposed to 2% compared to 0% NaCl, and stem thickness of the internodes

region was also reduced (Wang *et al.* 1997). The combined effect of shorter internode length and reduced thickness are likely to be the cause of the reduced stem weights, leading to a higher leaf-to-stem ratio under the higher salinity treatment, although the reason for these changes is unclear.

Like the leaf-to-stem ratios, concentrations of crude protein in the shoots were also increased with the high salinity treatment. The likely cause of this increase is the accumulation of glycinebetaine, a non-protein nitrogen compound that is synthesized by the plant as a compatible solute (Marcum 1999). A compatible solute is required within the cytoplasm to achieve osmotic adjustment. Marcum (2006) has shown that glycinebetaine accounts for about 74% of the cytoplasmic osmotic adjustment in *D. spicata*. The accumulation of non-protein nitrogen compounds have been shown to contribute significant amounts of nitrogen to the plant tissue, which subsequently is reported as crude protein, with contributions equivalent to 2-4% of the total crude protein concentrations (Masters *et al.* 2001; Storey *et al.* 1977).

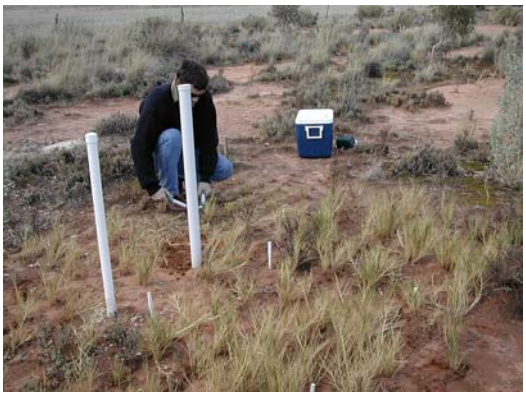
The measurements of *in-vitro* digestibility and acid-detergent fibre in the shoots enabled an assessment of the effects of the nitrogen, phosphorus and salt treatments on digestibility of *D. spicata*. Care needs to be taken when interpreting the results of halophytic pastures due to the higher soluble ash component of the pasture, which can lead to errors in the actual digestibility value (Masters *et al.* 2001). As a result, these values can not be used to predict the animal performance from such a pasture. Instead, they can only provide a relative ranking between the treatments. A key finding in this study was the marked interaction between nitrogen and phosphorus supply on the i*n-vitro* digestibility of the *D. spicata* shoots in phase I, where the digestibility values increased from 45 to 55% when adequate phosphorus was applied with the high nitrogen rates. It is likely that several factors were responsible, including an increase in individual leaf areas that occurred in phase I and II, where larger leaves occurred with the higher nitrogen and phosphorus treatments. In addition, there was a significantly lower concentration of acid detergent fibre in shoots of *D. spicata*, receiving adequate phosphorus and high nitrogen (Fig. 8.8A).

This present study also showed that high salt loads and high phosphorus supply decreased the concentration of acid detergent fibre in stems, but not leaves (Fig. 8.8B, C, D). Similar findings have been found in *Atriplex prostrata*, where the cross-sectional area of lignin from transverse sections of stems, decrease in the third and fourth internodes when salinity was increased from 0 to 0.5 and 1.0% (Wang *et al.* 1997). Hagège *et al.* (1988) showed lignin concentration reduced from 5.99 to 1.64 mg g^{-1} in *Suaeda maritime* when NaCl concentration was increased from 0 to 130 mM. Wang *et al.* (1997) hypothesized from the anatomical studies of *A. prostrata* that a reduction in lignin was due to a reduction in the cross-sectional area of xylem and an associated

increase in the phloem tissue under saline conditions. The reduction in ADF concentration observed in the stems of *D. spicata*, may possibly be due to a reduction in lignin concentration. However, this can not be verified from the present work, and further research is required to determine the cause of this reduction.

We also see a double benefit with the high nitrogen and phosphorus treatments not only producing a higher feed quality, but also increasing the amount of shoot dry matter. Increased shoot dry matter yields occurred in both phases of the experiment with higher phosphorus and nitrogen. In contrast, the high level of salt (33 dS m^{-1}) halved shoot dry matter production. It was also shown, that a high nutrient supply can overcome the negative effect of high salt load on the shoot dry matter. It is interesting to note that the nitrogen requirement for adequate growth was 3.2 mM, which was lower than that for maximum forage quality, where the high nitrogen treatment (16 mM) generally produced the higher forage quality.

The results of this experiment demonstrate that nitrogen, phosphorus and salt, all improved different aspects of the forage quality of *D. spicata* under the conditions of this experiment. The effects of high nutrient supply on feed quality under field conditions will be investigated in the following chapter. Under high nitrogen and phosphorus regimes, increases in crude protein, *in-vitro* dry matter digestibility and a lowering in acid detergent fibre concentrations were observed. These improvements can be attributed to morphological changes, most notable larger leaf area, and higher leaf-to-stem ratios. The findings highlight how nutrient management will be an important consideration if *D. spicata* is to be grown as a forage on saline land.



Chapter 9

Management guidelines for *Distichlis spicata* – field trial

9.1 Introduction

The salt tolerant grass *Distichlis spicata* cv. yensen-4a (NyPa Forage), was first introduced into southern Australia in the mid 1990s. It has grown on a trial basis to determine its suitability for forage production in saline discharge sites (Leake *et al.* 2002). It has shown promise as a suitable halophytic grass for forage production at these sites. Observations of the growth of *D. spicata* on a saline discharge site in Western Australia, showed dry matter yields of around 14 t DM ha^{-1} can accumulate on saline land over the growing season (M. Sargeant, personal observation).

Distichlis spicata is unlike any other pasture grass used in the mixed farming zone of Australia. Unlike most other conventional pastures, *D. spicata* cv. Yensen-4a does not produce a seed, and consequently requires vegetative establishment. This species also spreads by rhizomes, which are storage organs for carbohydrate reserves within the plant (Smith 1968). Being a C_4 species, it is most productive throughout the summer months, under conditions of high temperature and light regimes.

There have been no studies on how to best manage this species for forage production on saline land in the mixed farming low rainfall areas of southern Australia. Initial observations by growers have suggested that the grass provides greatest benefits when grazed rotationally, responds well to fertilizers and can provide significant feed reserves throughout the summer/autumn period. These observations provide a valuable insight into some of the important management factors for a *D. spicata* sward. The importance of these observations is also supported by the scientific literature for other pasture systems, where grazing frequency has been linked to persistance and productivity (McKenzie 1996; 1997a; b) and the importance of nutrient inputs to improve persistence and dry matter production (McKenzie *et al.* 2002; Singh *et al.* 1999).

The supply of green forage in the summer months would be extremely valuable on such farms to maintain livestock condition. Currently, this is achieved with the use of supplementary feeding, or by running low stock numbers. Thus *D. spicata* may be able to produce valuable forage on

land that has previously been considered unproductive or worthless, by using saline moisture at discharge sites. The grass therefore provides an economic opportunity for managers of saline land.

The field experiment documented in this chapter aims to test the hypothesis that grazing frequency and nutrient inputs affect plant persistence, dry matter production and feed quality. The approach was to establish plots of *D. spicata* on land irrigated with saline water. Varying rates of fertilizer treatments (containing nitrogen and phosphorus), together with clipping frequency treatments, were applied to the *D. spicata* plots. Plants were measured for survival, rate of spread, dry matter production and feed quality.

9.2 Materials and Methods

9.2.1 Site description

The field site was located at Donald (36°17' 45" S, 143 °01' 28" E) in the Wimmera region of Victoria, on a grey sandy loam. The soil pH (1:5) $_{(soil:water)}$ was 5.5 and the electrical conductivity ($EC_{(1:5)}$), was 0.33 dS m^{-1} in August 2005. The site was established vegetatively in August 2005 with bare rooted *Distichlis spicata* cv. yensen-4a plants which were planted at 1m spacing.

9.2.2 Rainfall / irrigation

The site was irrigated by a traveling irrigator in the 2005/06 growing season with saline ground-water obtained from a bore on the property. Irrigations occurred approximately every 14 days, and applied about 30 mm per irrigation. The electrical conductivity of the bore water ranged between 30 and 35 dS m^{-1}. Irrigation water was not available during the 2006/07 growing season and consequently the site was not irrigated in the second season. The long-term average (1885-2007) rainfall of the area is 400 mm per year, which is predominately received through winter and spring (Fig. 9.1).

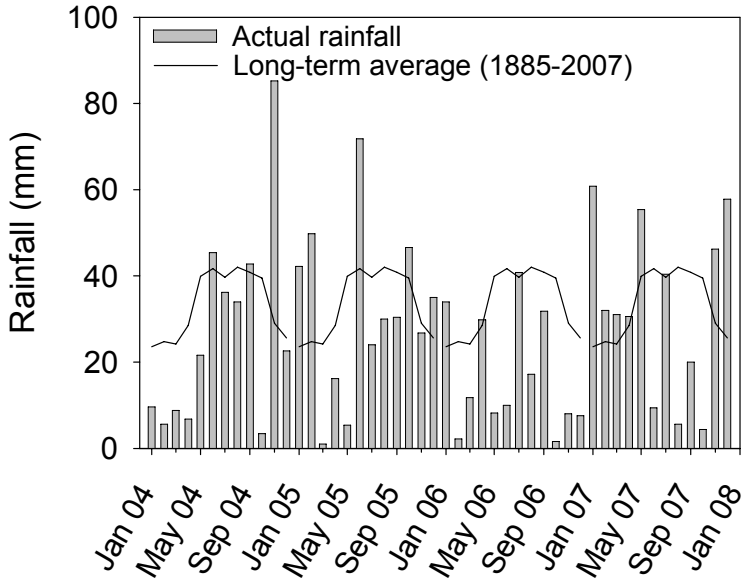


Figure 9.1. The monthly rainfall received throughout the experimental period along with the long-term average (1885-2007).

9.2.3 Experimental design and treatments

The experiment was set up using a randomized split-plot design, replicated 5 times with 2 plants per subplot. The treatments consisted of four fertilizer main plot treatments (Table 9.1) and three subplot clipping frequency treatments. The fertilizer treatments involved the annual application of 0, 75 and 300 kg ha^{-1} di-ammonium phosphate (DAP), with a further treatment involving the highest rate of DAP together with 5 additional applications of 280 kg ha^{-1} ammonium nitrate (equivalent to 100 kg N ha^{-1} in each of the 5 applications) applied regularly during the growing season (Table 9.1). The high rate of fertilizer inputs for this treatment was to ensure that both nitrogen and phosphorus were not limiting throughout the growing season. The 3 clipping frequency subplot treatments involved cutting the plants to a height of 2 cm, 2, 3 or 5 times at regular intervals during the spring, summer and autumn period.

Table 9.1. Fertiliser treatments showing the amount of di-ammonium phosphate (DAP) applied, and the resulting amount of phosphorus, nitrogen and additional nitrogen applied during both growing seasons.

DAP	Phosphorus applied as DAP	Nitrogen applied as DAP	Additional nitrogen applied as NH_4NO_3
(kg ha^{-1} yr^{-1})	(kg P ha^{-1} yr^{-1})	(kg N ha^{-1} yr^{-1})	(kg N ha^{-1} yr^{-1})
0	0	0	0
75	15	15	0
300	60	60	0
300	60	60	100 × 5

9.2.4 Measurements and analysis

Field observations were conducted in September, November, January, March and May in the 2005/06 growing season, and in September, November, January, March and June in the 2006/07 season. At each of these times, plant survival (percentage of plants with green growth), the maximum distance of emerged tillers from the mother plant (plant spread), and the number of tillers per plant, were recorded.

At each harvest, plants from the clipped or defoliated treatments were clipped to a height of 2 cm from the soil surface, and then dried in an oven at 70°C until a constant weight was achieved. Samples were then divided into leaf and stem portions and weighed separately, to calculate the leaf-to-stem ratios and total dry matter production.

Plant material was ground using a coffee grinder, and mortar and pestle. Approximately 35 mg of oven-dried material was weighed out for nitrogen analysis. The total nitrogen was analysed by dry combustion using a CNHS auto-analyzer (Elementar, Varios EL) and the concentration was then multiplied by 6.25 to give crude protein concentration of the plant material. Nitrogen analysis was not conducted on plots clipped 5 times per season.

