SALT AVOIDANCE AND TOLERANCE IN BEACH PEA (Lathyrus maritimus (L.) BIGELOW): A STUDY OF THE EFFECTS OF SALINITY ON GERMINATION, GROWTH, PHOTOSYNTHESIS, AND RESPIRATION IN COMPARISON TO COMMON FORAGE LEGUMES

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Salt avoidance and tolerance in beach pea (*Lathyrus maritimus* (L.) Bigelow):

A study of the effects of salinity on germination, growth, photosynthesis, and respiration in comparison to common forage legumes.

by

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Abstract

Beach pea (*Lathyrus maritimus* (L.) Bigelow) is a lush green legume which grows along the shores of the island of Newfoundland. It is a circumpolar plant found on both sea shores and the shores of freshwater lakes. Beach pea is a fleshy perennial with an extensive rhizome system. The roots form nodules that contain bacteria (*Rhizobium* sp.) which provide fixed nitrogen to the legume for incorporation into proteins of the host plant. Beach pea form large persistent stands on beaches and has been studied for its potential as an alternate forage crop. The plants that grow on salt water beaches are exposed to salt water spray inferring some level of salt tolerance in the plant. The objectives of the thesis were to compare salinity effects on beach pea germination relative to other forage crops and to study salinity effects on growth.

Slightly increased salt concentration delayed beach pea germination more so than that of alfalfa and red clover, contrary to the initial hypothesis that beach pea germination would show higher tolerance for saline environments. Red clover and alfalfa were affected only at salt concentrations of 1.75 and 2.0 percent whereas beach pea germination was affected at 0.50 percent salinity. Final germination percentage relative to germinating salinity followed a sigmoidal pattern in all crops, dropping rapidly around 1.0 percent NaCl. Growth in saline environment effectively stopped the growth of beach pea by limiting stem elongation, new stem initiation, and new leaf initiation. Alfalfa mortality was observed as a result of the saline environment with a 50 percent mortality in the 2 percent saline environment 10 weeks

after planting and 100 percent at 15 weeks. The 1 percent saline environment also increased mortality in the alfalfa to 60 percent by week 16. Control plants showed no mortality over the 16 week experiment.

The delay of germination and inhibition of new growth seen in the beach pea did not support the hypothesis of the experiment. Salinity measurements of the natural beach environment showed low soil salinity level relative to salinity in the tide line. At the high tide line salinity was 20 times higher than the salinity in the beach pea stands. The experiments show that the environment in which beach pea grows is not saline. Salinity delays germination but the plants, once established, are salt tolerant. In a natural stand of beach pea exposure to a saline environment would be temporary as fresh water from rain or seeps would remove the salt before any accumulation and thus plant damage could occur.

Acknowledgements

I would first like to thank my supervisors Dr. Roger Lee and Dr. David McKenzie for all their help and patience while I completed this thesis. Thanks should also be given to the technicians at Agriculture and Agri-food Canada's Atlantic Cool Climate Crop Research Centre, namely Todd Power, Darryl Martin, Tony Mulrooney, and Leanne Conroy for their assistance in my experiments. Thanks also to Frank Ralph and Jim Flynn for the set up of the greenhouse environment and for their patience in the many lighting changes before I was happy. Thanks also to Dr. Dean Spaner for advice on the statistics and interpretation of the numbers.

Most importantly thanks to my wife Tracy Reid for patience and understanding during the past three years. The worst is almost over.

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1. Introduction

Agriculture crops used as livestock feed other than separated grain are referred to as forages along with most fields containing grass and/or legumes. Forage legumes and grain supply most of the plant protein in the feed of ruminant livestock. The high protein content of legumes is related to the fact that legumes form a symbiotic relationship with the soil bacteria *Rhizobium*. The bacteria fix atmospheric nitrogen into ammonia and ammonium usable by the plant. The ammonia and ammonium are incorporated into amino acids which are then polymerized into proteins. (Somasegaran and Hoben, 1994)

Lathyrus maritimus (L.) Bigelow, commonly known as beach pea, is a lush green legume which grows along the shores of Newfoundland and has been suggested as a potential forage crop (McKenzie *et al.*, 1997). There have been difficulties in moving the plant from the beach to the field (McKenzie, personal communication). Beach pea has been the object of study for several years at the Atlantic Cool Climate Crop Research Centre in St. John's, Newfoundland. Preliminary studies show that the plant has promise as a crop (McKenzie *et al.*, 1997) either for cattle feed or as a food source for humans. This research was undertaken in an attempt to have better understanding of the plant, by defining some of the growth parameters.

1.1 Beach Pea

Beach pea is a circumpolar plant found on shorelines in the arctic, sub-arctic and temperate regions. It is geographically variable with varieties found on the shorelines of freshwater lakes including the Great Lakes. Its distribution extends to Greenland as well as the Alaskan coasts in the west as far south as California. (Fernald, 1950)

Beach pea is a member of the Leguminosae family of flowering plants. The genus *Lathyrus* has 10 species and are commonly called the vetchling or wild pea genus. The beach pea (*Lathyrus maritimus*) is a fleshy perennial plant which has an extensive rhizome system. The stems are up to 1.5 m in length and stiffly branching. The plants form large mats using their long stems to intertwine with themselves and tendrils at the apex of their branched leaves for gripping neighbouring plants. The flowers are purple to blueish in arched peduncles consisting of 3 to 10 flowers. (Fernald, 1950)

The fruit is a fibrous legume, up to 3 cm in length (Fernald, 1950). The peas change from a light green to brown as they mature (Chavan *et al.*, 1999). At maturity the testa of the seed is very hard and seeds remain dormant, as a consequence of not being able to imbibe water. The seeds must be scarified to enable imbibition and thus germination (Gurusamy *et al.*, 1999). In the natural environment movement of small stones and sand found on the beaches, due to splashing of waves during storms, would provide the necessary abrasion to allow for the imbibition of beach pea seed.

Recent research has shown the seeds to be high in soluble protein, soluble sugars and phenolic acids prior to maturity, with levels decreasing as the seed matured. (Chavan *et al.*, 1999). Minerals such as potassium, sodium, calcium, and phosphorus were also high prior to maturity and decreased as the seed matured. Iron levels in the seed were highest at maturation as compared to earlier stages of growth (Chavan *et al.*, 1999).

The use of beach pea as a feed crop for cattle also shows promise (McKenzie *et al.*, 1997). Some farmers in rural areas of Newfoundland have used beach pea stands to supplement the diet of their livestock (McKenzie, personal communication). The plant forms large stands on the shore line which will persist for many years, showing resistance to frost and drought in the wild. The symbiotic relationship formed with the nitrogen fixing bacteria also makes it attractive as a forage crop. The nodules formed by the bacterium are indeterminate and have been shown to fix nitrogen at relatively low temperatures (Bal and Barimah-Asare, 1993).

1.2 Salinity

Beach pea grows on ocean side beaches where one can expect the plant to be exposed to sea water spray. The initial assumptions which prompted this and other investigations of beach pea were that one would expect a level of salt tolerance to be present in the beach pea in order to germinate, survive, and thrive in such an environment.

Inorganic salts are in the soil and water table in some concentration at all times. Many are necessary as nutrients for plants growing in these environments. A salinity problem occurs when the concentration of sodium salts or magnesium salts (chloride, sulphate, and carbonate) is too high. The exact level of salinity where problems occur depends on the crop being grown. Some plants can tolerate very high levels of salinity (halophytes) while others cannot (glycophytes) (Chapman, 1975). An excess of salt in the soil or water table can cause a variety of stresses on the plant.

The ions in the soil cause a decrease in the water potential of the soil making it harder for the plants to take up water. The process is slowed and can cause problems for the plant if water requirements cannot be met. Eventually the plants will have taken up enough of the ions in the soil solution to balance out the water potential gradient. An excess of ions in the tissue however are not good for the plant. Membrane damage can occur in some areas, and nutrient balances can be affected. The main effects of internal damage can be seen as colour changes in the leaves, tip-burn, or marginal necrosis. (Volkmar, *et al.*, 1998)

Colour changes in the leaves often occur as a yellowing, caused by loss of chlorophyll content in the leaves, and can be quantified, non-destructively, using a handheld Minolta chlorophyll meter (model SPAD 502) (Peterson *et al.*, 1993). The chlorophyll meter has been used to help assess nitrogen efficiency in several crops; for example: timothy (Virtanen and Peltonen, 1996), rice (Peng *et al.*, 1996), corn (Piekielek *et al.*, 1995), and turnip tops (Spaner and Lee, 2001). Using the handheld chlorophyll metre to study nitrogen use allows a realistic view of nitrogen requirements as a plant will not produce excess chlorophyll, even in the presence of excess nitrogen (Peterson *et al.*, 1993).

Salt tolerant plants are called halophytes which may have special structures, in the leaves, to aid in the removal of excess salts. These structures have either an active or passive method of emitting water and solutes on to the surface. Passive structures are directly connected to vascular system and act as a filter, using the vascular pressure to exude the salts. Active structures utilise cellular energy to pump the solutes out of the leaf. Both the passive and active structures, which include the salt gland take on a variety of forms from sunken into the leaf, to protruding above the leaf like hairs or trichomes. (Thomson, 1976)

Salt tolerant plants lacking a gland to excrete excess salt from the leaves are not easily distinguished from salt sensitive plants (Yeo, 1998). Resistence to salinity by a plant lies in it's ability to exclude salt from the cytoplasm and maintain a favourable osmotic gradient within the plant tissue (Yeo, 1998). This does not mean that salt exclusion from the entire plant is necessary for salt tolerance. Some salt sensitive varieties of corn have been found with lower shoot Na^+ concentration, implying a better exclusion method, than more salt tolerant species (Alberico and Cramer, 1993).

There are three main mechanisms of acquiring salt tolerance. The first is exclusion from the vital areas of the plants. Leaf exclusion has been linked with salt tolerance however shoot exclusion may not be occurring (Kumar, 1984). The second method is osmotic adjustment where the plant will produce compounds with high solubility, low polar charge, and a large hydration shell, to balance any osmotic effects the salts are having on the cell.

The compounds, often simple sugar alcohols and heat shock proteins, are energetically expensive and tie up carbon which could be used elsewhere by the plant. The plant may use an inorganic source from the substrate for it's osmotic adjustment but there is a risk of ion toxicity and damage to the metabolic processes (Volkmar *et al.*, 1998). The third method is through Na^+/K^+ discrimination. The two ions are thought to use similar pathways through the plasma membrane and exclusion of Na+ from plant cells can be a form of tolerance (McKimmie and Dobrenz, 1991). This discrimination is not required for salt tolerance as some salt tolerant species cannot discriminate between the two ions. The tolerance in those cases is often due to increased efficiency of one or both of the other methods (Volkmar *et al.*, 1998).

Excess salt concentrations in the water table can also have deleterious effects on the soil itself which would have an impact on the plants growing there. Particles of clay in the soil can swell and decrease the macroprosity of the soil limiting the amount of water which can flow through the soil, decreasing aeration and slowing the leeching process (Quirk, 1971). This means that the soils, once affected, will cause an increase in the salt levels by slowing the processes which can remove the salts. Van Hoorn *et al.* (1993) found an increase in sodium ions displacing calcium ions and magnesium ions in the water table of soil irrigated with salt water over a three year study.

For the farmer, irrigation over time can cause an increase in the salinity of the soil. In arid regions, where irrigation is common, a farmer must minimize the cost of the water while still getting a high yield from the crops, and often water must be conserved in spite of damage to the soil (Gardner, 1985). The small amount of dissolved salts found in all irrigation water combined with a high evaporation rate, low soil permeability, poor drainage, or low rainfall amounts can lead to salt build up in the soil, causing salinity and crop problems (Yaron, 1981).

1.3 Objectives

The fact that beach pea grows on ocean beaches lead logically to the objectives of this thesis. The first objective was to compare salinity effects on beach pea germination relative to other forage crops hypothesizing that adaptation to a sea beach environment would favour the ability to germinate under high salinity conditions. The second objective was to study the salinity effects on growth of beach pea in comparison to alfalfa hypothesizing that normal growth of beach pea would not be inhibited by exposure to a saline environment and that alfalfa growth would be adversely affected.