9.2.5 Statistical analysis

The data were analysed with GenStat 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). A split-plot analysis of variance was used to test the significance of the treatment effects and treatment interactions at the 5% confidence level. Because of the variability between individual *D. spicata* plants, significant treatment effects at the 10% level ($P<0.1$) are also reported. The 4 fertilizer treatments are considered to be increasing levels of nutrient inputs, in assessing fertilizer by clipping frequency interactions.

9.3 Results

The performance of *Distichlis spicata* in this 2-year field study was affected more by clipping than by the level of nutrient supply. The main effects of clipping were generally significant ($P<0.01$ to $P<0.1$) for growth measurements, including distance of plant spread, leaf-to-stem ratio, number of tillers and dry matter yield in the first and second year of the study (Table 9.2). In contrast, significant effects of fertilization only occurred with distance of plant spread ($P<0.1$) and leaf to stem ratio ($P<0.05$). However, there were a series of important interactions between clipping and fertilizer treatments for plant growth and morphology measurements in the second year.

Table 9.2. Significant main effects and interaction terms for clipping and fertilizer treatments when the analysis of variance is performed on plant survival, growth, morphology and crude protein measurements.

	Distance of plant spread		Leaf-to-stem ratio		Crude protein		Number of tillers		Dry matter	
Season ending	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Clipping		**	**	*	***	**	*	***	*	#
Fertilizer	#		*			#				
Clip × Fert		#						*		#

	Plant survival								
	Sept 2005	Jan 2006	March 2006	May 2006	Sept 2006	Nov 2006	Jan 2007	March 2007	June 2007
Clipping			#						#
Fertilizer								*	*
Clip × Fert									

*** p<0.001, ** p<0.05, * p<0.05, # p<0.1

9.3.1 Establishment of the sward

Distichlis spicata survival was affected by seasonal clipping frequency in March 2006 (P<0.1), and by the fertilizer and clipping treatments in June 2007 (P<0.05 and P<0.1) respectively (Table 9.2). The marginal effect in March 2006 was caused by a higher plant survival of more than 90% when clipped three times, compared to two clippings at the beginning and end of the growing season (Fig. 9.2A). However, the effect had disappeared by the end of the second growing season when five clippings per season reduced plant survival to less than 40% of initial plant numbers (Fig. 9.2B). The other clipping treatments maintained plant numbers at around 60%. In June 2007, fertilizer had a significant effect on plant survival, with survival increasing from less than 40% with no fertilizer inputs to about 70% with high inputs (Fig. 9.2D). Significant differences occurred between the highest nutrient input of 300 kg DAP ha^{-1} + N with 73% survival and the 75 kg DAP ha^{-1} treatment with 45% survival. Likewise, the 300 kg DAP ha^{-1} treatment had a higher survival than the 0 kg DAP ha^{-1} treatment.

A small increase in *D. spicata* spread, that was significant at the 10% level, occurred in the 2005/06 growing season. Tillers emerged 10 cm from the original plant with the 300 kg DAP ha^{-1}

+N treatment, which was approximately 50% greater than the spread for plants that received 75 kg DAP ha^{-1} at the beginning of the 2005/06 growing season (Fig. 9.3A).

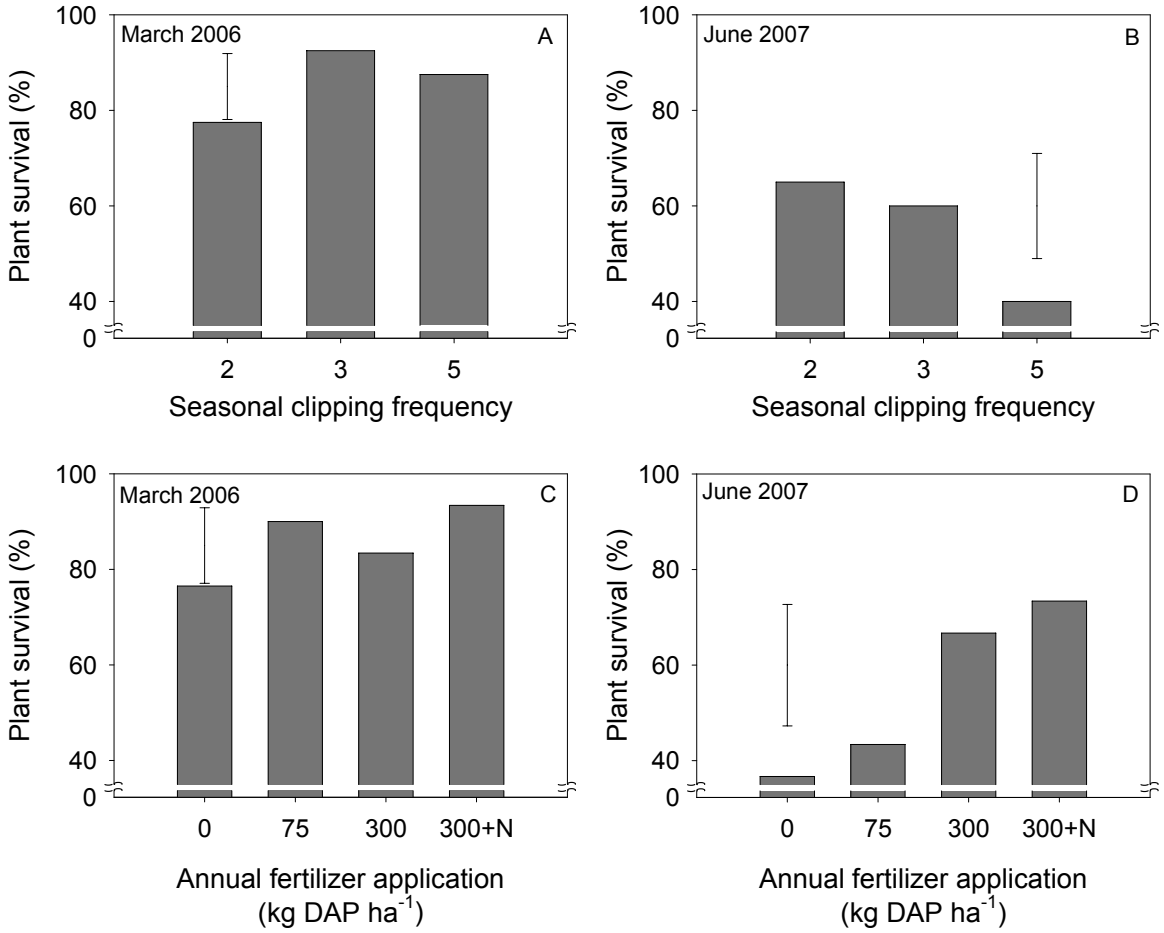


Figure 9.2. Plant survival at the end of the 2005/06 (A, C) and 2006/07 (B, D) growing seasons in response to clipping frequency and fertilizer application. DAP and +N represents di-ammonium phosphate and additional nitrogen applied as ammonium nitrate throughout the growing season. Error bars represent LSD (P=0.05). A significant clipping frequency main effect (P<0.1) occurred in March 2006, and significant clipping frequency and fertilizer main effects (P<0.05) in June 2007.

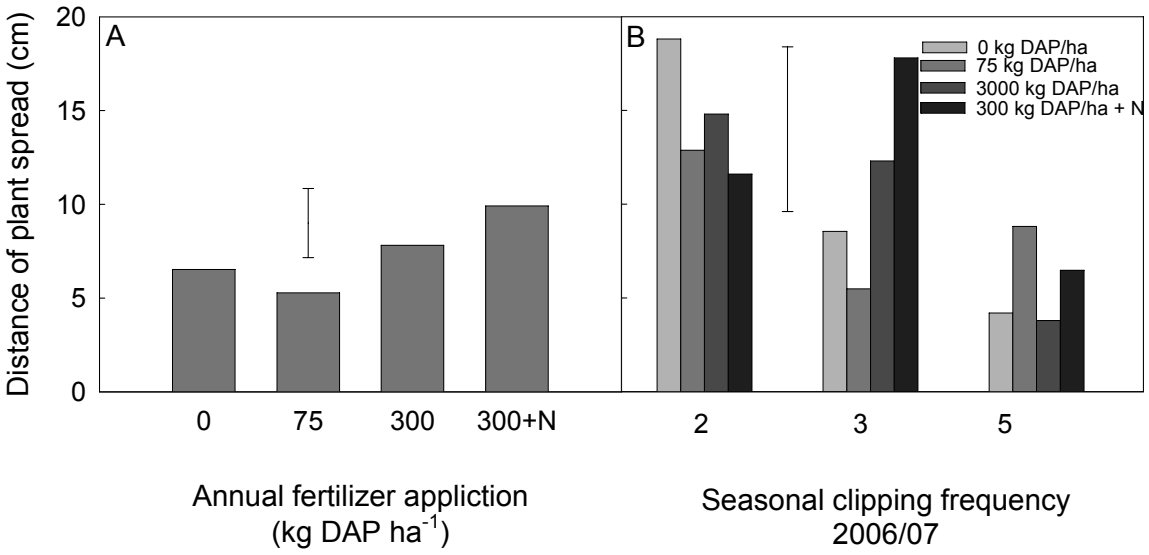


Figure 9.3. Distance spread from original plant at the end of the 2005/06 (A) and 2006/07 (B) growing seasons as a function of the nutrient supply and clipping treatments. DAP and +N represents di-ammonium phosphate and additional nitrogen applied as ammonium nitrate throughout the growing season. Error bars represent LSD (P = 0.05) for the fertiliser treatment main effect (A) and the clipping × fertiliser interaction (B).

Seasonal clipping frequency had a marked effect on *D. spicata* spread at the end of the 2006/07 season (Table 9.2, Fig. 9.3B). Five clippings during 2006/07 significantly reduced the spread of tillers from the original plant. However, a significant interaction between fertilizer input and clipping frequency occurred at the 10% level during the second growing season. The basis for the interaction occurred with the moderate clipping frequency of 3 cuts per season: the nil and 75 kg DAP ha^{-1} treatments resulted in 7 and 5 cm, whereas the 300 kg DAP ha^{-1} with additional N applications resulted in a spread of around 18 cm from the original plant.

9.3.2 Growth of D. spicata

The number of tillers per plant declined significantly ($P<0.05$ and $P<0.001$) in the first and second growing seasons, respectively, with increased clipping frequency (Table 9.2, Fig. 9.4). In the first season, plants that were clipped twice per season had 11.5 tillers per plant, whereas plants clipped five times produced only 4.2 tillers per plant. However, during the second growing season, there was a significant interaction ($P<0.05$) between clipping frequency and fertilizer treatment. The interaction resulted from the marked increase in tiller numbers, to more than 50 tillers per plant, with the highest level of nutrient input (300 kg DAP + N) when the *D. spicata*

plants were clipped three times in the growing season. Low nutrient addition with three clippings resulted in less than 10 tillers per plant. This response to high nutrient addition did not occur with two or five clippings in the growing season.

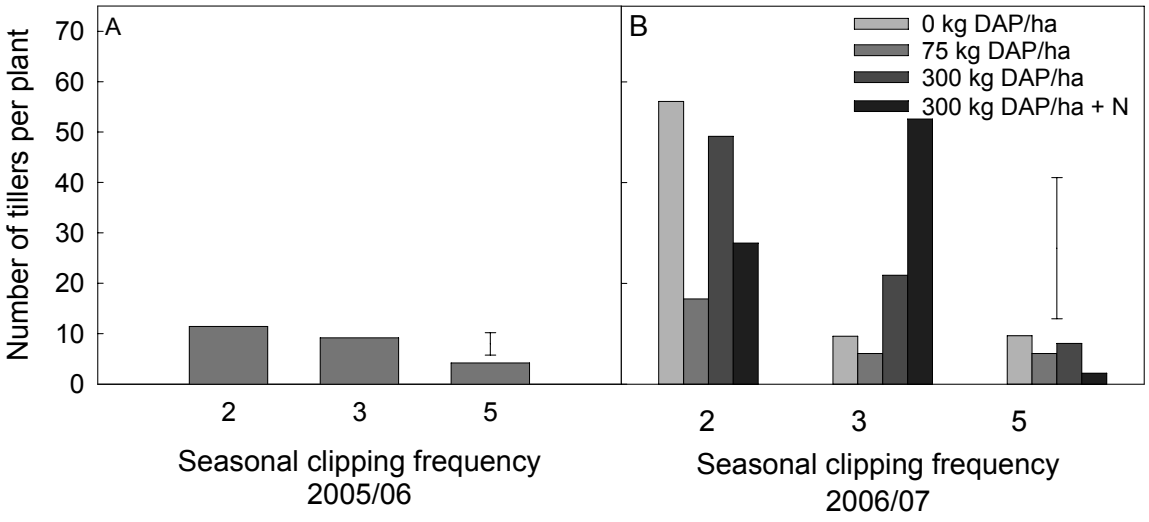


Figure 9.4. The number of tillers per plant at the end of the 2005/06 (A) and 2006/07 (B) growing seasons as a function of seasonal clipping frequency and fertilizer inputs. DAP and +N represents di-ammonium phosphate and additional nitrogen applied as ammonium nitrate throughout the growing season. Error bars represent LSD (P = 0.05) for the seasonal clipping frequency main effect (A), or the seasonal clipping frequency × fertilizer interaction (B).

Dry matter yield of *D. spicata* shoots responded to clipping in a similar manner to tiller numbers. In the season of 2005/06, the yield declined significantly (P<0.05) when clipping frequency increased from two and three cuts to five cuts per season (Fig. 9.5A). In 2006/07, another interaction between clipping and fertilizer treatments occurred. The basis for this was the 4-5-fold increase in shoot dry matter with the highest fertilizer treatment where shoots had been clipped three times in the season. Such response to high nutrient supply did not occur with two or five clippings. There was minimal shoot dry matter produced when plants were clipped five times in the growing season.

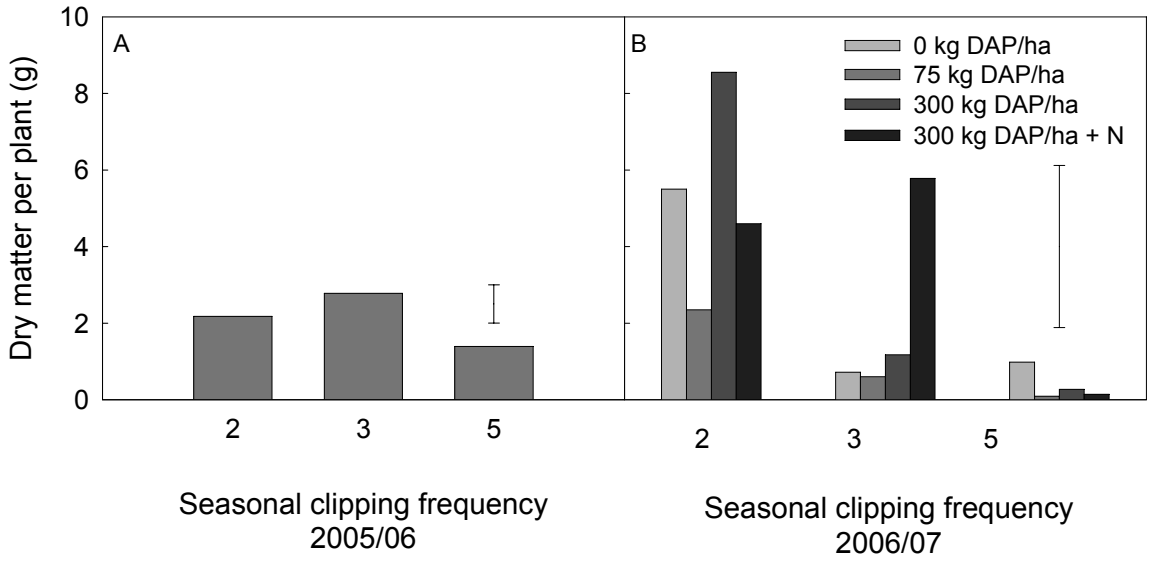


Figure 9.5. Seasonal dry matter production for the 2005/06 (A) and 2006/07 (B) growing seasons as a function of seasonal clipping frequency and fertilizer inputs. DAP and +N represents di-ammonium phosphate and additional nitrogen applied as ammonium nitrate throughout the growing season. Error bars represent LSD (P=0.05) for the seasonal clipping frequency main effect means (A), and the seasonal clipping frequency × fertilizer interaction means (B).

9.3.3 Quality of D. spicata forage

The leaf-to-stem ratio of *D. spicata* shoots, measured on a dry matter basis, was significantly affected by clipping frequency in both growing seasons (Table 9.2). The ratio was increased from around 1.5 with two clippings to a ratio of 2.3-2.5 with 3 and 5 clippings per season (Fig. 9.6).

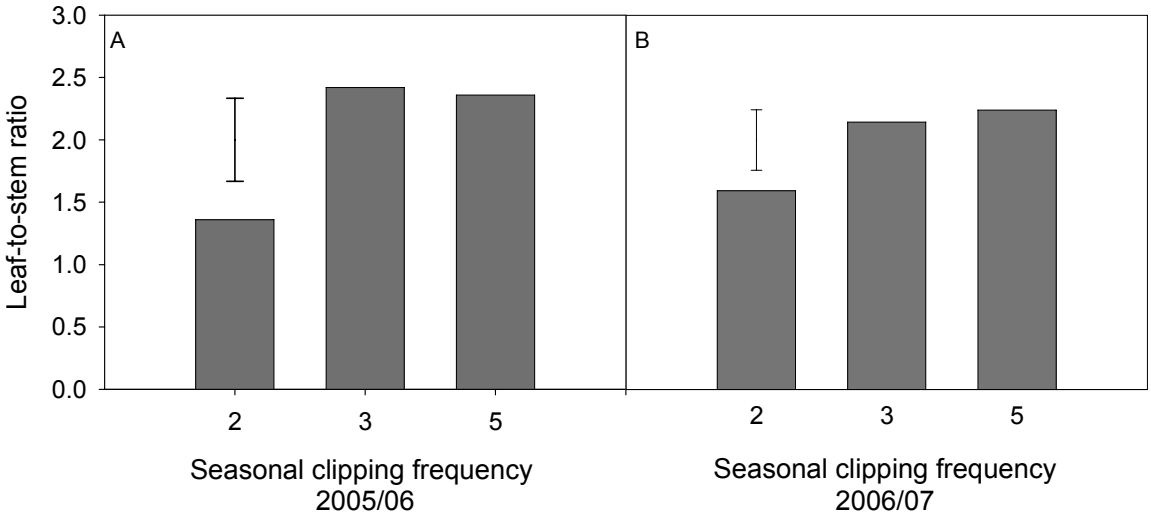


Figure 9.6. Leaf-to-stem ratio in the 2005/06 (A) and 2006/07 (B) growing seasons with respect to the number of clippings per growing season. Error bars represent LSD (P = 0.05) for the clipping frequency main effect.

The concentration of crude protein in *D. spicata* shoots increased in the second growing season and also increased with more frequent clipping. The combined effect of time and clipping frequency meant that the crude protein concentration in *D. spicata* shoots increased from 5 to 8.5% in 2005/06 to 8 and 12% in 2006/07 for 2 and 3 clippings per season respectively (Fig. 9.7A,B). There was also a significant increase in crude protein concentration in the second season to 13% with the 300 kg DAP ha^{-1} +N treatment (Fig. 9.7D), compared to the concentration of around 9% for the nil fertilizer treatment. There were no fertilizer effects on crude protein in the 2005/06 season.

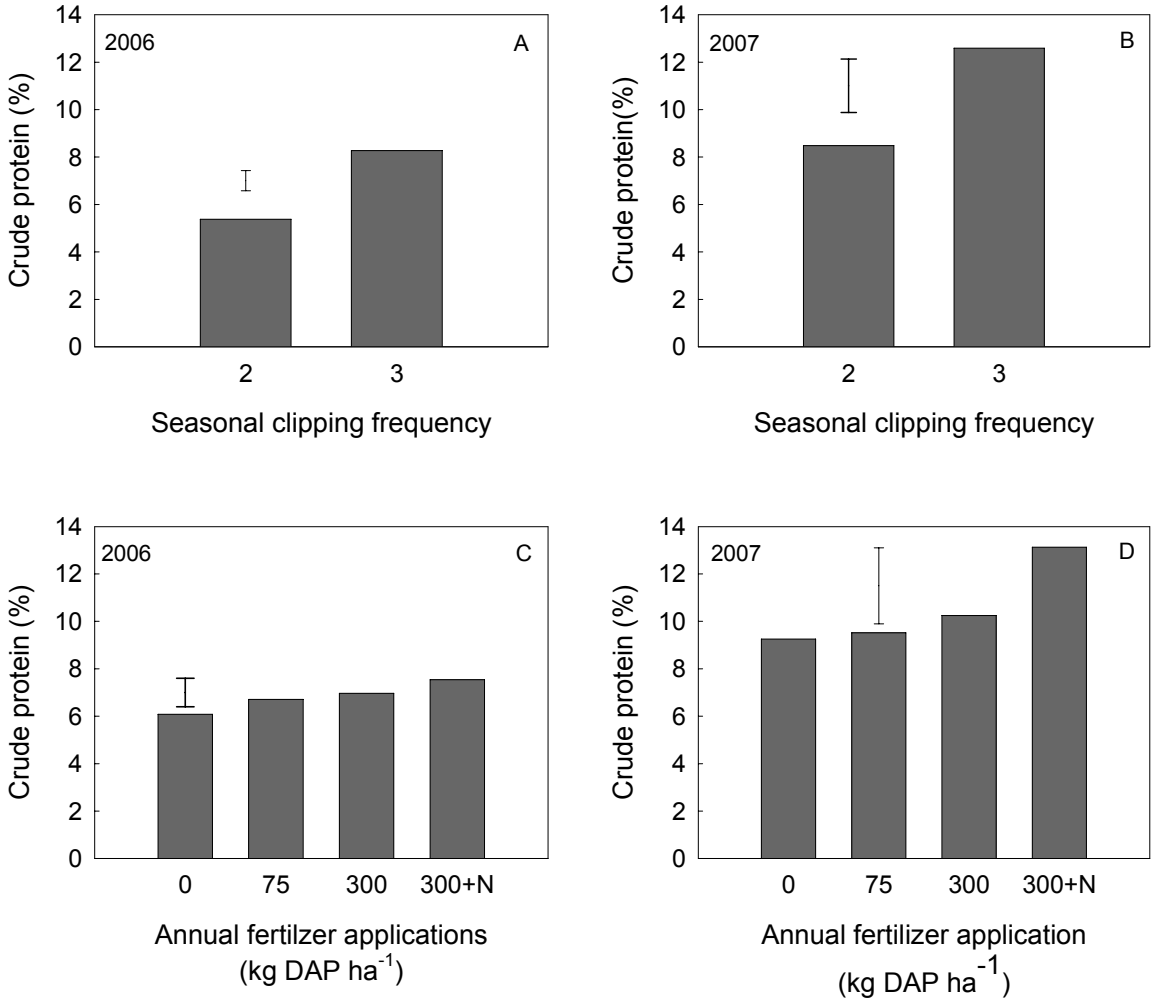


Figure 9.7. Crude protein concentrations in *D. spicata* shoots at the end of the 2005/06 (A,C) and 2006/07 (B,D) growing seasons, as a function of clipping frequency and fertilizer application. DAP and +N represents di-ammonium phosphate and additional nitrogen applied as ammonium nitrate throughout the growing season. Error bars represent the LSD (P = 0.05) for the clipping frequency and fertilizer main effects.

9.4 Discussion

It has previously been suggested that *Distichlis spicata* cv. yensen (NyPa Forage) is ideally suited to saline discharge zones throughout the mixed farming zone in southern Australia (Leake *et al.* 2002). In this farming system, *D. spicata* is able to provide standing green forage throughout the summer months when it is actively growing: this is a time of year when green forage is scarce and supplementary feeding of stock is common to maintain live weights. However, for *D. spicata* to be ideally suited to these areas, it is important that forage production and its feed quality are able to match the grazing and feeding requirements of livestock in this system. It can be argued that grazing would need to occur in the spring to utilize any winter growth from companion annual grasses, and again in late summer to utilize remaining *D. spicata* feed. A key issues is whether *D. spicata* can tolerate a third grazing in mid summer. The results of this field experiment demonstrate that this is possible if adequate nutrients are supplied, and that this third grazing also markedly improves the feed quality of the *D. spicata* shoots.