2. Materials and Methods

All experiments took place at the Atlantic Cool Climate Crop Research Center, in St. John's, Newfoundland. Beach pea seed was collected from a natural stand of beach pea on Bellevue Beach, Newfoundland, in the fall of 1997 and were scarified prior to use. Beach pea is a wild species and there is limited information on the amount of variation inherent in the species. In a effort to reduce the amount of variation in the experiment the seeds used came from one collection and one source.

A Forsberg mechanical scarifier (Forsberg Inc. Thief River Falls, Minn. USA), consisting of an electric motor connected to four metal bars located within a sandpaper lined metal drum, was used for all scarification. Seeds were placed in the drum and spun for five seconds at 1725 rpm. This was sufficient time to produce visible scratches in the seed coat to the depth of the endosperm. Experiments were designed to study salt effects on germination and seedling growth.

NaCl was used in the experiments as the primary salt because it is the most abundant salt found in the ocean. In a sample of sea water with 35 ppt salinity, the concentration of chloride ions was 19.353 ppt and the concentration of sodium ions was 10.760 ppt (Jakš, 1994). Given the proximity of the ocean to natural beach pea stands it was thought that NaCl would be the best salt to use.

2.1 Germination

The methods used in this experiment were modeled after Allen *et al.* (1985). Three species of seeds; beach pea (*Lathyrus maritimus* (L.) Bigelow), Apica alfalfa (*Medicago sativa* L.), and Marino red clover (*Trifolium pratense* L.) were germinated in saline solution at concentrations of 0%, 0.25%, 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% NaCl by weight of water. Two petri dishes, size 100 x 15 mm, of 50 seeds each were used at the 9 saline concentrations. The seeds were placed between two filter papers, saturated with the appropriate solution in a petri dish. Petri dishes were then wrapped with Para-film to prevent loss of water and placed in the dark at room temperature for the duration of the experiment. The number of seeds germinated were recorded each day for 14 days. Germinated seeds were removed from the dishes to minimize competition within the dish. This experiment was replicated a month after completion.

2.2 Growth Study

Natural growth parameters were assessed in seedlings of beach pea germinated, grown, and observed for 12 weeks. Seeds of beach pea were inoculated with *Rhizobia* broth culture prior to planting. The *Rhizobia* was originally isolated from the root nodules of a plant growing on Bellevue Beach and cultured at the Atlantic Cool Climate Crop Research Center (Martin *et al.*, 1999). Seeds were planted in Promix potting mixture in Styroblock planting trays containing 45 cells per tray. Trays were planted with 4 seeds per cell and thinned to one plant per cell one week after emergence. Trays were watered as necessary with tap water. Ten plants were sampled each week for 12 weeks.

2.3 Growth in Saline Environment

Styroblock planting trays containing 45 planting cells each were filled with a Number 2 coarse sand. The drainage hole at the bottom of each cell was filled with a small square cut from a chemical spill pillow. This allowed the nutrient solution to pass through while holding the sand in place. The trays were placed on a metal rack in a green house at the Atlantic Cool Climate Crop Research Center with day temperature of 20°C and a night temperature of 10°C. The day length was set to 14 hours with supplemental lighting supplied by six 400 W sodium lights 1.3m above and angled toward the 6 trays providing a mean par of 470.3 $\mu mol \cdot s^{-1} \cdot m^{-2}$. The experiment compared Apica alfalfa and beach pea using three salinity treatments of 0%, 1%, and 2% NaCl by weight of water. Red clover was not included in these experiments due to limited greenhouse space available at the time of the trials.

Several seeds were planted per cell, the sand was wetted with tap water and covered with wet spill pillows until the seedlings emerged. One week after planting a regular watering schedule was started. The trays were watered to field capacity every Monday, Wednesday, and Friday with 2 L of full strength Hoaglands solution (Downs and Hellmers, 1975). When there was stem elongation in the alfalfa (week 6 after planting), the trays were thinned to one plant per cell. Measurements were started on week 8 and were taken on ten randomly selected plants in each tray. The measurements were height, number of leaves, number of stems, leaflet length and width, and chlorophyll content. Chlorophyll content was recorded with a non-destructive field chlorophyll meter SPAD 502 (Minolta, Japan).

Salt treatments, made by adding the appropriate weight of NaCl to the Hoagland solution prior to watering each tray, were also started on week 8 following the measurement recording. All measurements were taken on Fridays prior to that days watering. Size and chlorophyll content were recorded for the second fully expanded leaf from the apical meristem.

Photosynthesis and respiration readings were taken using the three beach pea trays. Photosynthesis and respiration rates were not measured on the alfalfa plants because of high mortality during the experiment. Three plants per tray were selected. The tray cells were covered over with Plastic wrap sealing off the underground portion of the plant.

A photosynthesis chamber was placed over the plant and sealed to the plastic wrap with putty (Figure 2.1). The chamber was made of clear plastic and was outfitted with two hoses to allow the cyclical flow of air through a loop and the infra-red gas analyzer (EGA Series Carbon Dioxide Analyzer, Analytical Development Company Limited). The chamber also contained a LiCor 190sb Quantum Sensor and a Campbell Scientific Temperature Probe (Model 107). The hoses and wire leads were all sealed with putty at their respective chamber exit points. Data from the infra-red gas analyzer (IRGA) and the quantum sensor were sent to one Campbell Scientific CR500 Data Logger with the temperature probe attached to a second Campbell Scientific CR500 Data Logger. Data were recorded every second from the IRGA and LiCor sensors with minute averages of temperature recorded (Figure 2.2).

The tray was placed between two 400 W sodium lamps. The chamber was covered at the start of the experiment and the respiration was recorded for 5.0 minutes. Lights were positioned 60 cm away from the chamber with a mean par of 363.6 $\mu mol \cdot s^{-1} \cdot m^{-2}$. The cover was removed and the chamber was exposed to the light for 5.0 minutes and the cycle was repeated.

Upon the completion of all the experiments the sand from each tray was sampled and sent to the Newfoundland and Labrador Department of Forest Resources and Agri-foods Soil, Plant and Feed Laboratory for soluble salts analysis. To relate the salinity experiments to the natural environment, soil samples from both wild beach pea stands and from the high tide line on Bellevue Beach (N 47° 38' 10" ; W 53° 46' 26") and Salmon Cove Sands (N 47° 46' 54" ; W 53° 09' 22") were also sent for soluble salts analysis.

Figure 2.1: Plant isolation chamber setup used for all carbon dioxide measurements. Below ground portions of the plant were isolated using plastic wrap and putty. Air moved from the outflow hose (A) to the intake hose (B). The light sensor (C) and the temperature sensor (D) were secured to the side of the chamber.

Figure 2.2: Data collection equipment for all carbon dioxide measurements. One datalogger collected data from the IRGA and light sensor (A) and the other collected data from the temperature sensor. Data collection was monitored via the led display of the IRGA (C) and by real time graphing on a laptop computer (D).


A
B
C
D

3. Results

3.1 Germination

Two aspects of the affects of salinity on germination were studied, 1) percent germination over the two week experiment and 2) final germination percentage. Non-linear regressions were performed on the data using Sigmaplot 5.0 (SPSS Inc. 1999). The results of the regressions and the equations used are presented in the appendix. The effect of salt exposure on germination percentage on a day by day basis can be seen for each crop tested. Beach pea show a delay in time taken to reach the final germination percentage percent with no effect on the final germination percentage obtained. At salt concentrations below 1.0 percent the final germination percentage ranged from 69 percent to 63 percent (Figure 3.1). At 1.0 percent salinity the rate and final germination percentage at two weeks was effected. Germination appeared to be in the exponential phase and the experiment may have been terminated too early with the final germination percentage recorded as 60 percent (Figure 3.1). More prominent effects seen at 1.5 percent with germination just starting at the very end of the experiment with the final germination percentage recorded as 6 percent (Figure 3.1).

The maximum germination percentage reached was decreased by increased salt concentration in alfalfa with no effect on the time taken to reach that percentage at salt concentrations below 1.5 percent (Figure 3.2). At 1.5 percent salinity a delay in time taken to reach the final germination percentage can be seen as well as a depression in the maximum germination percentage from 91 percent germination at 0.0 percent salinity to 38 percent germination at 1.5 percent salinity (Figure 3.2).

Salinity had little effect on the time taken to reach the maximum germination percentage in red clover and at salt concentrations below 1.0 percent there was no significant difference in the maximum achieved (Figure 3.3). As salinity increased to 1.0 percent the maximum germination percentage reached was decreased from 65 percent to 31 percent with a further decrease to 6 percent germination seen at 1.5 percent salinity (Figure 3.3).

Final percent germination of all species was affected by salt and followed a sigmoidal pattern with beach pea showing a rapid drop in percentage at 1.0 percent NaCl (Figure 3.4). Alfalfa was most tolerant of salt in the germination phase but had a steep decline at NaCl concentrations of 1.25 to 1.75 percent (Figure 3.4). The red clover had a more gradual decline which occurred at 1.0 percent NaCl (Figure 3.4). Under optimal conditions of the control with no salt, beach pea was slower to germinate than either alfalfa or clover even though the final germination percentage was comparable to the alfalfa (Figure 3.5).

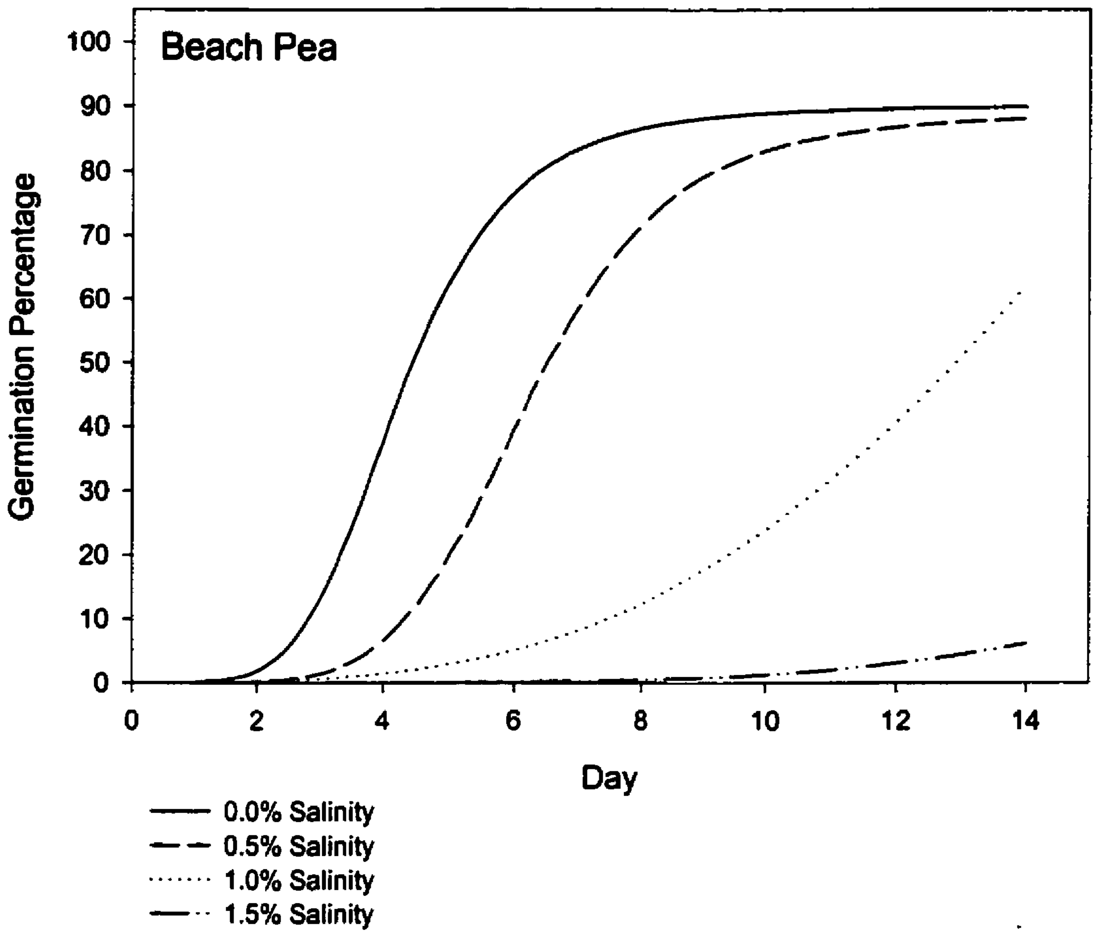


Figure 3.1: Beach pea germination percentage over the 14 days of the experiment. 0.0% salinity is represented by a solid line, 0.5% salinity is represented by a dashed line, 1.0% is represented by a dotted line, and 1.5% is represented by a dash-dot line. The 2.0% salinity remained too close to zero to be represented graphically.