Distichlis spicata survival was directly related to fertilizer input and clipping frequency under the conditions of this field experiment. However, it was not until late in the second growing season that these effects become apparent, with the effects more noticeable towards the end of the growing season (Table 9.2). It is likely that the plants had sufficient energy reserves to survive in the first year, and it was not until the second year that these energy reserves were depleted and management factors started to make their impact. For example, plant persistence at the end of the 2006/07 growing season decreased significantly with the five clippings per season, but increased with the higher fertilizer rates. It has been suggested by Stoll *et al.* (1998) that rhizome reserves of *Solidago altissima* are depleted under frequent mowing regimes, and that this is a suitable method for the control of this species. The same appears to be happening in the current experiment with *D. spicata*, where reduced survival with frequent clippings could be attributed to the depletion of rhizome reserves.

While higher clipping frequency decreased *D. spicata* survival, higher nitrogen and phosphorus fertilizer inputs increased plant survival. Such results have been observed by other researchers, as applications of both nitrogen (Fulkerson *et al.* 1993) and phosphorus (Singh *et al.* 1999) have both been linked to increasing plant persistence. Fulkerson *et al.* (1993) found that the application of 46 kg N ha^{-1} as urea at monthly intervals increased the persistence of perennial ryegrass (*Lollium perenne*) over 2 seasons, and speculated that this was due to increased root growth. Singh *et al.* (1999) working with phosphorus, found that paddocks that recieved 140 kg P ha^{-1} applied per year retained a high proportion of white clover (*Trifolium repens)*, compared to paddocks that received nil phosphorus. The increased persistence of white clover was attributed

to the increased carbohydrate reserves within the stolons (Singh and Sale 1997b) along with the more effective stomatal control to minimize transpirational water loss (Singh 1998). It is likely that enhanced nutrient supply to *D. spicata* in this study was able to stimulate or increase photosynthesis, translocation and the storage of carbohydrate reserves, enabling the plant to survive and tolerate the clippings in mid summer. In the current field experiment, the effect of nitrogen and phosphorus could not be separated as both nutrients were applied together.

While plant survival declined with increasing clipping frequency, dry matter production of *D. spicata* shoots could be maintained with three clippings per season, provided high rates of fertilizers were applied to the sward. The production of *D. spicata* is a function of (i) the underground spread of rhizomes, (ii) the number of tillers that grow from the spreading rhizomes, and (iii) the shoot growth produced by each tiller. All of these components of sward growth responded in a similar manner to clipping and fertilizer treatments in the second growing season. In general, each component such as rhizome spread (Fig. 9.3B), tiller number (Fig. 9.4B) and total tiller shoot growth (Fig. 7.5B) was greater when clipped twice per season. However, the third clipping in mid summer was possible without yield loss provided that high rates of fertilizer, nitrogen and phosphorus were applied in the growing season. When five clippings occurred during the second season, high fertilizer rates were unable to overcome the negative effects of the frequent clippings. Singh and Sale (1997a) also found that high rates of phosphorus supply increased dry matter production over a range of defoliation frequencies. Indeed, high rates of phosphorus were essential to enable frequently defoliated white clover to survive, particularly under moisture deficit stress. Further work carried out by Singh and Sale (1997b) demonstrated an interaction between phosphorus supply and defoliation, where total non-structural carbohydrate concentrations in the stolons increased with high rates of phosphorus supply under frequent defoliation. Although non-structural carbohydrate reserves in the *D. spicata* rhizomes and tiller bases were not measured in the current field experiment, it is possible that high fertilizer inputs enabled carbohydrate reserves to be replenished from the shoots, which in turn enabled the grass to better tolerate the effects of 3 clippings per season.

The associated improvement in feed quality with the more frequent clippings resulted from an increase in the leaf-to-stem ratio when clipped three or more times per season (Fig. 9.6). This more frequent clipping was able to increase production of leaves relative to stems; this in turn will increase nutritive value as stems are more fibrous and less digestible than leaves (Terry and Tilley 1964). Frequent grazing also ensures that the tillers are of a younger physiological age. Terry and Tilley (1964) reported that the *in-vitro* dry matter digestibility of all plant parts is highest in young growth, but then declines with age, with the decline being greatest in stems. Maintaining a high leaf-to-stem ratio with more frequent grazing will therefore maintain the

nutritional value in *D. spicata*. Apart from the leaf-to-stem ratio, more frequent clipping also increased crude protein concentration (Fig. 9.7). Again, this is most likely due to the increase in leaf-to-stem ratio, as leaves have considerably higher crude protein concentrations than stems (Polan *et al.* 1968; Wales *et al.* 1999).

Marginal increases in crude protein concentrations in *D. spicata* shoots, occurred when the highest fertilizer rate (300 kg DAP ha^{-1} + N) was applied in the second season. The different rates of annual DAP at the beginning of the season had no effect on crude protein concentration. It is not surprising that the repeated heavy applications of nitrogen (100 kg N ha^{-1}) increased crude protein, because nitrogen is a major constituent of protein. It is uncertain whether such high rates of nitrogen during the growing season would be economic, or whether such high nitrogen rates are necessary to increase crude protein concentration. According to Milton *et al.* (2001), the crude protein requirement by adult sheep to meet their maintenance requirement is 8%, and to achieve weight gain is 12-15%. The results obtained in this field experiment during the first year were below that required for maintenance, but all crude protein concentrations in the second year were adequate for maintenance. Where *D. spicata* was clipped three times and additional nitrogen was applied throughout the growing season, then the crude protein concentrations were in the range to meet requirements for weight gain.

The results of this field experiment demonstrate that *D. spicata* swards can be managed effectively to provide forage under saline conditions in a mixed farming system in southern Australia. Guidelines for managing *D. spicata* under these conditions would first require that phosphorus and nitrogen fertilizers be applied to the plants during the establishment phase to ensure that maximum plant survival is achieved. A second guideline would be that *D. spicata* swards be grazed three times per growing season, and that additional nitrogen be applied throughout the season to ensure that dry matter production and feed quality are maximized. It is important to note that very high rates of DAP and additional nitrogen were used in this study. Further research is required to define the phosphorus and nitrogen response, in terms of *D. spicata* shoot growth for an economic return. The soil salinity in this current experiment was also relatively low and further field trials should be conducted under more saline conditions.

Chapter 10

General discussion

Soil salinity is a major constraint that limits agricultural production both within Australia and throughout the world (Anonymous 1999; Oldeman *et al.* 1991). Conventional agricultural crop and pasture species are not able to tolerate high concentrations of salts within the rootzone, due to their inability to manage high salt loads into the plant. However, halophytes are a group of plants that are able to complete their life cycle in saline environments, and have been considered as potential crop and pasture species for saline environments. One of the more promising halophytes that is being considered in Australia as a forage species is *Distichlis spicata* cv. yensen-4a. However, very little research had been conducted on the management and sustainability of *D. spicata* within Australia. The focus of this thesis was to (1) investigate the sustainability of a farming system utilizing *D. spicata* as a forage, (2) to determine the constraints to successful establishment, and then try to develop some techniques for overcoming these constraints, and (3) to provide some basic management guidelines for grazing and nutrient management to maximise productivity.

The farming system where *Distichlis spicata* could be used

Distichlis spicata is a C_4 halophytic grass that is ideally suited to wet saline discharge sites within the mixed farming regions of southern Australia. These saline discharge sites are often abandoned to agricultural production and are characterized by shallow saline water tables, saline soils, poor soil structure and a lack of vegetation. Within these areas, rainfall is generally winter dominant, and results in a lack of green feed through the summer and autumn period. The use of productive salt-tolerant pastures in saline sites has been shown to be as productive as pasture adjacent on non-saline land (Thomas *et al.* 2009). Such pastures would enable green feed to be produced during the summer and autumn period, which would be particularly valuable to grazing enterprises when feed is scarce and most valuable, helping to minimize supplementary feeding.

Although the loss of agricultural production is a major issue associated with salinity (McLarty and I'Anson 2002), increasing production is not necessarily the major reason for re-vegetating saline discharge sites. Social aspects can be some of the more powerful drivers for re-vegetating these areas, with landholders gaining a lot of satisfaction, pride and confidence from successfully

establishing salt-tolerant pastures (Bennett and Price 2007). These can flow from achieving personal and family goals for the farm, meeting social responsibilities for improving catchment health, along with the satisfaction of generally improving the aesthetic value of the land. Bennett and Price (2007) concluded that these social benefits often out-weigh the economic considerations in re-vegetating saline land. An established sward of *D. spicata* in a saline discharge site provides a marked improvement in aesthetic value, but also increases the production potential from these sites. As was pointed out by Thomas *et al.* (2009), the out-of-season forage production from salt-land pastures is a very valuable resource, and these areas are potentially as productive as adjacent non-saline land. The photographs in plate 10.1 show what an established area of *D. spicata* can look like, compared to a bare salt scald prior to planting.

Plate 10.1. Photographs of a saline discharge site at Wickepin in Western Australia when *D. spicata* was first established (a), the same site 2 years after planting (b), and an adjacent area that was planted over 5 years earlier (c).

Not only does a *D. spicata* sward provide many social and production benefits, but it is also a sustainable use of saline discharge land. Previously, questions have been raised about the long-term sustainability of saltland pastures after it was found that salt accumulates within the root-zone of *Atriplex* stands, with the chloride concentrations increasing from 0.17 to 0.25% of the soil dry weight at 0.5 m over a 2 year period (Barrett-Lennard and Malcolm 1999). However, no evidence was found for any salt accumulation under *D. spicata* swards over an 8 year period (Fig. 3.7 and 3.8). It should be pointed out that some level of caution needs to be exercised here, as there were no base line data for the areas where the *D. spicata* was planted. Nevertheless, three sites were investigated at Wickepin in Western Australia, and each site had an adjacent control area that was not planted with *D. spicata*. The main site was also sampled over two seasons to give a greater level of confidence in these results. The lack of any observed salt accumulation within the root zone of *D. spicata* can most likely be attributed to a number of factors including its ability to take up salt and excrete it on to the leaf surface, where it is then able to wash, blow or fall back to the soil surface. In addition, the extensive network of roots were also shown to

increase the saturated hydraulic conductivity of the soil (Fig. 3.6), which in turn would aid the movement of salts down the soil profile and so prevent accumulation in the root zone.

Apart from the lack of salt accumulation within the root-zone, we also see a number of soil chemical and physical improvements that lead to an overall improvement in soil health. These include an increase in soil carbon and nitrogen concentrations, and physical improvements from increased aggregation and increased saturated hydraulic conductivity (Chapter 3). All of these changes can be traced back to the extensive root system, with total dry matter yields of 1.9 t ha^{-1} being recorded in the top 30 cm (Chapter 3). The addition of organic matter into the soil profile improves soil aggregation, and adds carbon and nitrogen to the soil. The death and renewal of this extensive root system also creates old root channels which along with the improved aggregation increase the saturated hydraulic conductivity. This improvement in soil health may allow for alternative uses for this saline land. The establishment of less salt-tolerant plants during the cooler and wetter winter months, such as balansa clover (*Trifolium michelianum)* and annual grasses or winter cereals would boost winter forage production. This would utilize the winter rainfall, at a time of year when the *D. spicata* sward is in a semi-dormant state. The large investment in root growth may qualify for carbon sequestration outcomes in the future. Assuming a bulk density of 1.3 g cm^{-1}, approximately 8.5 t ha^{-1} of carbon has been sequestered in to the top 50 cm of soil at the Wickepin site in Western Australia.