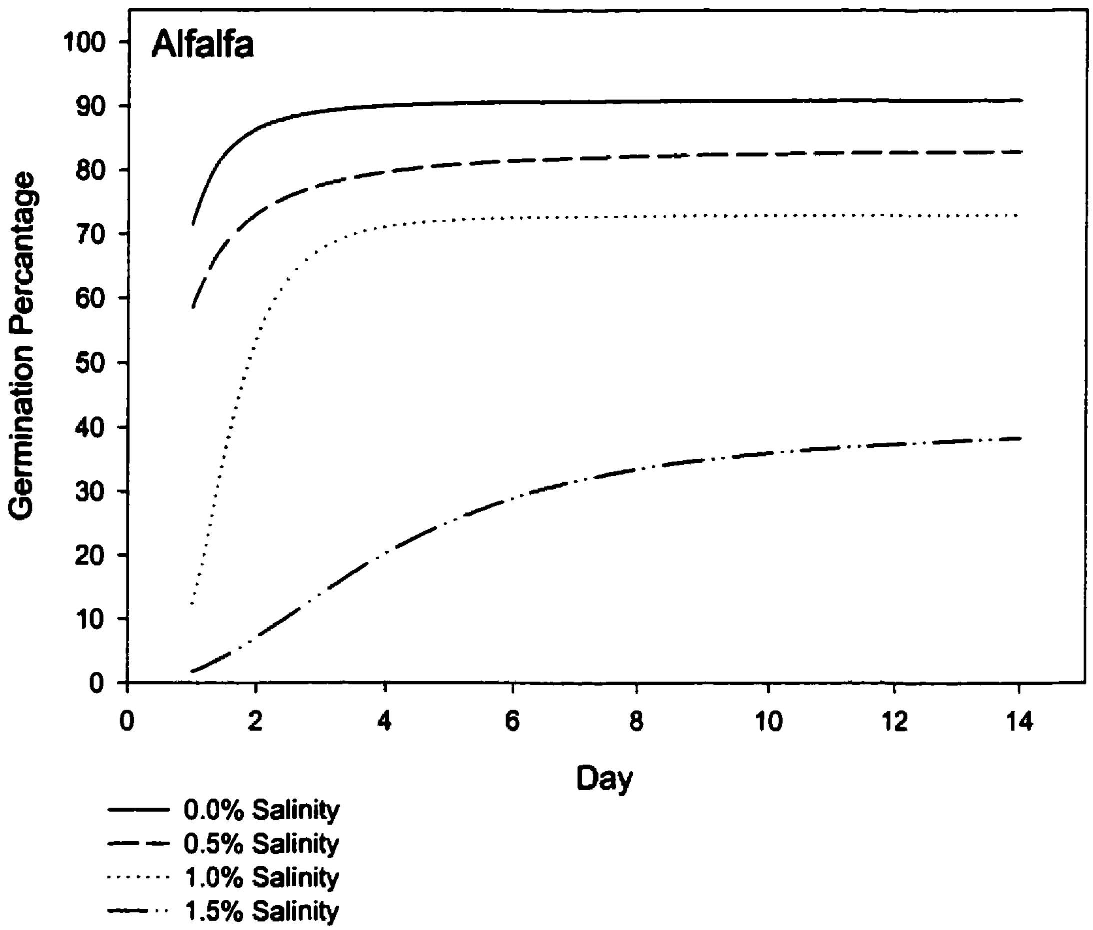


Figure 3.2: Alfalfa germination percentage over the 14 days of the experiment. 0.0% salinity is represented by a solid line, 0.5% salinity is represented by a dashed line, 1.0% is represented by a dotted line and 1.5% is represented by a dash-dot line. The 2.0% salinity remained too close to zero to show a graphed line.

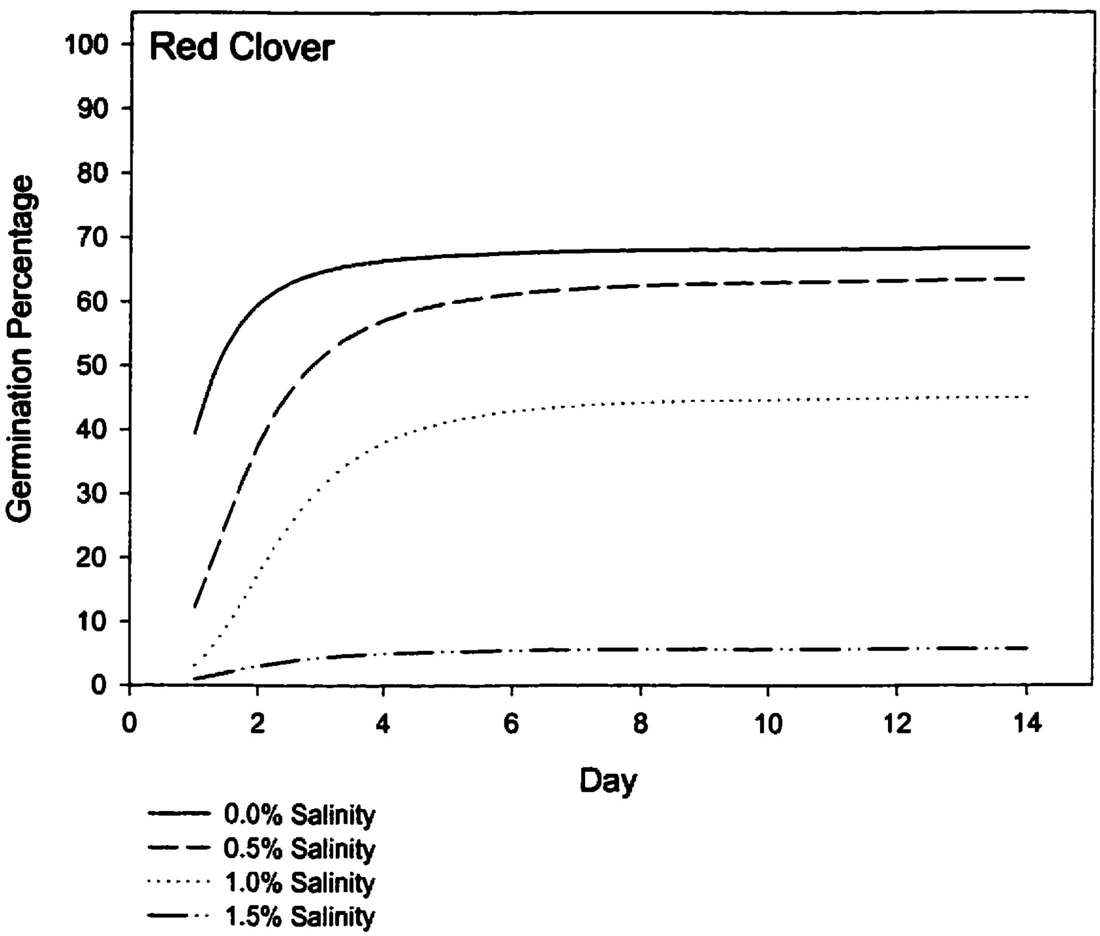


Figure 3.3: Red clover germination percentage over the 14 days of the experiment. 0.0% salinity is represented by a solid line, 0.5% salinity is represented a dashed line, 1.0% is represented by a dotted line and 1.5% is represented by a dash-dot line. The 2.0% salinity remained too close to zero to be represented graphically.

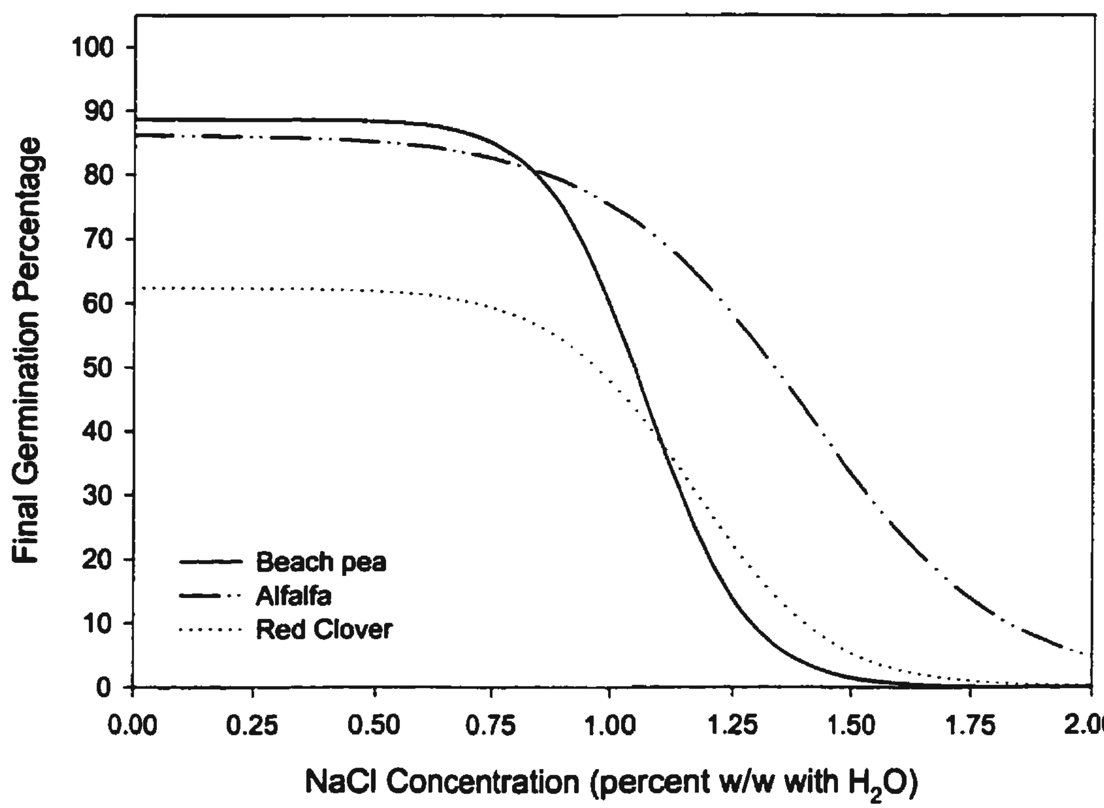


Figure 3.4: Final germination percentages for beach pea, alfalfa and red clover at each salinity concentration. Beach pea is represented by a solid line, alfalfa by a dash-dotted line, and red clover by a dotted line. Concentration is given in percent NaCl determined weight per weight with water.