Establishing *Distichlis spicata* swards

The successful establishment of *D. spicata* is one of the major factors that limits the broad scale adoption of this species within the mixed farming system in southern Australia. Establishment has previously been identified as problematic (Leake *et al.* 2002), due to the patchy nature of past attempts in establishment. There were a number of attempts to establish *D. spicata* at the Manangatang site documented in this thesis, which were also problematic. The first attempt resulted in successful establishment adjacent to the discharge area, where soil salinity was lower, and soil moisture also lower, but no establishment in the dry saline discharge soil. The second attempt aimed to reduce the salinity in the saline discharge soil by forming raised beds prior to the winter rains, and then plant into these beds. However, the construction of the beds actually increased the soil EC in the beds due to the use of more saline soil to construct the beds, with the winter rainfall being insufficient to leach this salt from the bed. The third attempt involved using salt-primed plants with intact root systems alongside bare-rooted tillers. This technique worked well at the Donald site, where soil moisture and salinity were high. This technique failed at Manangatang where the soil was drier, despite soil salinity being similar. All of these results can be explained by the moisture and salinity interaction. When plants are first exposed to salts,

growth is limited by the reduced water availability, rather than the toxic effect of salts (Munns 2002; Passioura and Munns 2000). The additive effect of low water availability just exacerbates the moisture deficit stress. This was demonstrated by Passioura and Munns (2000) when leaf elongation rates of barley were maintained in the short term by pressurizing the saline root solution to maintain the leaf water relations, compared to a decrease in leaf elongation rates when the saline root solution was not pressurized.

Salt priming was also used to try to improve the establishment under glasshouse conditions (Chapter 4) and field conditions (Chapter 6). In the glasshouse, this technique proved to be very successful, with salt-primed plants producing 6 times more dry matter than non salt-primed plants (Fig. 4.4), along with improved survival (Fig. 4.2). However, when trialed in the field with bare-rooted tillers, no benefit was observed. However, the seasonal conditions encountered were very unfavourable, with only 1 rainfall event greater than 2 mm falling in the 3 weeks after planting, along with rising temperatures (Fig. 6.2). Salt-priming was also used on the plants with intact root systems. This system proved very successful at the moist Donald site with 100% of the plants surviving, compared to only 20% of the bare-rooted non salt-primed tillers (Table 6.4). However, at Manangatang, these intact root system plants failed to establish. As there were no control plants with intact root systems that had no salt priming, the successful establishment at Donald can not be attributed just to salt-priming. Further research needs to be conducted to determine how effective salt-priming is under such moist, saline field conditions.

Traditionally *D. spicata* has been established with bare-rooted tillers that have been transplanted into surface soil (Leake *et al.* 2002). However, little research has been conducted on the suitability of using lengths of rhizomes to establish the species, despite rhizome growth being the natural method of spreading. As with planting tillers, there also appears to be the delicate salinity, moisture balance, with low moisture and high salinity impeding tiller initiation, and presumably establishment (Fig. 7.1). Under moist conditions, between 40 and 80% of the rhizome fragments produced emerged tillers (Fig. 7.1 and 7.2). By planting the rhizome fragments deeper in the soil at discharge sites, it may also be possible to place the fragments in really moist soil that is saline. The depth that these could be planted would be determined by the ability of the tillers to emerge to the surface. Presumably larger fragments could be successfully planted deeper. There would also be the added advantage of planting into warmer soil at depth, for late winter and early spring planting, which would aid establishment of this C_4 grass. The timing of rhizome harvesting and prior management may also be important to avoid any bud dormancy within the rhizomes, as has been found in *Agropyron repens* (Leakey *et al.* 1977a; McIntyre 1970). Leakey *et al.* (1977b) found that bud dormancy was present during spring, whereas rhizomes harvested during autumn did not exhibit bud dormancy. It was also found that bud dormancy in *A. repens* was due to low

nitrogen concentrations within the rhizomes (Leakey *et al.* 1977a). Further research needs to be conducted to determine whether dormancy does exist in *D. spicata,* and if so, how this may be managed to improve bud burst. The utilization of rhizome fragments for establishment provides an opportunity to reduce establishment costs of this species, and hence increase adoption on a broad acre scale.

The success of the salt-primed plants with intact root systems at Donald gives some optimism for field establishment. The survival of these plants at a similar soil EC to the Managatang site where similar plants failed, demonstrates that the success was due to the abundant soil moisture at Donald. The ironic part to this story is that both of these saline discharge sites are saline due to excess saline water being present. However, the saline water at the Manangatang discharge site is deeper in the soil, than at the Donald site. The depth to the saline groundwater varies greatly across the landscape. A recent survey of saltland pasture sites in Western Australia had saline watertable depths ranging from 0.2 to -3.8 m (Thomas *et al.* 2009). These depths vary between seasons and years depending on seasonal conditions, with fluctuations of over 1 m been reported between summer and winter at the same site (Ferdowsian *et al.* 2002). Given the variation in seasonal and regional watertable depths, along with the large variability in seasonal rainfall, it may be appropriate to irrigate *D. spicata* plants with saline water during the establishment phase to ensure adequate soil moisture. Given the large reservoir of saline water that is present below the surface of drier saline discharge sites, then the installation of an automatic, low-cost solar pump may provide a technique for establishing *D. spicata* in these sites. Once the grass has been established, its deep root system will be able to access to saline groundwater and thus survive. While this technique would not be cost-effective for landholders taking an economic view towards salt land pastures, it may provide an option for landholders who are willing to make modest investments in *D. spicata* establishment for social or environmental reasons.

A key finding in this thesis is that the timing of establishment must occur when there is adequate moisture in the root zone, as is the case for other crop and pastures. Moisture has consistently been shown to be the most critical factor affecting the success or failure of a planting, whether using bare rooted tiller, plants with intact roots, or rhizome fragments. This suggests that planting should occur during the cooler winter to early spring months when rainfall is expected and overcast conditions and short days prevail. It does appear that conditions of low light intensity do not adversely affect the salt tolerance of *D. spicata* (Chapter 5). In fact, conditions of low light intensity may assist establishment with tillers, due to the reduced transpiration rate (Fig. 5.10) under these conditions, which would lower the water requirement from the soil, and reduce the salt load arriving in the shoots via the xylem stream.

Managing *Distichlis spicata* swards

Adequate supplies of nutrients are required, especially nitrogen and phosphorus to ensure that *D. spicata* swards are productive. This was demonstrated in Chapters 8 and 9, where higher rates of nitrogen and phosphorus increased shoot dry matter production (Fig. 8.2 and 9.5). High levels of nutrients also increased the rate of spread by the plants, which would ensure a faster establishment of a sward (Fig. 9.3). It was also shown that a high nutrient supply was able to overcome the negative effect of high salinity on the dry matter production (Fig. 8.2). Increasing the nitrogen supply from 0.4 to 3.2 mM had the largest effect in overcoming the high salinity load (Fig. 8.2), which may be due to the supply of nitrogen for the synthesis of glycinebetaine, a non-protein nitrogen compound that the plant uses to osmoregulate (Marcum 1999).

Correct grazing management is also an important factor in a successful *D. spicata* sward. If a mid summer grazing is to be achieved, it is essential that additional nitrogen be applied to ensure maximum production is achieved. Without this additional nitrogen, dry matter production following defoliation is very low. More than three grazings per summer were found to have a negative effect on plant survival (Fig. 9.2), and dry matter production of the sward (Fig. 9.5), regardless of the fertilizer inputs. Applications of nitrogen and phosphorus have both been identified as improving plant persistence under grazed conditions (Fulkerson *et al.* 1993; Singh *et al.* 1999). In the field experiment described in Chapter 9, no differentiation can be made between the effectiveness of nitrogen and phosphorus separately as both nutrients were applied together.

One of the major benefits of adequate nutrient supply for *D. spicata* swards is the improved feed quality. The adequate supply of nutrients primarily improved the feed quality by increasing the leaf size. Leaves are known to be of a higher nutritive value than stems (Terry and Tilley 1964), so any factor that increases the leafiness will improve the feed quality. The adequate supply of nutrients decreased the acid detergent fibre concentrations (Fig. 8.8), increased digestibility (Fig. 8.7) and increased the crude protein concentration (Fig. 8.5). Grazing management also affected feed quality, with at least 3 grazings per season ensuring that the plant leafiness was high. There were also a number of unexpected benefits of the highly saline conditions, with the higher salt loads creating higher crude protein concentrations in the shoot tissue, and an increased leafiness.

Given that the adequate supply of nutrients are important for the successful management of *D. spicata* swards, then issues such as how to best apply these nutrients, and how much nutrient needs to be applied will arise. Saline discharge zones are often characterised by waterlogged conditions Applications of nitrogen fertiliser increase the risk of denitrification occurring under these conditions, generating more nitrous oxide which is a problematic greenhouse gas (Chen *et*

al. 2008). The form in which nutrients are applied and the method of application that avoids losses to the environment need to be researched further.

Future research needs

The research presented in this thesis provides a basic understanding of how *D. spicata* may fit into the mixed farming system of southern Australia. However, there is a large amount of research to be conducted to fine tune its use. These include:

- More research on field establishment techniques under a wider range of field and seasonal conditions. In what environments and conditions may salt priming be successful? Can difficult sites be established with intact root system plants that are irrigated with a cheap solar pump system?
- Can rhizomes be used successfully under field conditions for establishment? What management factors may affect rhizome health prior to harvesting?
- What forms of nutrients should be applied, how much should be applied, and in what way should they be applied to minimise nitrogen losses to the environment?
- Are there more salt tolerant *D. spicata* populations available for breeding more salt tolerant and productive cultivars?

There is a large under-utilised resource lying below saline discharge sites throughout southern Australia, in the form of saline water. It is quite ironical that a lack of water is the major limiting factor to Australian agriculture, yet in these areas there is an excess of water – except that it is saline. *Distichlis spicata* allows farmers to utilise this resource in a sustainable and productive manner. The future adoption of this grass in these areas will be determined by improvements in establishment techniques that will allow successful stands of *D. spicata* to develop in these hostile environments. The work presented within this thesis provides a useful indication of how *D. spicata* might be established, and managed to ensure forage production and quality is maximised.

References

AACC (1995) 'American Association of Cereal Chemists Approved Methods.' (American Association of Cereal Chemists Inc.: St Paul, Minnesota).

Ajmal Khan M, Gul B, Weber DJ (2002) Improving seed germination of *Salicornia rubra* (Chenopodiaceae) under saline conditions using germination-regulating chemicals. *Western North American Naturalist* **62**, 101-105.

Ajmal Khan M, Zaheer Ahmed M, Hameed A (2006) Effect of sea salt and L-ascorbic acid on the seed germination of halophytes. *Journal of Arid Environments* **67**, 535-540.

Akhter J, Mahmood K, Malik KA, Ahmed S, Murray R (2003) Amelioration of a saline sodic soil through cultivation of a salt-tolerant grass *Leptochloa fusca*. *Environmental Conservation* **30**, 168-174.

Akhter J, Murray R, Mahmood K, Malik KA, Ahmed S (2004) Improvement of degraded physical properties of a saline-sodic soil by reclamation with kallar grass (*Leptochloa fusca*). *Plant and Soil* **258**, 207-216.

Amarasinghe V, Watson L (1989) Variation in salt excretory of microhairs in grasses. *Australian Journal of Plant Physiology* **16**, 219-229.

Anonymous (1999) National Dryland Salinity Program Annual Report.

AOAC (1995) 'Official methods of Analysis of AOAC International.' (AOAC International: Arlington, Virginia).

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt Tolerance Conferred by Overexpression of a Vacuolar [Na.sup.+]/[H.sup.+] Antiport in *Arabidopsis*. *Science* **285**, 1256.

Arieli A, Naim E, Benjamin RW, Pasternak D (1989) The effect of feeding saltbush and sodium chloride on energy metabolism in sheep. *Animal Production* **49**, 451-457.

Australian Fodder Industry Association Inc. (2005) 'Fodder Analyst's Laboratory Manual: A reference manual of standard methods for the analysis of fodder.' (New Generation Print and Copy: Melbourne).

Awad AS, Edwards DG, Campbell LC (1990) Phosphorus enhancement of salt tolerance of tomato. *Crop Science* **30**, 123-128.

Babu S, Thirumurugan T (2001) Effect of NaCl priming for increased salt tolerance in sesame (*Sesamum indicum*). *J. Ecobiol.* **13**, 309-311.

Badawy N, Anderson H, G. W (1994) Response to N and P Fertilisers by saltbush grown in south west Victoria. In 'Productive Use of Saline Lands'. Echuca, Victoria pp. 168-169.

Barrett-Lennard E, Frost F, Vlahos S, Richards N (1991) Revegetating salt-affected land with shrubs. *Journal of Agriculture, Western Australia* **32**, 124-129.

Barrett-Lennard EG (2003a) The interaction between waterlogging and salinity in higher plants: causes, consequences and implication. *Plant and Soil* **253**, 35-54.

Barrett-Lennard EG (2003b) 'Saltland Pastures in Australia: A practical guide.' (Land, Water and Wool Sustainable Grazing on Saline Lands Sub-program: Canberra).

Barrett-Lennard EG, Malcolm CV (1999) Increased concentrations of chloride beneath stands of saltbushes (*Atriplex* species) suggest substantial use of groundwater. *Australian Journal of Experimental Agriculture* **39**, 949-955.

Batanouny KH (1993) Adaptation of plants to saline conditions in arid regions. In 'Tasks for Vegetation Science 27: Towards the rational use of high salinity tolerant plants'. (Eds H Lieth, AA Masoom) pp. 387-401. (Kluwer Academic Publishers: Dordrecht).

Bathgate AD, O'Connell M (2001) The case for animal production from saline land. In '7TH National PUR$L Conference: Wanted Sustainable futures for saline land' pp. 167-169. (Conference Design Pty. Ltd.: Launceston, Tasmania).

Bennett SJ, Price R (2007) 'Saltland prospects: prospects for profit and pride from saltland.' (Future Farm Industries CRC: Perth).

Berger-Landefeldt U (1959) Beiträge zur Ökologie der Pflanzen nordafrikanischer Salzpfannen. *Vegetatio* **2**, 1-48.

Binzel ML, Hes FD, Bressan RA, Hasegawa PM (1988) Intracellular compartmentation of ions in salt adapted tobacco cells. *Plant Physiology* **86**, 607-614.

Black RF (1954) The leaf anatomy of Australian members of the genus *Atriplex*. I. *Atriplex vesicaria* Heward and *A. nummularia* Lindl. *Australian Journal of Botany* **2**, 269-286.

Bohra JS, Dorffling K (1993) Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. *Plant and Soil* **152**, 299-303.

Botella MA, Martinez V, Pardines J, Cerda A (1997) Salinity Induced Potassium Deficiency in Maize Plants. *Journal of Plant Physiology* **150**, 200-205.

Bradley PM, Morris JT (1991) Relative Importance of Ion Exclusion, Secretion and Accumulation in *Spartina alterniflora* Loisel. *J. Exp. Bot.* **42**, 1525-1532.

Brizuela MA, Cid MS, Minon DP, Fernandez Grecco R (1990) Seasonal utilization of saltgrass (*Distichlis* spp.) by cattle. *Animal Feed Science and Technology* **30**, 321-325.

Bruce C (2003) SGSL Producer Network - Kingston SE, South Australia. In '9th PUR$L National Conference: Salinity under the sun - investing in prevention and rehabilitation of salinity in Australia'. (Eventcorp Pty Ltd: Rydges Capricorn Resort, Yeppoon).

Bruce R, Langdale GW, West LT, Miller WP (1992) Soil surface modification by biomass inputs affecting rainfall infiltration. *Soil Science Society of America Journal* **56**, 1614-1620.

Cervinka V, Chaloupka C, Davis D, Diener J, Erickson J, Grimes R, Hayes D, Jenkins B, Ketelhut B, Shannon M, Sun G, Swanson K (1999) Utilization of salt and selenium harvested from agricultural drainage water. The San Joaquin Valley Drainage Implementation Program and The University of California Salinity/Drainage Program, Salt Utilization Technical Committee.

Chapman VJ (1968) Vegetation under saline conditions. In 'Saline Irrigation for Agriculture and Forestry'. (Ed. H Boyko) pp. 201-216. (Dr W. Junk Publishers: The Hague).

Chen D, Suter H, Islam A, Edis R, Freney JR, Walker CN (2008) Prospects of improving efficiency of fertiliser nitrogen in Australian agriculture: a review of enhanced efficiency fertilisers. *Australian Journal of Soil Research* **46**, 289-301.

Chow WS, Ball MC, Anderson JM (1990) Growth and photosynthetic response of spinach to salinity: Implications of K^+ nutrition for salt tolerance. *Australian Journal of Plant Physiology* **17**, 563-578.

Clarke AL, Greenland DJ, Quirk JP (1967) Changes in some physical properties of the surface of an impoverished red-brown earth under pasture. *Australian Journal of Soil Research* **5**, 59-68.

Close DC, Bail CL, Clasen QC (2003) Physical and nutritional characteristics and performance after planting of *Eucalyptus globulus* Labill. seedlings from ten nurseries: implications for seedling specifications. *Australian Forestry* **66**, 145-152.

Close DC, Beadle CL, Brown PH (2005) The physical basis of containerised tree seedling 'transplanting shock': a review. *Australian Forestry* **68**, 112-120.

Colmer TD, Epstein E, Dvorak J (1995) Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt tolerant wheat x *Lophopyrum elongatum* (Host) A. Love Amphiploid. *Plant Physiology* **108**, 1715-1724.

Colmer TD, Fan TWM, Lauchli A, Higashi RM (1996) Interactive effects of salinity, nitrogen and sulphur on the organic solutes in *Spartina alterniflora* leaf blades. *Journal of Experimental Botany* **47**, 369-375.

Cornwall D, Mann L, Broadfoot K (2002) Managing Saline Groundwater to Improve Farm Productivity and Enhance Biodiversity. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)'. Freemantle - Kojonup - Kattaning p. 343. (Promaco Conventions Pty. Ltd.).

Cribb J (2004) The man who defeated famine. *Ecos* **120**, 16-20.

Dalhaus PG, MacEwan RJ (1997) Dryland salinity in south west Victoria challenging the myth. In 'Collected case studies in Engineering Geology, Hydrogeology and Environmental Geology.'. (Ed. G McNally) pp. 165-177. (Geological Society of Australia: Sydney).

Darling J (1999) Plants for saline environments: restora sweet grass (rsg) - *Puccinellia ciliata*. In 'Productive Use and Rehabilitation of Saline Lands National Conference'. Naracoorte, South Australia p. 41. (Sapro Conference Management).

Davies A (1977) Changes in growth rates and morphology of perennial ryegrass swards at high and low nitrogen levels. *Journal of Agricultural Science, Cambridge* **77**, 123-134.

Davison T, Cowan R, Shepherd R, Martin P (1985) Milk production from cows grazing on tropical grass pastures. 1. Effects of stocking rate and level of nitrogen fertilizer on the pasture and diet. *Australian Journal of Experimental Agriculture* **25**, 505-514.

Drennan P, Pammenter MW (1982) Physiology of salt excretion in the mangrove *Avicennia marina* (Forsk.) Vierh. *New Phytiologist* **91**, 597-606.

Dumsday RG, Pegler R, Oram DA (1989) Is broadscale revegetation economic and practical as a groundwater and salinity management tool in the Murray-Darling Basin? *BMR Journal of Australian Geology and Geophysics* **11**, 209-218.

Fahn A (1988) Secretory tissues in vascular plants. *New Phytologist* **108**, 229-257.

Fenton ML, Edwards NJ, McFarlane JD, Craig AD, Abraham EA, Hocking Edwards JE (2004) Urea applied to Puccinellia pastures increases sheep production. In 'Proceedings of the 25th Biennial Conference of the Australian Society of Animal Production'. University of Melbourne, Victoria.

Ferdowsian R, Pannel DJ, Lloyed M (2002) Explaining groundwater depths in saltland: Impacts of saltbush, rainfall, and time trends. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)'. Freemantle - Kojonup - Kattaning p. 343. (Promaco Conventions Pty. Ltd.).

Fernandez ON (2003) Establishment of *Cynodon dactylon* from stolon and rhizome fragments. *Weed Research* **43**, 130-138.

Flowers TJ, Troke PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology* **28**, 89-121.

Flowers TJ, Yeo AR (1986) Ion relations of plants under drought and salinity. *Australian Journal of Plant Physiology* **13**, 75-91.

Forster SM (1979) Microbial aggregation of sand in an embyro dune system. *Soil Biology & Biochemistry* **11**, 537-543.

Franklin-McEvoy J, Bellotti WD, Revell DK (2007) Supplementary feeding with grain improves the performance of sheep grazing saltbush (*Atriplex nummularia*) in autumn doi:10.1071/EA06149. Australian Journal of Experimental Agriculture **47**, 912-917.

Fulkerson WJ, Slack K, Moore K, Rolfe C (1993) Management of *Lolium perenne/Trifolium repens* Pastures in the Subtropics. I. Effect of Defoliation Interval, Seedling Rate and Application of N and Lime. *Australian Journal of Agricultural Research* **44**, 1947-1958.

Ghassemi F, Jakeman AJ, Nix HA (1995) 'Salinisation of Land and Water Resources: Human causes, extent, management and case studies.' (University of New South Wales Press: Sydney).

Glenn E (1987) Relationship between cation accumulation and water content of salt-tolerant grasses and a sedge. *Plant Cell and Environment* **10**, 205-212.

Glenn E, Felger R (1994) Saltwater Agriculture. *The World & I* **March**, 194-201.

Glenn E, Olsen M, Frye R, Moore D (1994a) Use of halophytes to remove carbon from the atmosphere: Results of a demonstration experiment. Electric Power Research Institute Project 8011-03.

Glenn EP, Squires V, Olsen MW, Frye R (1993) Potential for carbon sequestration in the drylands. *Water, Air and Soil Polution* **70**, 341-355.

Glenn EP, Swingle RS, Riley JJ, Mota CU, Watson MC, Squires VR (1994b) North American halophytes: Potential use in animal husbandry. In 'Halopytes as a resource for livestock and for rehabilitation of degradeable lands'. (Eds VR Squires, AT Ayoub) pp. 165-174. (Kluwer Academic Publishers).

Glenn EP, Watson MC, Maas EV, Lesch SM (1992) Comparison of salt tolerance and osmotic adjustment of low-sodium and high-sodium subspecies of the C4 halophyte, *Atriplex canescens*. *Plant Cell and Environment* **15**, 711-718.

Gorham J (1996) Mechanisms of salt tolerance of halophytes. (Eds R Choukr-Allah, CV Malcolm, A Hamdy) Halophytes and biosaline agriculture.

Grable AR (1966) Soil aeration and plant growth. *Advances in Agronomy* **18**, 57-106.

Grattan SR, Grieve CM (1992) Mineral element acquisition and growth response of plants grown in saline environments. *Agriculture, Ecosystems and Environment* **38**, 275-300.

Greenway H (1968) Growth stimulation by high chloride concentrations in halophytes. *Israel Journal of Botany* **17**, 169-177.

Greenway H, Osmond CB (1972) Salt Responses of Enzymes from Species Differing in Salt Tolerance. *Plant Physiol.* **49**, 256-259.

Groenevelt PH, Grant CD, Murray RS (2004) On water availability in saline soils. *Australian Journal of Soil Research* **42**, 833-840.

Hagège D, Kevers C, Boucaud J, Gaspar T (1988) Activités peroxydasiques, production d'éthylène, lignification et limitation de croissance chez *Suaeda maritime* cultivé en l'absence de NaCl. *Plant Physiology and Biochemistry* **26**, 609-614.

Hajibagheri MA, Yeo AR, Flowers TJ, Collins JC (1989) Salinity resistance in *Zea mays*: fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipids. *Plant, Cell and Environment* **12**, 753-757.

Hansen DJ, Dayanandan P, Kaufman PB, Brotherson JD (1976) Ecological adaptations of salt marsh grass, *Distichlis spicata* (Gramineae), and environmental factors affecting its growth and distribution. *Am. J. Bot.* **63**, 635-650.

Harward HE, McNulty I (1965) Seasonal changes in ionic balance in *Salicornia rubra*. *Journal of the Utah Academy* **42**, 65-68.

Hassidim M, Braun Y, Lerner HR, Reinhold L (1990) Na+/H+ and K+/H+ Antiport in Root Membrane Vesicles Isolated from the Halophyte Atriplex and the Glycophyte Cotton. *Plant Physiol.* **94**, 1795-1801.