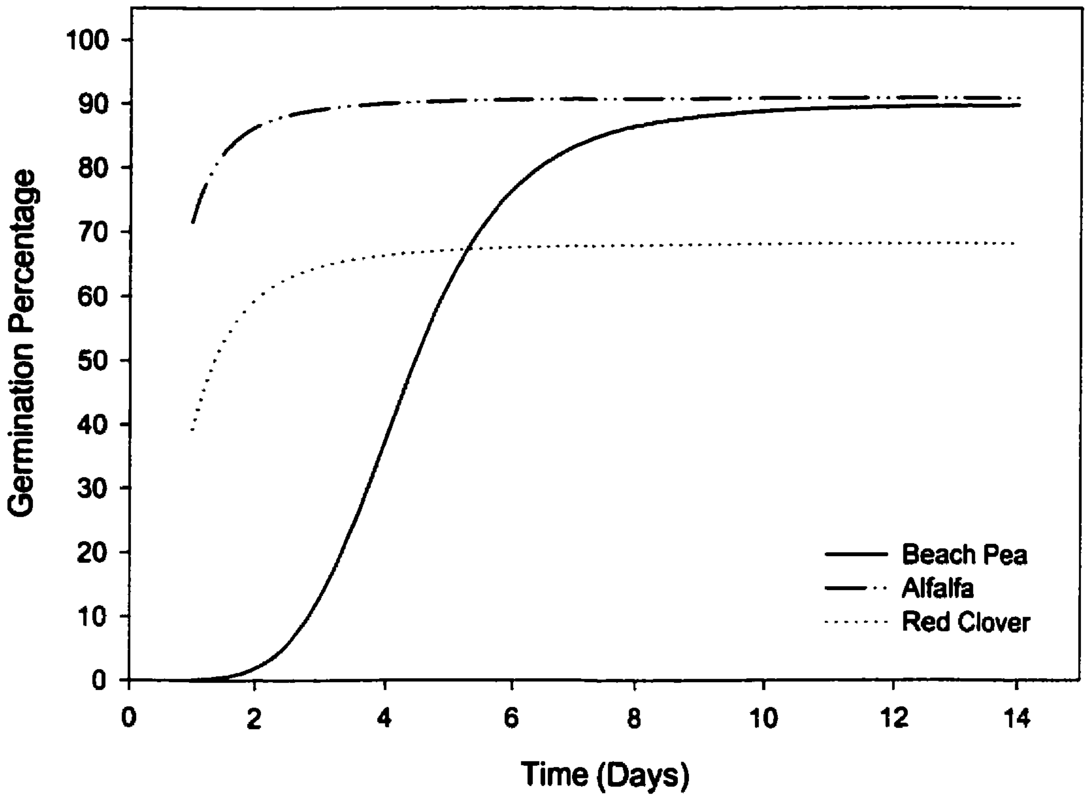


Figure 3.5: Germination percentage for the control seeds over the 14 days of the experiment. Beach pea is represented by a solid line, alfalfa by a dash-dotted line, and red clover by a dotted line.

3.2 Seedling Growth Study

The growth study established basic data for the beach pea growth cycle. After emergence, main shoot length and stem number increased until week eight when the length and stem number plateaued (Figure 3.6). Other measured traits (leaflet length and width, and leaf greenness) showed no change over the growth period. Plants did not flower during the 12 week period of the experiment. Non-linear regressions were performed on the data using Sigmaplot 5.0 (SPSS Inc. 1999). The results of the regressions and the equation used are presented in the appendix.

3.3 Growth in Saline Environment

Although salinity had less effect on the germination of alfalfa rather than red clover or beach pea, alfalfa is significantly impaired by salinity as it grows older. Comparisons were made between the three salinity treatments for main shoot height (Figure 3.7), number of stems (Figure 3.8), number of leaves (Figure 3.9), leaflet length (Figure 3.10), leaflet width (Figure 3.11), leaf greenness (Figure 3.12), and plotted from the start of salt treatments to the end of the experiment after 8 weeks exposure to the saline environment. All beach pea survived the entire experiment at all salinities. Salinity treatments affected alfalfa survival with control alfalfa plants having 100 percent survival while 2 percent salinity treatment caused 50 percent death (LD-50) at week 10 and 100 percent death at week 7. The 1 percent solution showed some mortality starting at week 6, however survival of the alfalfa had dropped to 60% by the termination of the experiment at week 8.

The average main shoot length of beach pea (Figure 3.7) showed a slower rate of elongation in the 1% salt treatment up to week 3 where stem elongation effectively stopped. The 2% treatment showed no stem elongation over the period of salt exposure. Elongation continued over the experiment in the control plants (Figure 3.7). The alfalfa had a similar trend with elongation stopping at week 4 for the 1% salt treatment and cessation of elongation coincident with the 2% salt treatment. Controls continued to gradually increase in length over the course of the experiment (Figure 3.7). The graphed data show significant differences between treatments at week 3 for beach pea and week 1 for alfalfa which continue to the end of the experiment.

The average number of stems for control beach pea increased over the course of the experiment while beach pea in the 1% and 2% salt stopped producing new stems and branches (Figure 3.8). Significant differences between treatments began at week 3 and continued to the end of the experiment. This indicates that the increased salinity interfered with shoot meristem activity. Similarly alfalfa plants stopped new stem production in the 1% and 2% salt treatments while average stem number increased over the course of the experiment in the control (Figure 3.8). Significant differences between treatments occurred at week 2 and continued to the end of the experiment.

Average leaf number in beach pea showed little variation after week 2 for the 1% and 2% salt treatments while the control showed an increase over the course of the experiment

(Figure 3.9). At week 3 all treatments were significant. Average leaf number for alfalfa increased in both the control and 1% salt treatment while the 2% salt treatment showed a decrease with all treatment differences becoming significant at week 3 (Figure 3.9).

Average leaflet length for beach pea varied little throughout the experiment while alfalfa showed an erratic trend with a steady decrease in the average leaflet length for all treatments (Figure 3.10). Similarly average leaflet width showed little variation for beach pea through the experiment with the same erratic trend as average leaflet length for the alfalfa (Figure 3.11).

Beach pea showed a slow decline in leaf greenness in the control for all the treatments over the course of the experiment (Figure 3.12). The 1 percent salt treatment was greener than the other treatment and the control in the last half of the experiment. With the exception of the 2% salt treatment to alfalfa (the plants that died), the alfalfa was always greener than the beach pea. Over all, alfalfa showed a decline in leaf greenness (Figure 3.12) in trays which were given the salt treatments. The control for alfalfa had the lowest average leaf greenness at the start of the experiment but increased as the experiment progressed. Overall, the data suggest that salinity had little effect on the parameters of leaf length, leaf width, and leaf greenness and such measurements can be excluded from further salinity work on beach pea or alfalfa.

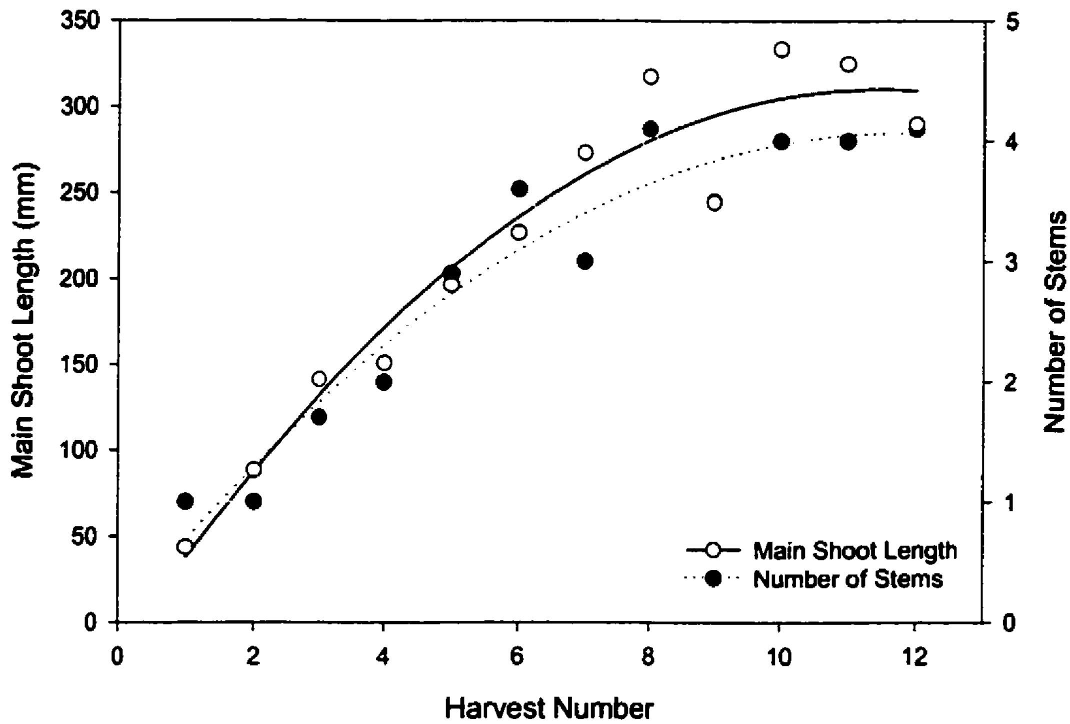
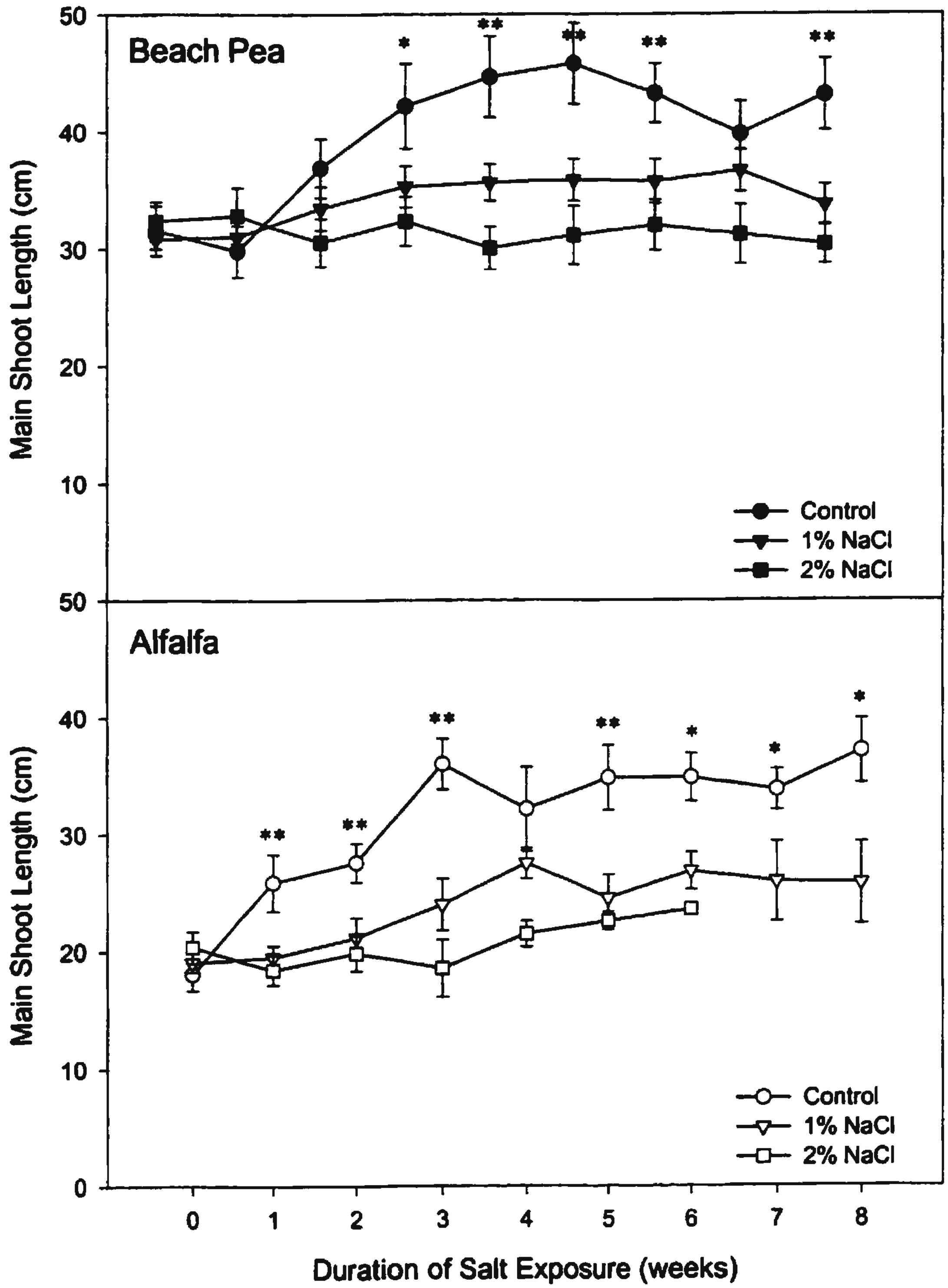


Figure 3.6: Main shoot length and number of stems for beach pea plants grown from seed in the greenhouse over a 12 week period. Main shoot length is represented by open circles with a solid trend line and the number of stems is represented by closed circles and a dashed trend line.