Haynes RJ, Francis GS (1993) Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *Journal of Soil Science* **44**, 665-675.

Hendricks RC, Bushnell DM (2008) Halophytes energy feedstocks: Back to our roots. In 'The 12th International Symposium on Transport Phenomena and Dynamics of Rotating Machiney'. Honolulu, Hawaii.

Heuperman A (1999) Value adding to serial biological concentration for improved environmental management. Institute of Sustainable Irrigated Agriculture Murray Darling Basin Commission project I6061, Tatura, Victoria.

ICBA (2007) Annual Report 2006 (1426-27H). International Center for Biosaline Agriculture, United Arab Emirates.

Jacobs JL, McKenzie FR, Ryan MJ, Kearney G (1999) Effect of rate and time of nitrogen application from autumn to midwinter on perennial ryegrass-white clover dairy pastures in western Victoria. 2. Pasture nutritive value. *Australian Journal of Agricultural Research* **50**, 1067-1072.

Jacobs JL, Ward GN, McKenzie FR, Kearney G (2006) Irrigation and nitrogen fertiliser effects on dry matter yield, water use efficiency and nutritive characteristics of summer forage crops in south-west Victoria. *Australian Journal of Experimental Agriculture* **46**, 1139-1149.

Jefferies RL (1981) Osmotic adjustment and the response of halophytic plants to salinity. *BioScience* **31**, 42-46.

John CD, Limpinuntana V, Greenway H (1977) Interaction of salinity and anaerobiosis in barley and rice. *Journal of Experimental Botany* **28**, 133-141.

Joshi GV, Mishra SD (1970) Photosynthesis and mineral metabolism in senescent leaves of *Clerodendron inerme* Gaertn. *Indian Journal of Experimental Biology* **8**, 41-43.

Jou Y, Wang Y, Yen HE (2007) Vacuolar acidity, protein profile, and crystal composition of epidermal bladder cells of the halophyte *Mesembryanthemum crystallinum* doi:10.1071/FP06269. *Functional Plant Biology* **34**, 353-359

Kemp PR, Cunningham GL (1981) Light, temperature and salinity effects on growth, leaf anatomy and photosynthesis of *Distichlis spicata* (L.) Greene. *Am. J. Bot.* **68**, 507-516.

Khan MA, Duke NC (2001) Halophytes - A resource for the future. *Wetlands Ecology and Management* **6**, 455-456.

Khan MA, Ungar IA, Showalter AM, Dewald HD (1998) NaCl-induced accumulation of glycinebetaine in four subtropical halophytes from Pakistan. *Physiologia Plantarum* **102**, 487-492.

Kilminster T, Burt E, Tipper G (2002) Grazing and feedlotting sheep on saline land. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)' pp. 123-128. (Promaco Conventions Pty. Ltd.: Fremantle - Kojonup - Kataning).

Klute A, Dirkson C (1986) Hydraulic conductivity and diffusivity: Laboratory methods. In 'Methods of soil analysis - Part 1'. (Ed. A Klute) pp. 687-732. (American Society of Agronomy: Madison, USA).

Kobayashi H, Masaoka Y, Takahashi Y, Ide Y, Sato S (2007) Ability of salt glands in Rhodes grass (*Chloris gayana* Kunth) to secrete Na^+ and K^+. *Soil Science & Plant Nutrition* **53**, 764-771.

Lal R, Pimentel D (2007) Biofuels from crop residues. *Soil and Tillage Research* **93**, 237-238.

Lazof D, Lauchli A (1991) The nutritional status of the apical meristem of *Lactuca sativa* as affected by NaCl salinization: An electron-probe microanalytic study. *Planta* **184**, 334-342.

Leake JE, Barrett-Lennard EG, Sargeant MR, Yensen NP, Prefumo J (2002) NyPa *Distichlis* cultivars: Rehabilitation of highly saline areas for forage, turf and grain. Rural Industries Research and Development Corporation Publication number 02/154.

Leakey R, Chancellor R, Vince-Prue D (1977a) Regeneration from rhizome fragments of *Agropyron repens* II. The breaking of 'late spring dormancy' and the influence of chilling and node position on growth from single-node fragments. *Annals of Applied Biology* **87**, 433-441.

Leakey R, Chancellor R, Vince-Prue D (1977b) Regeneration from rhizome fragments of *Agropyron repens.* 1. The seasonality of shoot growth and rhizome reserves in single-node fragments. *Annals of Applied Biology* **87**, 423-431.

Levitt J (1972) 'Responses of Plants to Environmental Stresses.' (Academic Press: New York).

Lieth H, Lohmann M (2000) 'Cashcrop Halophytes for future halophyte growers.' (Institute of Environmental Systems Research, University of Osnabruck).

Liphschitz N, Waisel Y (1982) Adaptations of plants to saline environments: salt excretion and glandular structure. In 'Tasks for Vegetation Science, Contributions to the Ecology of Halophytes'. (Eds DN Sen, KS Rajpurohit) pp. 197-214. (W. Junk Publishers: The Hague).

Ludwig JR, McGinnes WJ (1978) Revegetation Trials on a Saltgrass Meadow. *Journal of Range Management* **31**, 308-311.

Mackay N (1990) 'The Murray.' (Murray Darling Basin Commission: Canberra).

Malcolm CV, Lindley VA, O'Leary JW, Runciman HV, Barrett-Lennard EG (2003) Halophyte and glycophyte salt tolerance at germination and the establishment of halophyte shrubs in saline environments. *Plant Soil* **253**, 171-185.

Marcum K (1999) Salinity tolerance mechanisms of grasses in the subfamily chloridoideae. *Crop Science* **39**, 1153-1160.

Marcum K (2006) Saline Tolerance Physiology in Grasses. In 'Ecophysiology of High Salinity Tolerant Plants'. (Eds. M Ajmal Khan, DJ Weber) pp. 157-172. (Springer: Netherlands).

Mass EV (1993) Plant Growth Response to Salt Stress. In 'Towards the raional use of high salinity tolerant plants'. (Eds H Lieth, A Masoom) pp. 279-291. (Kluwer Academic Publishers: London).

Masters DG, Norman HC, Dynes RA (2001) Opportunities and limitations for animal production from saline land. *Asian-Australian Journal of Animal Science* **14**, 199-211.

Masters DG, Rintoul AJ, Dynes RA, Pearce KL, Norman HC (2005) Feed intake and production in sheep fed diets high in sodium and potassium
doi:10.1071/AR04280. Australian Journal of Agricultural Research **56**, 427-434.

McIntyre G (1970) Studies on bud development in the rhizome of *Agropyron repens.* 1. The influence of temperature, light intensity, and bud position on the pattern of development. *Canadian Journal of Botany* **48**, 1903-1909.

McKenzie FR (1996) The influence of grazing frequency and intensity on the vigour of *Lolium perenne* L. under subtropical conditions. *Australian Journal of Agricultural Research* **47**, 975-983.

McKenzie FR (1997a) Influence of grazing frequency and intensity on the density and persistence of *Lolium perenne* tillers under subtropical conditions. *Tropical Grasslands* **31**, 219-226.

McKenzie FR (1997b) Influence of grazing frequency and intensity on tiller appearance and death rates of *Lolium perenne* L. under subtropical conditions. *Australian Journal of Agricultural Research* **48**, 337-342.

McKenzie FR, Jacobs JL, Kearney G (2002) The long-term impact of nitrogen fertiliser on perennial ryegrass tiller and white clover growing point densities in grazed dairy pastures in south-western Victoria. *Australian Journal of Agricultural Research* **53**, 1203-1209.

McLarty A, I'Anson P (2002) Social Issues of Salinity. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)'. Freemantle - Kojonup - Katanning p. 343. (Promaco Conventions Pty. Ltd.).

McLeary BV, Solah V, Gibson TS (1994) Quantitative measurement of total starch in cereal flours and products. *Journal of Cereal Science* **20**, 51-58.

Milton JTB, Paterson J, Roberts D (2001) Principles of Ruminant Nutrition. In 'The Good Food Guide for Sheep: Feeding sheep for meat production in the agricultural areas of Western Australia'. (Eds K Croker, P Watt) pp. 13-18. (Department of Agriculture: South Perth).

Mozafar A, Goodin JR (1970) Vesiculated Hairs: A Mechanism for Salt Tolerance in Atriplex halimus L. *Plant Physiol.* **45**, 62-65.

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell and Environment* **16**, 15-24.

Munns R (2002) Comparative physiolgy of salt and water stress. *Plant, Cell and Environment* **25**, 239-250.

Munns R, Passioura JB (1984) Effect of prolonged exposure to NaCl on the osmotic pressure of leaf xylem sap from intact, transpiring barley plants. *Australian Journal of Plant Physiology* **11**, 497-507.

Myers BA, Morgan WC (1989) Germination of the salt-toleant grass *Diplachne fusca.* II*. Salinity responses. *Australian Journal of Botany* **3**, 239-251.

National Land and Water Resources Audit (2001) Australian Dryland Salinity Assessment 2000. Extent, impacts, processes, monitoring and management options. Commonwealth of Australia, Canberra.

Nelson W (1996) Container types and containerised stock for New Zealand afforestation. *New Zealand Journal of Forestry Science* **26**, 184-190.

Norman HC, Dynes RA, Masters DG (2002) Nutritive value of plants growing on saline land. In 'Wake Up Australia! Saltland Opportunities:Profit for our Communities and the Environment 8th National Conference on the Productive Use and rehabilitation of Saline Lands'. Freemantle-Kojonup-Katanning p. 343. (Promaco Conventions Pty. Ltd.).

Norman HC, Friend C, Masters DG, Rintoul AJ, Dynes RA, Williams IH (2004) Variation within and between two saltbush species in plant composition and subsequent selection by sheep. *Australian Journal of Agricultural Science* **55,** 999-1007.

O'Connell M, Young JA (2002) The role of saltland pastures in the farming system - A whole-farm bio-economic analysis. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)'. Freemantle - Kojonup - Kattanging p. 343. (Promaco Conventions Pty. Ltd.).

O'Connell M, Young J, Kingwell R (2006) The economic value of saltland pastures in a mixed farming system in Western Australia. *Agricultural Systems* **89,** 371-389.

O'Leary JW, Glenn EP (1994) Global distribution and potential for halophytes. In 'Halophytes as a resource for livestock and for rehabilitation of degraded lands'. (Eds VR Squires, AT Ayoub) pp. 7-17. (Kluwer Academic Publishers).

Oldeman LR, Hakkeling RTA, Sombroek WG (1991) The extent of human-induced soil degradation. In 'World Map of the Status of Human-Induced Soil Degradation: An Explanatory Note'. (Eds LR Oldeman, RTA Hakkeling, WG Sombroek) pp. 27-33. (International Soil Reference and Information Centre (ISRIC): Wageningen).

Oross JW, Thomson WW (1982) The ultrastructure of the salt glands of *Cynodon* and *Distichlis* (Poaceae). *American. Journal of Botany* **69**, 939-949.

Pallaghy CK (1970) Salt relations of *Atriplex* leaves. In 'The Biology of *Atriplex* '. (Ed. R Jones) pp. 57-62. (CSIRO Australia: Canberra).

Passioura JB, Munns R (2000) Rapid enviornmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. *Australian Journal of Plant Physiology* **27**, 941-948.

Pavlicek KA, Johnson GV, Aldon EF (1977) Vegetative propagation of desert saltgrass rhizomes. *Journal of Range Management* **30**, 377-380.

Payne L (2002) Green feed in dry times. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)' pp. 129-131. (Promaco Conventions Pty. Ltd.: Fremenatle - Kojonuo - Katanning).

Pearce KL (2006) Carcass and eating quality of sheep grazing saltbush based saline pasture systems. PhD Thesis, Murdock University.

Pearce KL, Jacob RH, Masters DG, Pethick DW, Power D, White G (2004) Lambs grazed on saltbush are more hydrated at slaughter. In 'Proceedings of the 25th Biennial Conference of the Australian Society of Animal Production'. University of Melbourne, Victoria.

Perfect E, Kay BD, van Loon WKP, Sheard RW, Pojasok T (1990) Factors influencing soil structural stability within a growing season. *Soil Science Society of America* **54**, 173-179.