Figure 3.7: Main shoot length in centimetres for beach pea and alfalfa plants measured over 9 weeks beginning with the start of salt treatments. Plants were grown in the green house for 7 weeks prior to the start of salt treatments. Control is represented by circles, 1% salinity is represented by triangles and 2% salinity is represented by squares. Significant differences between treatments are denoted with a * ($p<0.05$) and a ** ($p<0.01$). Alfalfa plants had 50% death (LD-50) at week 2 and 100% death at week 7 for the 2% NaCl. Alfalfa with 1% NaCl had some mortality starting at week 6 and survival had dropped to 60% by the termination of the experiment at week 8.



Beach Pea
Alfalfa
Main Shoot Length (cm)
Duration of Salt Exposure (weeks)
Control
1% NaCl
2% NaCl
0
1
2
3
4
5
6
7
8
10
20
30
40
50

Figure 3.8: Number of stems for beach pea and alfalfa plants over 9 weeks beginning with the start of salt treatments. Plants were grown in the green house for 7 weeks prior to the start of salt treatments. Control is represented by circles, 1% salinity is represented by triangles and 2% salinity is represented by squares. Significant differences between treatments are denoted with a * ($p<0.05$) and a ** ($p<0.01$). Alfalfa plants had 50% death (LD-50) at week 2 and 100% death at week 7 for the 2% NaCl. Alfalfa with 1% NaCl had some mortality starting at week 6 and survival had dropped to 60% by the termination of the experiment at week 8.

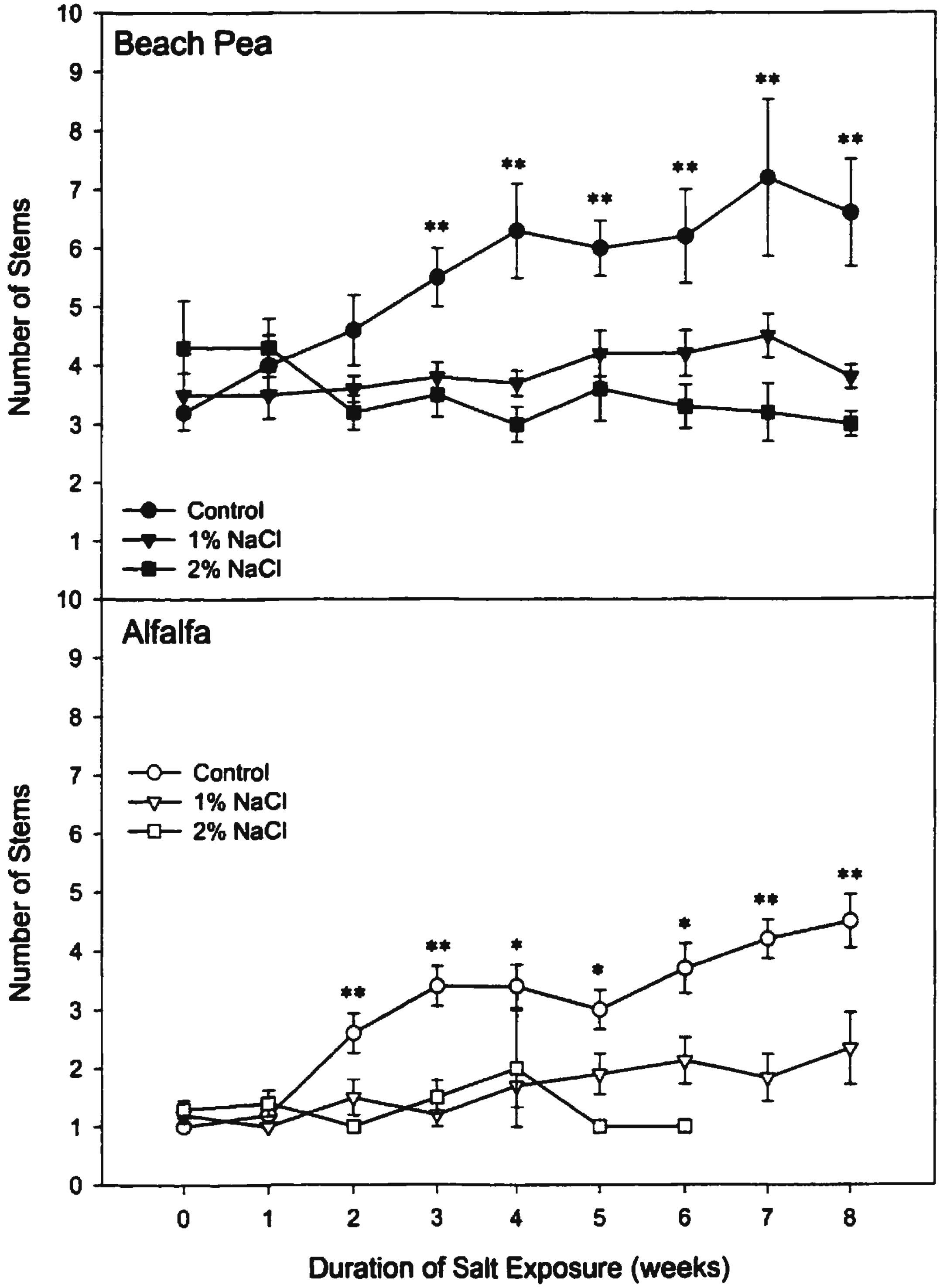

Beach Pea
Number of Stems
Control
1% NaCl
2% NaCl
Alfalfa
Number of Stems
Control
1% NaCl
2% NaCl
Duration of Salt Exposure (weeks)

Figure 3.9: Number of leaves for beach pea and alfalfa plants measured over 9 weeks beginning with the start of salt treatments. Plants were grown in the green house for 7 weeks prior to the start of salt treatments. Control is represented by circles, 1% salinity is represented by triangles and 2% salinity is represented by squares. Significant differences between treatments are denoted with a * ($p<0.05$) and a ** ($p<0.01$). Alfalfa plants had 50% death (LD-50) at week 2 and 100% death at week 7 for the 2% NaCl. Alfalfa with 1% NaCl had some mortality starting at week 6 and survival had dropped to 60% by the termination of the experiment at week 8.

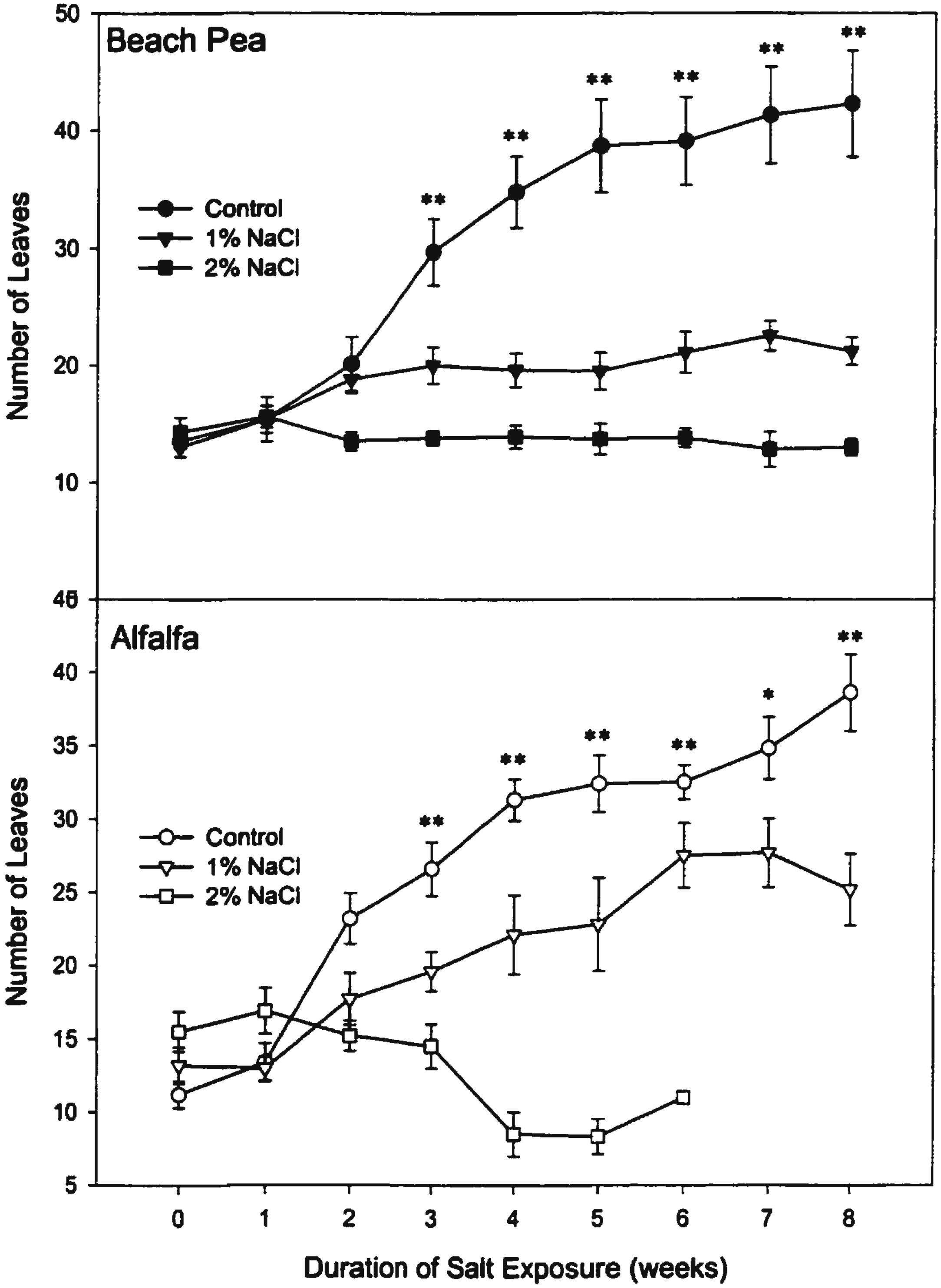
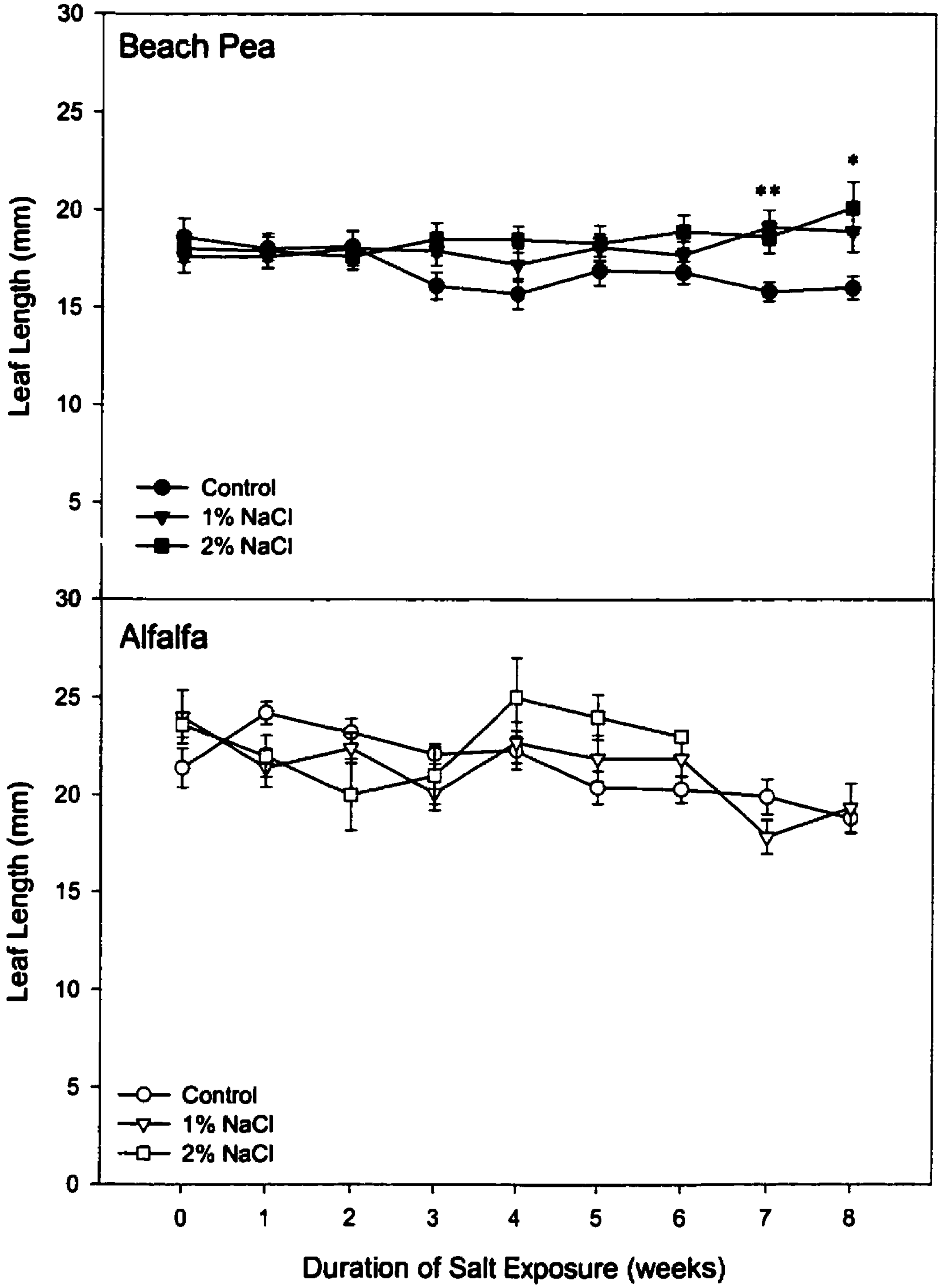

Beach Pea
Control
1% NaCl
2% NaCl
Number of Leaves
Alfalfa
Control
1% NaCl
2% NaCl
Number of Leaves
Duration of Salt Exposure (weeks)
**
*

Figure 3.10: Leaf length in millimetres for beach pea and alfalfa plants measured over 9 weeks beginning with the start of salt treatments. Plants were grown in the green house for 7 weeks prior to the start of salt treatments. Control is represented by circles, 1% salinity is represented by triangles and 2% salinity is represented by squares. Significant differences between treatments are denoted with a * ($p<0.05$) and a ** ($p<0.01$). Alfalfa plants had 50% death (LD-50) at week 2 and 100% death at week 7 for the 2% NaCl. Alfalfa with 1% NaCl had some mortality starting at week 6 and survival had dropped to 60% by the termination of the experiment at week 8.



Beach Pea
Leaf Length (mm)
Control
1% NaCl
2% NaCl
Alfalfa
Leaf Length (mm)
Control
1% NaCl
2% NaCl
Duration of Salt Exposure (weeks)

Figure 3.11: Leaf width in millimetres for beach pea and alfalfa plants measured over 9 weeks beginning with the start of salt treatments. Plants were grown in the green house for 7 weeks prior to the start of salt treatments. Control is represented by circles, 1% salinity is represented by triangles and 2% salinity is represented by squares. Significant differences between treatments are denoted with a * ($p<0.05$) and a ** ($p<0.01$). Alfalfa plants had 50% death (LD-50) at week 2 and 100% death at week 7 for the 2% NaCl. Alfalfa with 1% NaCl had some mortality starting at week 6 and survival had dropped to 60% by the termination of the experiment at week 8.

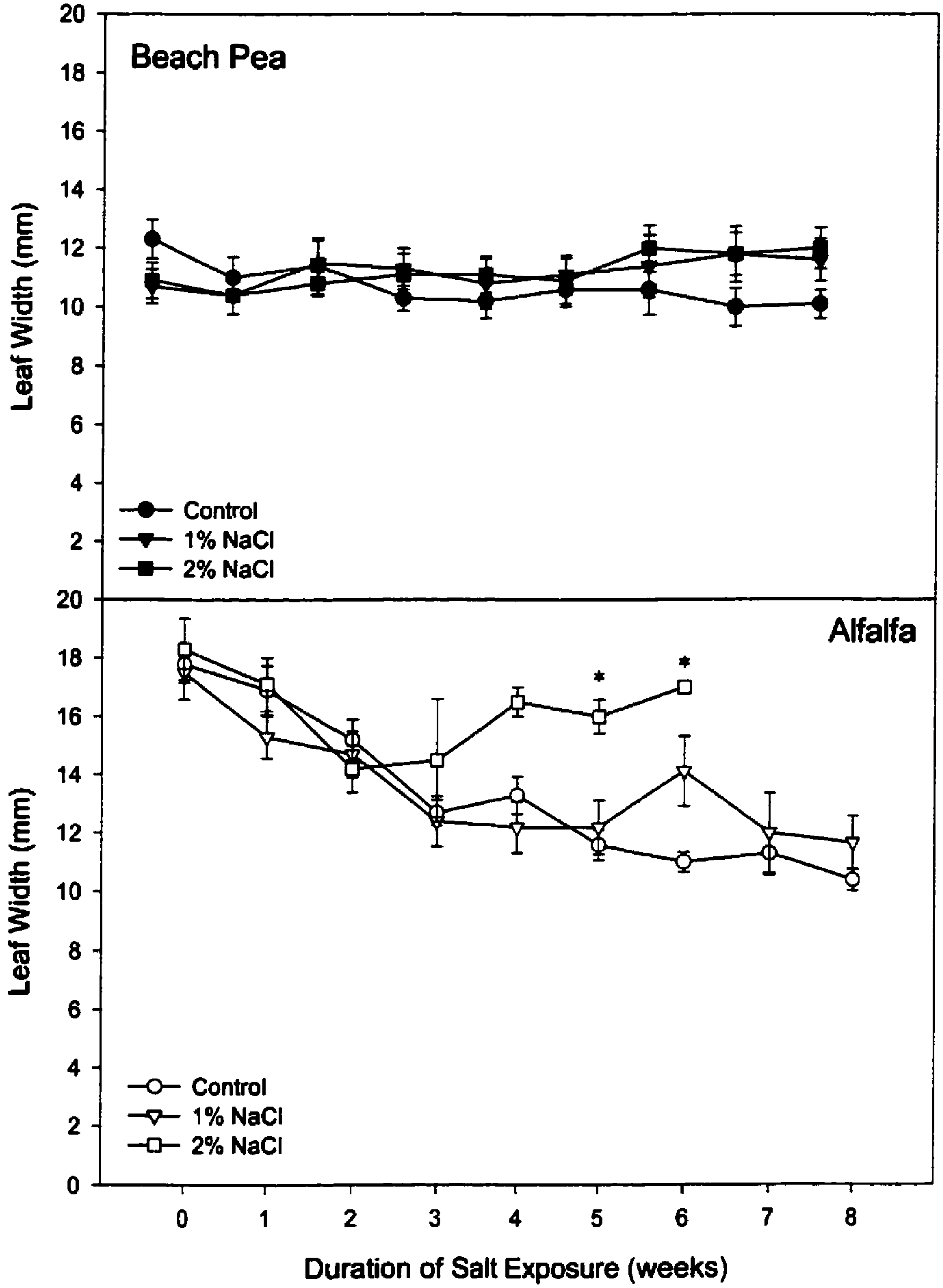
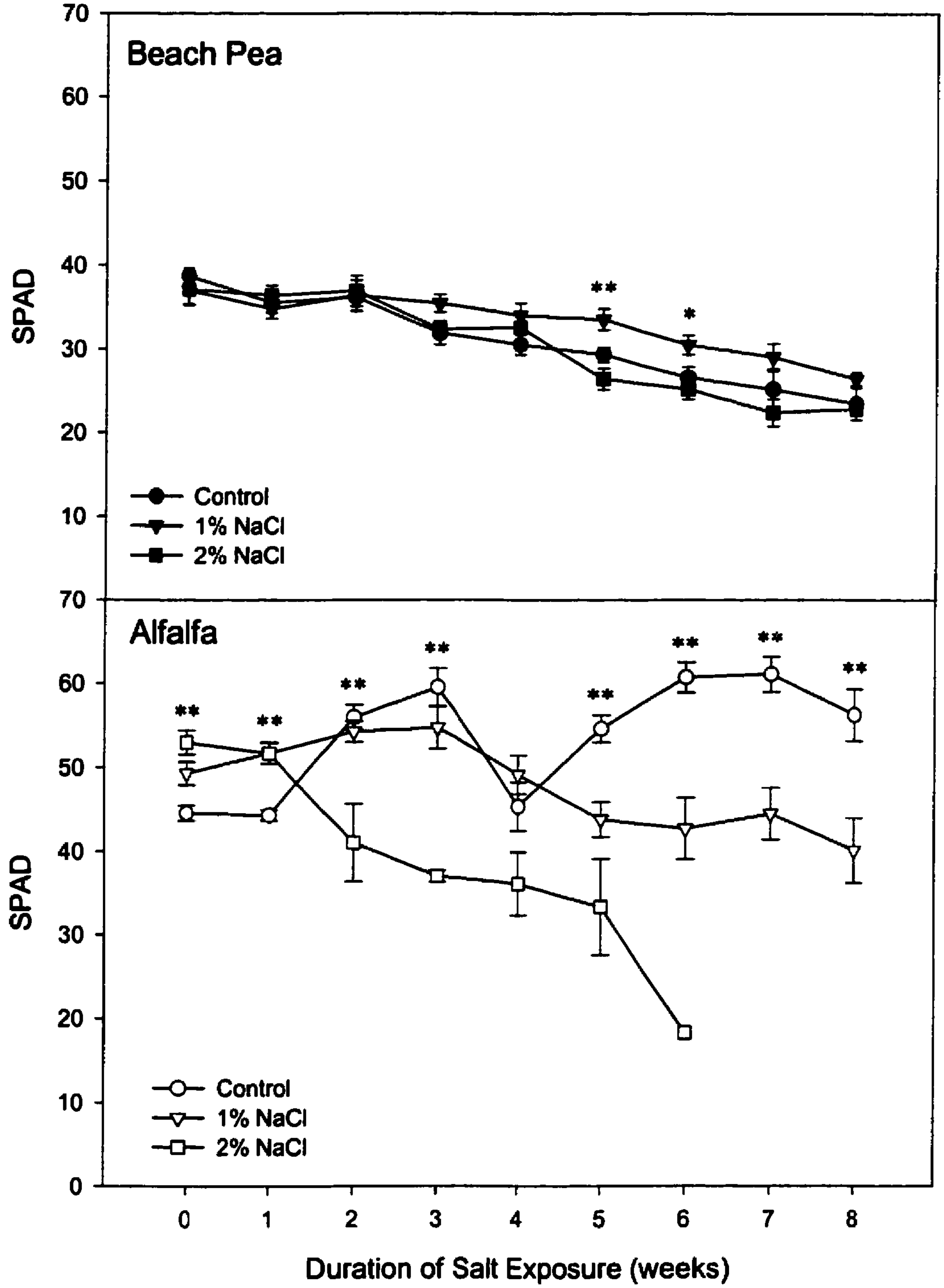

Beach Pea
Leaf Width (mm)
Control
1% NaCl
2% NaCl
Alfalfa
Leaf Width (mm)
Control
1% NaCl
2% NaCl
Duration of Salt Exposure (weeks)
*
*
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
8

Figure 3.12: Leaf greenness (SPAD) for beach pea and alfalfa plants measured over 9 weeks beginning with the start of salt treatments. Plants were grown in the green house for 7 weeks prior to the start of salt treatments. Control is represented by circles, 1% salinity is represented by triangles and 2% salinity is represented by squares. Significant differences between treatments are denoted with a * ($p<0.05$) and a ** ($p<0.01$). Alfalfa plants had 50% death (LD-50) at week 2 and 100% death at week 7 for the 2% NaCl. Alfalfa with 1% NaCl had some mortality starting at week 6 and survival had dropped to 60% by the termination of the experiment at week 8.