Polan CE, Starling TM, Huber JT, Miller CN, Sandy RA (1968) Yields, Compositions, and Nutritive Evaluation of Barley Silages at Three Stages of Maturity for Lactating Cows. *J. Dairy Sci.* **51**, 1801-1805.

Pollak G, Waisel Y (1970) Salt Secretion in Aeluropus litoralis (Willd.) Parl. *Annals of Botany* **34**, 879-888.

Pollak G, Waisel Y (1979) Ecophysiology of Salt Excretion in *Aeluropus litoralis* (Graminae). *Physiologia Plantarum* **47**, 177-184.

Potter BJ (1961) The renal response of sheep to prolonged ingestion of sodium chloride. *Australian Journal of Agricultural Research* **12**, 440-445.

Qureshi RH, Barrett-Lennard EG (1998) 'Saline Agriculture for Irrigated Land in Pakistan: a handbook.' (Australian Centre for International Agricultural Research: Canberra).

Radin JW, Eidenbock MP (1984) Hydraulic Conductance as a Factor Limiting Leaf Expansion of Phosphorus-Deficient Cotton Plants. *Plant Physiol.* **75**, 372-377.

Ramadan T (1998) Ecophysiology of salt excretion in the xero-halophyte *Reaumuria hirtella*. *New Phytologist* **139**, 273-281.

Raven JA (1985) Regulation of pH and generation of osmolarity in vascular plants: a cost benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytologist* **101**, 25-77.

Rengasamy P, Olsson KA (1993) Irrigation and Sodicity. *Australian Journal of Soil Research* **31**, 821-837.

Rhodes D, Hanson AD (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 357-384.

Robinson MF, Very A, Sanders D, Mansfield TA (1997) How Can Stomata Contribute to Salt Tolerance? *Annals of Botany* **80**, 387-393.

Rodriguez D, Keltjens WG, Goudriann J (1998) Plant leaf area expansion and assimilate production in wheat (*Triticum aestivum* L.) growing under low phosphorus conditions. *Plant and Soil* **200**, 227-240.

Rozema J, Gude H, Pollak G (1981) An ecophysiological study of the salt secretion of four halophytes. *New Phytologist* **89**, 201-217.

Russ P (1995) 'The Salt Traders: A history of salinity in Victoria.' (The Department of the Premier and Cabinet, State of Victoria: Melbourne).

Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant, Cell and Environment* **25**, 163-171.

Sargeant M (2003) Establishment and Comparitive Performance of *Distichlis spicata* var. yensen-4a grown in saline soil. M Agr Sci, Thesis University of Melbourne.

Sargeant MR (1999) The effect of salinity and temperature on the growth and nutritional value of *Distichlis spicata* var. yensen-4a. B. Agr. Sc. Thesis, La Trobe University.

Satorre E, Rizzo F, Arias S (1996) The effect of temperature on sprouting and early establishment of *Cynodon dactylon*. *Weed Research* **36**, 431-440.

Schirmer U, Breckle S (1982) The role of bladders for salt removal in some Chenopodiaceae (mainly *Atriplex* species). In 'Tasks for Vegetative Science 2: Contributions to the Ecology of Halophytes'. (Eds DN Sen, KS Rajpurohit) pp. 215-231. (Dr W. Junk Publishers: The Hague).

Scholander PF, Hammel HT, Hemmingsen E, Garey W (1962) Salt Balance in Mangroves. *Plant Physiology* **37**, 722-729.

Semple WS, Koen TB (2004) Characteristics of saline seepage scalds: what to measure and when? In 'Proceedings of the Conference "Salinity Solutions: Working with Science and Society'. Bendigo, Victoria. (Eds A Ridley, P Feikema, S Bennet, MJ Rogers, R Wilkinson, J Hirth). (CRC for Plant-Based Management of Dryland Salinity: Perth).

Shannon MC, Grieve CM, Wilson C, Poss J, Suarez DL, Lesch S, Rhoades JD (1998) Growth and water relations of plant species suitable for saline drainage water reuse sytems. U.S. Salinity Laboratory, Agricultural Research Service, USDADWR B-59922, Riversdie, California.

Sharma ML (1982) Aspects of salinity and water relations of Australian chenopods. In 'Tasks for Vegetative Science 2: Contributuons to the Ecology of Halophtes'. (Eds DN Sen, KS Rajpurohit) pp. 155-172. (Dr W. Junk Publishers The Hague).

Singh DK (1998) Phosphorus supply and the tolerance of frequently defoliated white clover (*Trifolium repens* L.) to water deficit stress. PhD Thesis, La Trobe University.

Singh DK, Sale PWG (1997a) Defoliation frequency and the response by white clover to increasing phosphorus supply 1. Leaf dry matter yield and plant morphology responses. *Australian Journal of Agricultural Research* **48**, 111-118.

Singh DK, Sale PWG (1997b) Defoliation frequency and the response by white clover to increasing phosphorus supply 2. Non-structural carbohydrate concentrations in plant parts. *Australian Journal of Agricultural Research* **48**, 119-124.

Singh DK, Sale PWG (2000) Growth and Potential Conductivity of White Clover Roots in Dry Soil with Increasing Phosphorus Supply and Defoliation Frequency. *Agron J* **92**, 868-874.

Singh DK, Sale PWG, Gourley CJP, Hasthorpe C (1999) High phosphorus supply increases persistence and growth of white clover in grazed dairy pastures during dry summer conditions. *Australian Journal of Experimental Agriculture* **39**, 579-585.

Sivritepe HO, Eris A, Sivritepe N (1999) The effects of priming treatments on salt tolerance in melon seeds. *Acta Hort.* **492**, 287-295.

Sivritepe HO, Sivritepe N, Eris A, Turhan E (2005) The effects of NaCl pre-treatments on salt tolerance of melons grown under long-term salinity. *Sci. Hort-Amsterdam* **106**, 568-581.

Sivritepe N, Sivritepe HO, Eris A (2003) The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Sci. Hort-Amsterdam* **97**, 229-237.

Smart RM, Barko JW (1980) Nitrogen nutrition and salinity tolerance of *Distichlis spicata* and *Spartina alterniflora*. *Ecology* **61**, 630-638.

Smith D (1968) Classification of Several Native North American Grasses as Starch or Fructans Accumulators in Relation to Taxonomy. *Grass and Forage Science* **23**, 306-309.

Stewart GR, Lee JA (1974) The role of proline accumulation in halophytes. *Planta* **120**, 279-289.

Stoll P, Egli P, Schmid B (1998) Plant foraging and rhizome growth patterns of *Solidago altissima* in response to mowing and fertilizer application. *Journal of Ecology* **86**, 341-354.

Storey R, Ahmad N, Wyn Jones RG (1977) Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants. *Oecologia* **27**, 319-332.

Storey R, Wyn Jones RG (1977) Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochemistry* **16**, 447-453.

Sullivan MJ (1981) Effects of canopy removal and nitrogen enrichment on a *Distichlis spicata* - edaphic diatom complex. *Estuarine, Coastal and Shelf Science* **13**, 119-129.

Tanji KK (1990) 'Agricultural Salinity Assessment and Management.' (American Society of Civil Engineers: New York).

Terry RA, Tilley JMA (1964) The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an *in vitro* procedure. *Grass and Forage Science* **19**, 363-372.

Thomas DT, White CL, Hardy J, Collins J, Ryder A, Norman HC (2009) An on-farm evaluation of the capability of saline land for livestock production in southern Australia. *Animal Production Science* **49**, 79-83.

Tiong MK, Masters DG, Norman HC, Milton JTB, Rintoul AR (2004) Variation in nutritive value between four halophytic shrub species collected from five saline environments. In 'Proceedings of the 25th Biennial Conference of the Australian Society of Animal Production'. University of Merlbourne.

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. *Journal of Soil Science* **33**, 141-163.

Tobe K, Li X, Omasa K (2000) Seed Germination and Radicle Growth of a Halophyte, Kalidium caspicum(Chenopodiaceae). *Ann Bot* **85**, 391-396.

Umezawa T, Shimizu K, Kato M, Ueda T (2000) Enhancement of salt tolerance in soybean with NaCl pretreatment. *Physiol. Plant.* **110**, 59-63.

Waisel Y (1972) 'Biology of halophytes.' (Academic Press: New York).

Wales WJ, Doyle PT, Stockdale CR, Dellow DW (1999) Effects of variations in herbage mass, allowance, and level of supplement on nutrient intake and milk production of dairy cows in spring and summer. *Australian Journal of Experimental Agriculture* **39**, 119-130.

Walsh I, Powell J, Lloyd M, Munday B, Brewin D (2002) Saltland revegetation - production and diversity farmer case study. In '8th National Conference and Workshop on the Productive Use and Rehabilitation of Saline Lands (PUR$L)' pp. 133-138. (Promaco Conventions Pty. Ltd.: Fremantle - Kojonup - Katanning).

Wang L, Showalter AM, Ungar IA (1997) Effect of salinity on growth, ion content, and cell wall chemistry in *Atriplex prostrata* (Chenopodiaceae). *Am. J. Bot.* **84**, 1247-1255.

White LM (1973) Carbohydrate Reserves of Grasses: A Review. *Journal of Rangeland Management* **26**, 13-18.

Wood WE (1924) Increase of salt in soil and streams following the destruction of the native vegetation. *Journal and Proceedings of the Royal Society of Western Australia* **10**, 35-47.

Yensen NP (1997) The agronomic production and nutritional characteristics of NyPa Forage, *Distichlis spicata* var. yensen-4a, (Poaceae) when grown on highly salinewater in arid regions of the world with respect to economic worth. In 'International Symposium on Sustainable Management of Salt Soils in Arid Ecosystems'. Cairo, Egypt. (Ed. AM Elgala) p. 363.

Yensen NP (1998) 'Halophytes of Latin America and the World: Their Use with Saline & Waste Waters and Marginal Soils.' (Tucson, Arizona).

Yensen NP (1999a) 'Salt-tolerant plants of Southern Africa and their uses.' (NyPa Interantional: Tucson, Arizona).

Yensen NP (1999b) 'Salt Tolerant Plants and their Uses.' (Tucson, Arizona).

Yensen NP (2002) New developments in the world of saline agriculture. In 'Prospects for saline agriculture' pp. 321-332. (Kluwer Academic Publishers: Netherlands).

Yensen NP (2004) The Success of NyPa International as a Saline Agriculture Outlet: Origin, Development and Philosophy. In 'Prospects of Saline Agriculture in the Arabian Peninsula'. (Eds FK Taha, S Ismail, A Jaradat). (Amherst Scientific Publishers).

Yensen NP, Bedell JL (1993) Considerations for the selection, adaptation, and application of halophyte crops to highly saline environments as exemplified by the long-term development of cereal and forage cultivars of *Distichlis* spp. (Poaceae). In 'Towards the rational use of high salinity tolerant plants'. (Eds H Leith, AA Masoom) pp. 305-313. (Kluwer Academic Publishers: Netherlands).

Yensen NP, Bedell JL, Yensen SB (1995) Domestication of *Distichlis* as a grain and forage. In 'Biology of Salt Tolerant Plants'. (Ed. MAKIA Ungar). (Department of Botany, University of Karachi, Pakistan: Karachi, Pakastan).

Yensen NP, Hinchman RR, Negri MC, Mollock GN, Settle T, Keiffer CS, Carty DJ, Martin R, Erickson R (1999) Using Halophytes to Manage Oilfield Saltwater: Disposal by Irrigation/Evapotranspiration and Remediation of Spills. In 'Sixth International Petroleum Environmental Conference: Environmental Issues and Solutions in Petroleum Exploration, Production and Refining'. Houston, USA.

Yensen NP, Yensen SB, Weber CW (1985) A Review of *Distichlis* spp. for Production and Nutritional VAlues. In 'Arid Lands: Today and Tomorrow. Proceedings of an International Research and Development Conference'. Tucson, Arizona. (Eds E Whitehead, C Hutchinson, B Timmermany, R Varady). (Westview Press, Boulder).

Yensen SB (1995) Characterization of the proteins and flour of *Distichlis palmeri* (Vasey) grain and *Distichlis* spp. fiber. The University of Arizona.

Zimmermann U (1978) Physics of turgor and osmoregulation. *Annual Review of Plant Physiology* **29**, 121-148.