Beach Pea
Control
1% NaCl
2% NaCl
Alfalfa
Control
1% NaCl
2% NaCl
SPAD
SPAD
Duration of Salt Exposure (weeks)

3.4 Comparison of Greenhouse Soil Salinity to the Natural Environment

Salinity concentrations created in the greenhouse were similar to those found in the natural environment. Controls for both beach pea and alfalfa had a soluble salt conductivity around 0.15 mmhos cm^{-1}, and both 1 percent salt treatments produced a soluble salt conductivity of 1.0 mmhos cm^{-1} (Figure 3.13). The 2 percent salt treatment produced a soluble salt conductivity of 1.8 mmhos cm^{-1} in the beach pea sand and 1.3 mmhos cm^{-1} in the alfalfa sand (Figure 3.13). These values show that the conductivity is proportional to the salt concentration applied in the experimental environment.

The distance of natural beach pea stands from high tide line and the stands height above the high tide line at two beach locations were recorded (Table 3.1). On Bellevue Beach the beach pea were horizontally closer to the high tide line then the beach pea growing on salmon cove sands, however Bellevue Beach, beach pea were higher in elevation then the beach pea in Salmon cove (Table 3.1) These measurements show that the beach pea does not grow in the surf of the ocean but some distance from the salt water and grows in an environment with low salinity (Table 3.1). Soils from the area where beach pea was growing ranged from small pebbles found on Bellevue beach to fine sand found in Salmon cove. The experimental soil used was a number 2 silica sand, more coarse than fine beach sand and not as large as pebbles.

The extremes of the experimental environment mirror the natural environment as the beach pea grow wild in an area with 0.1 mmhos cm^{-1} soluble salt conductivity while the high tide mark sand produced a soluble salt conductivity of 1.8 mmhos cm^{-1} (Table 3.1). The sand in which alfalfa was grown had a lower soluble salt conductivity (1.3 mmhos cm^{-1}) than the sand found at the high tide line on both beaches. Only single soil samples were taken from the natural environment and from trays in the experimental environment. This did not allow for statistical analysis to be performed.

3.5 Effects of Salinity on Photosynthesis and Respiration in Beach pea

At the termination of the salinity growth experiment, respiration and photosynthesis were measured to see if the treatments produced any change in these physiological processes. Rates were calculated as microgram of carbon dioxide taken up or removed per minute per gram dry weight of the plant being tested. The respiration rate first dropped at 1 percent salinity but increased above the level of the control with 2 percent salinity (Figure 3.14). Both the net and gross photosynthesis rates decreased with an increase in salinity to 1.0 percent NaCl. At 2.0 percent NaCl the net photosynthesis rate was further decreased while gross photosynthesis increased above the level of the control.

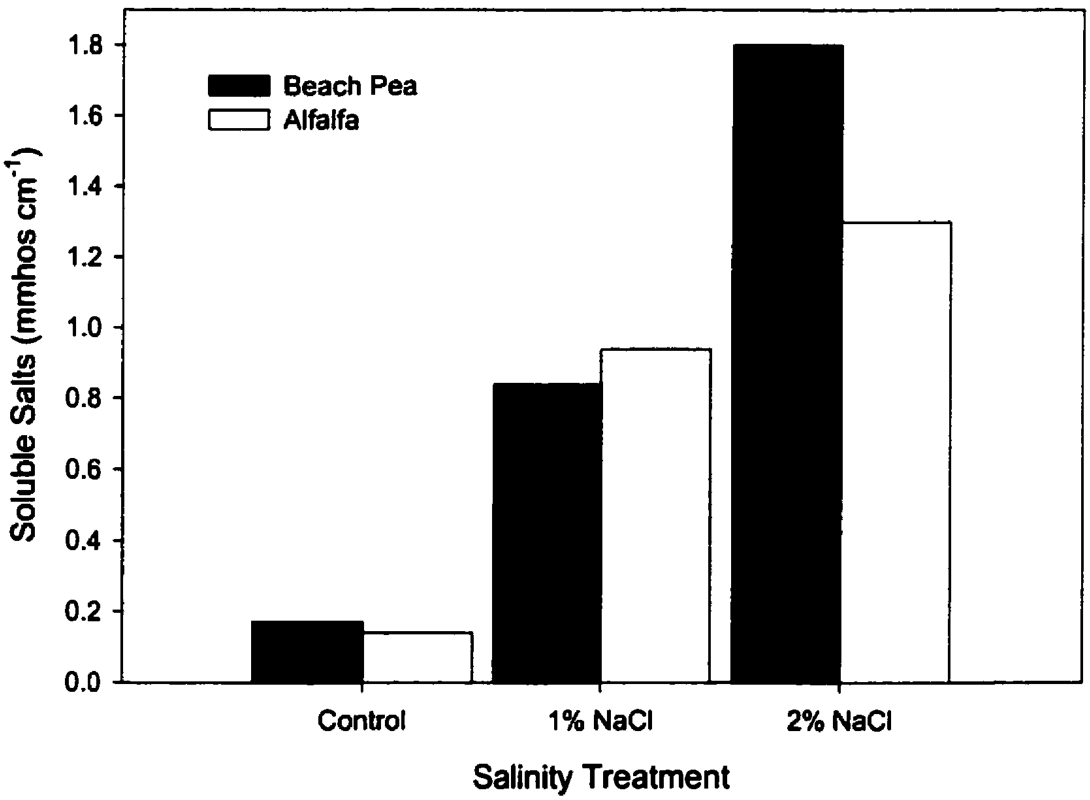


Figure 3.13: Bar chart of the level of soluble salts found in silica sand after 8 weeks of watering with NaCl solution during the saline environment growth experiment. The sand which contained beach pea plants are represented by the black bars and the sand containing alfalfa plants are represented by the white bars. Soluble salts analysis was completed by the Newfoundland and Labrador Department of Forest Resources and Agrifoods Soil, Plant and Feed Laboratory.

Table 3.1: Soluble salts analysis from soil samples taken at two different beaches in Newfoundland. Distance and elevation of the beach pea from the high tide line are also given. Analysis was completed by the Newfoundland and Labrador Department of Forest Resources and Agri-foods Soil, Plant and Feed Laboratory.

Beach	Environment	Soluble Salts (mmhos/cm)	Distance (m)	Elevation (m)
Bellevue Beach	High Tide	1.8		
	Beach Pea	0.1	35.7	3.35
Salmon Cove Sands	High Tide 1	2.0		
	High Tide 2	1.8		
	Beach Pea 1	0.2	52.4	0.59
	Beach Pea 2	0.1	63.1	0.36

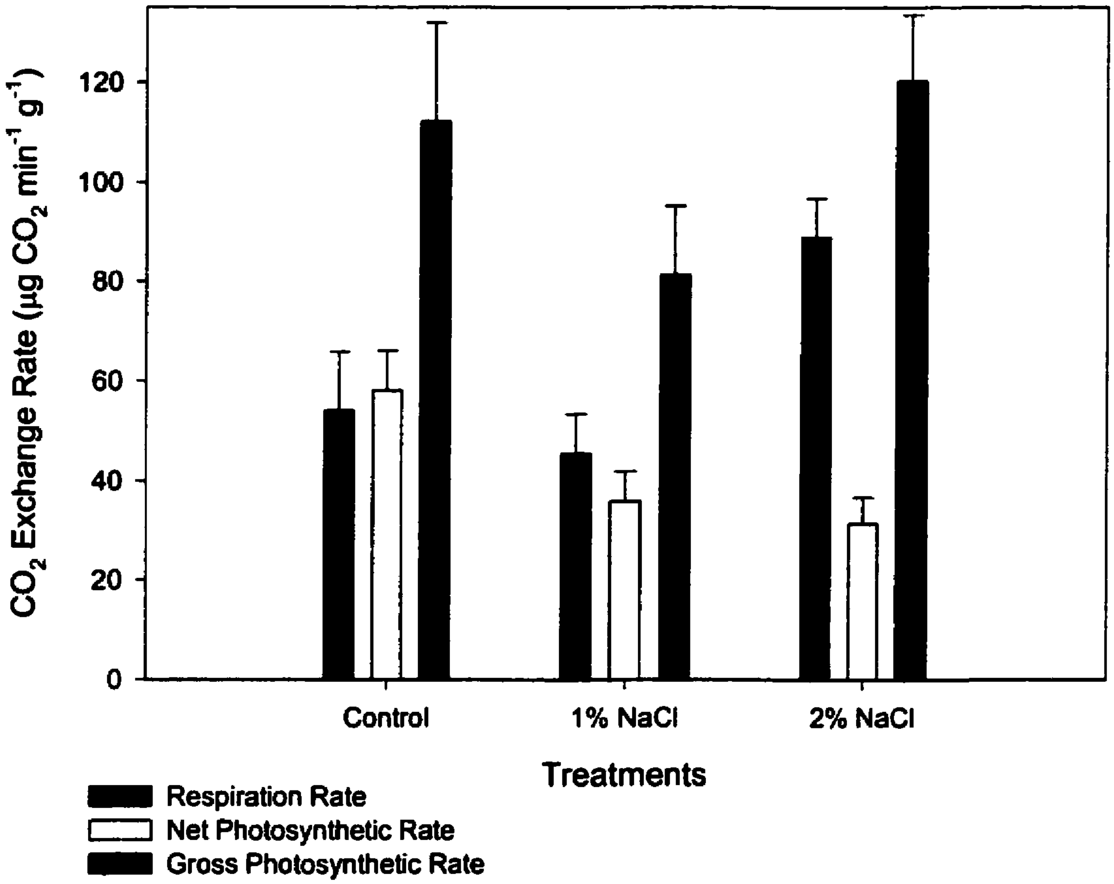


Figure 3.14: Average respiration, net photosynthesis, and gross photosynthesis rates of beach pea grown in saline environments. Average respiration is shown in black bars, average net photosynthesis is shown in light grey bars and average gross photosynthesis is shown in the dark grey bars. Standard error of the mean is shown by the error bars above the figures. Treatments were prepared in full strength Hoaglands solution with NaCl added to the appropriate treatments as a percentage of weight.

4. Discussion

4.1 Saline Environment

4.1.1 *Germination of Beach pea, Alfalfa and Red Clover in Saline Treatments*

Salinity treatments affected germination rate and final germination percentage for all species tested. Over the two weeks of the experiment, concentrations of 1.0 percent salinity and above caused a delay and reduced the final germination percentage of beach pea (Figure 3.1). Beach pea germinated in 1.0 percent did appear to be in the exponential stage of germination with the 1.5 percent salinity starting to increase in germination percentage suggesting a premature termination of the experiment (Figure 3.1). These findings contravene the initial expectation that salinity would have little or no affect on germination, but shows that the likelihood of beach pea germinating in a harsh environment is not very high. This contrasts sharply with both alfalfa and red clover. The results imply that the seeds of beach pea has some salt tolerance, but germination responds fast to low saline conditions.

Increasing salinity did not delay germination but caused a decrease in final germination percentage for both the alfalfa (Figure 3.2) and red clover (Figure 3.3). These seeds would be able to germinate at higher soil salinities than beach pea. Final germination percentage was reduced by increased salinity for beach pea, alfalfa, and the red clover (Figure 3.4).

Germination inhibition and delay, as a consequence of increased NaCl concentration, has been reported in chickpea (Muthukumarasamy *et al.*, 1997), different varieties of corn (Begum *et al.*, 1996), tomato (Foolad, 1996 and Jones *et al.*, 1985), cotton (Tort, 1996), and semi-dwarf and durum wheat (Francois *et al.*, 1986) as well as the halophyte *Atriplex patula* (Ungar 1996).

Delays in germination by increased salt concentration may be explained by the osmotic potential of the solution. Reduced osmotic potential of the solution the seed is in results in decreased cell elongation. Studies have shown that initial radical emergence maybe more susceptible to changes in the osmotic potential than would be found at a later growth stage in some species. (Bradford, 1995)

On salt water beaches, not all areas of the beach are suitable for growth. The soluble salts analysis (Table 3.1) showed a large difference in the electro-conductivity of the soil in the beach pea stand when compared to the high tide line for both beaches. Beach pea seeds exposed to the harsh conditions and high salinity of areas close to the waters edge would not germinate. The data suggests a salt avoidance mechanism in beach pea. In the control environment of 0.0 percent NaCl beach pea show a sigmoidal pattern to their germination percentages (Figure 3.1). The addition of 0.5 percent NaCl to the environment pushes germination to later in the experiment but the pattern is still evident with the same maximum achieved (Figure 3.1). At 1.5 percent NaCl there was very little germination seen in the beach pea however germination did appear to be increasing at the end of the two week experiment (Figure 3.1). A salt induced germination delay would enable the beach pea seed to survive accidental exposure to the high salt environment and may improve overall germination percentage once favourable conditions are found.

Ungar (1996) found that seeds of the halophyte *Atriplex patula* were still able to germinate in distilled water after the seeds were exposed to high saline conditions. Yoon *et al.* (1997) found that pansy seeds primed in a salt solution had higher germination percentages at high temperatures than unprimed seeds. Upon reaching a favourable environment, the beach pea may germinate and once established would be able to withstand short exposure to increased saline conditions. Further research would be of benefit to uncover the physiological mechanism behind the salt avoidance observed.

4.1.2 *Growth of Beach pea and Alfalfa with Saline Treatments*

Delay during germination in beach pea caused by salinity does not necessarily predict salt sensitivity at later growth stages. Measured growth parameters; main shoot length, number of stems, and number of leaves (Figures 3.7, 3.8, and 3.9) were affected by the increased salinity. The treated plants in those cases differed significantly from the control two weeks after the start of salinity treatments.

The difference seen in main shoot length between the control and two treatments show a decrease in internode elongation. This is most likely an osmotic affect of the salt on the beach pea. Decreased water potential in the soil reduces the pressure potential in the cells of the plant resulting in decreased cell expansion. However stem elongation is not a very sensitive part of the growth of the beach pea as the 1 percent salt treatment was able to continue main shoot elongation for an additional two weeks before levelling (Figure 3.7).

Beach pea stem number increased over the course of the experiment in the control plants but increased salinity stopped new stem initiation (Figure 3.8). New stem initiation was very sensitive to increased salt concentration as there were no significant differences between the 1 percent and 2 percent treatments. Decreased osmotic potentials would cause severe stress in the meristem of a growing stem. *Atriplex patula*, a halophyte, has been shown to have decreased biomass as a result of exposure to increased salinity (Ungar, 1996). A decrease in growth or, in the case of beach pea, the shut down of new stem production could be a way to survive short term saline exposure without exposing newly formed tissue to the harsh conditions.

Leaf number increased in the beach pea control plants over the course of the experiment. New leaf formation was effected by increased salinity. The 1 percent treatment continued to produce new leaves for two weeks after the start of salt treatments and then stopped while the 2 percent treatment stopped new leaf formation immediately upon addition of the salt. (Figure 3.9). The laying down of new leaf tissue may have stopped upon the addition of NaCl to the system, however the leaf primordia that were already differentiated in the apical meristem may have been able to over come the stress of the increased salinity and expand into new leaves.

Leaf size in the beach pea, both length and width (Figures 3.10 and 3.11), showed little change over the course of the experiment and leaf greenness (Figure 3.12) showed no significant differences for all the beach pea treatments. As a consequence of the unexpected termination of new leaf growth with increased salinity, the sampling method used could not provide changing data as measurements were often taken using the same leaf. The cessation of new growth means that further experiments involving salinity and beach pea can exclude these measurements from data collection as there would be no useful information provided.

The effects of salinity on alfalfa growth was also apparent. The most important difference between the beach pea and alfalfa was seen in the death of 40 percent of the alfalfa plants exposed to the 1 percent salinity at the termination of the experiment after 8 weeks of salt treatment and the death of all the alfalfa in the 2 percent salinity after 7 weeks of salt treatment. None of the beach pea plants exposed to these environments died during the experiment. Also of note is the increase in leaf number seen in the 1 percent NaCl treatment. In both beach pea salt treatments there was a cessation of new leaf formation however only the 2 percent NaCl treatment caused a similar cessation in alfalfa. Interesting to note is the higher soil conductivity of the sand in which beach pea was grown as compared to the sand where alfalfa was grown (Table 3.1) This means that the beach pea was exposed to a more stressful environment than the alfalfa.

The deleterious effects on the growth of forages by increased salinity has been noted in numerous studies (Pasternak *et al.*, 1993, and Asraf *et al.*, 1987). Salt tolerant forage species are always looked for in the wild and by breeding established forage crop species for salt tolerant traits. Two wild plants which have been shown to have potential as forages for saline environments are *Kochia* (*Kochia scoparia* (L.) Roth) and Russian thistle (*Salsola iberica*).

Kochia is a annual plant, high in protein, which has been shown to produce good stands of forage in saline soils (Steppuhn *et al.*, 1987). Russian thistle is a weed common on fallow land which produces an improved quality forage under saline conditions rather than under normal field conditions (Fowler *et al.*, 1992).

Breeding forage crops for salt tolerant characteristics is another way of improving the quality of forage grown on salt affected soils. Beach pea was able to tolerate and survive salinity treatments while the alfalfa showed a sensitivity to salts. Alfalfa has variable salt tolerance within different individuals. McKimmie and Dobrenz (1991) identified tolerant alfalfa plants as having greater inter-node elongation, larger leaf area and more leaves than sensitive alfalfa plants.

Shannon and Noble (1995) identified variability in the salt tolerance of different cultivars of subterranean clover (*Trifolium subterraneum* L.), namely differences in the ion accumulation of the shoots and leaves. Sodium ion accumulation in the leaves has been known to damage stomata function in non-halophytes (Thiel and Blatt, 1991). The ability to use sodium ions, in addition to potassium ions, for stomatal control has been found in some halophytes and such a mechanism may help identify potentially salt tolerant crops (Robinson *et al.*, 1997).

Beach pea, although able to survive longer than the alfalfa, did not thrive in the saline conditions. This provides evidence of an ability to withstand transient saline conditions, possibly until more favourable conditions are present. This maybe a further adaptation to survival on salt water beaches. The artificial condition of the experiment are probably more severe than any plants in nature would naturally see. A short immersion in sea water or salt spray would produce transient stress and any rainfall would translate into dilution of salts in the root environment.

The relationship between the beach environment and the artificial environment created in the greenhouse can be seen in the controls which provided an environment similar in salinity to the natural beach pea stand. The higher salinity sands were akin to moving closer to the salt water in terms of salinity and the 2 percent salt treatments were similar in salt conductivity and thus concentration to the high tide line at both beaches and (Table 3.1 and Figure 3.14).

Beach pea is not found adjacent to the high tide line but on the upper reaches of the beach where soil conductivity (salinity) is significantly lower (Table 3.1). Beach pea in their natural environment were found to have seedlings with thinner stems and smaller leaves than the adult plants and produced compound leaves of only one or two leaflet pairs along with many axillary stems and rhizomes (Bublitz 1982). Beach pea that were grown from seed in the green house started with the elongation of the main shoot but quickly started to produce branching stems from the base of the plant (Figure 3.7 and 3.8). Beach pea seedlings spread rapidly in the wild (Bublitz, 1982) providing evidence that beach pea have to establish quickly in their habitat. The results of the germination experiment show the seeds do not germinate in saline conditions and the growth in saline environments show that while not killed, the beach pea does not thrive under saline conditions once established, but can tolerate salinity to the level of the tide wash for periods up to 8 weeks.

4.1.3 *Photosynthesis and Respiration in Beach pea Grown in a Saline Environment*

In the saline environments respiration rates at the 1% treatment were less than the control but the 2% treatment produced respiration rates that were higher than the control (Figure 3.14). Gross photosynthesis does not appear to be affected by the salinity (Figure 3.14). In the 2% saline environment the respiration rate was double the respiration rate of the control. The consequence of this is that those plants have less carbon available for growth and storage, as a greater proportion of fixed carbon is needed to supply respiration requirements.

Respiration rates in leaves have been shown to increase in relation to increased salt concentration in the salt tolerant mangrove plants (Fukushima *et al.*, 1997). In the salt tolerant plant *Spartina alterniflora* there is also a decrease in gross photosynthesis rates as well as an increase in respiration (Hwang and Morris, 1994).

Some species of plant show no affect on respiration rates under increased salt concentrations. This can bee seen in the salt tolerant sapodilla which has been reported to show a drop in carbon accumulation with increased salinity (Mickelbart and Marler 1996). As well the salt sensitive *Annona squamosa* shows no increase in respiration rates with a similar drop in carbon accumulation (Marler and Zozor, 1996). The increase in respiration in beach pea may mean that there is a mechanism for salt tolerance but the increase alone is not conclusive.

A decrease in photosynthesis rates at increased salinity has been shown for other species. Sharma (1997) found that increased salinity had an adverse effect on the rate of photosynthesis in chick peas. Declines in net photosynthesis rates may not reflect the salt tolerance of the plant. Tattini *et al.* (1997) found salt stress reduced net carbon dioxide assimilation to a greater extent in a salt tolerant variety of olive than in a salt sensitive variety. The drop in carbon dioxide assimilation occurred at a lower leaf sodium ion concentration in the salt tolerant variety as opposed to the sensitive variety (Tattini *et al.*, 1997). Favourable conditions may bring about a rebound of photosynthesis and associated metabolic pathways. Delfine *et al.* (1998) found that in spinach, mesophyll conductance and photosynthesis recovered, following salinity induced drops, by watering with freshwater.

An increase in respiration may be reversed after the salinity level in the environment decreased. Given the transitory nature of beach pea salt exposure in the wild such a recovery would be likely. Future research should investigate the mechanisms involved in the respiration increase in beach pea and determination of the conditions whereby recovery of normal respiration levels would be possible. Comparisons of rates of respiration between beach pea and other forage crops, such as alfalfa and red clover, would also be of benefit.

4.2 Summary

Contrary to the initial hypothesis upon which this work was based, beach pea do not grow in a saline environment. The germination study has shown that increased salinity does cause a delay in germination in beach pea but did not appear to effect final germination percentage at concentrations equivalent to one half sea water.

Increased salinity caused an inhibition of new growth in beach pea. All processes linked to leaf and internode formation in beach pea appear to be inhibited by the salt treatments. The alfalfa showed the same response in the 2% treatment but continued leaf formation in the 1% salinity treatment. Increased salinity caused an increase in respiration rates which causes a reduction in th amount of assimilated carbon available to the plant for growth. There was no mortality seen in beach pea during the growth experiment, while at the same time the increased salinity did cause the death of alfalfa plants. These results provide evidence that once established beach pea can tolerate transient exposure to high saline conditions.

In the natural environment, established beach pea stands are not exposed to a saline environment for extended periods. The soluble salt levels found in the natural stands were comparable to those used in the greenhouse controls and ten times lower than the soluble salt level found at the high tide line. This means that fresh water is held in the soil in these areas, despite their proximity to the salt water. The preferred environment of beach pea is the fresh water seeps and upper reaches of the sea beaches where they can tolerate, but neither require nor desire a highly saline environment.

The ability of beach pea to survive the saline conditions shows that it has potential as a crop for salt affected areas. Further research into the mechanism of salt tolerance used by beach pea would lead to a better understanding of the plant and possibly more applications for the plant as a crop.

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6. Appendix

Table 6.1: Regression equation and r^2 values for Figures 3.1 to 3.3 and 3.5. Regressions were completed with Sigmaplot 5.0 (SPSS Inc. 1999).

Figure	Crop	Salinity	r^2	Regression Equation
3.1	Beach pea	0.00%	0.97	$f = \dfrac{a}{\left(1 + \left\lvert\left(\dfrac{x}{x_o}\right)\right\rvert^{b}\right)}$
		0.50%	0.90	
		1.00%	0.71	
		1.50%	0.31	
3.2	Alfalfa	0.00%	0.46	
		0.50%	0.71	
		1.00%	0.96	
		1.50%	0.77	
3.3	Red clover	0.00%	0.29	
		0.50%	0.80	
		1.00%	0.77	
		1.50%	0.11	
3.5	Beach pea	0.00%	0.97	
	Alfalfa		0.46	
	Red clover		0.29	

Table 6.2: Regression equation and r^2 values for Figure 3.4. Regressions were completed with Sigmaplot 5.0 (SPSS Inc. 1999).

<table>
<tr><th>Crop</th><th>r^2</th><th>Equation</th></tr>
<tr><td>Beach pea</td><td>0.95</td><td rowspan="3">$f = \frac{a}{\left(1+e^{-\frac{(x-x_o)}{b}}\right)}$</td></tr>
<tr><td>Alfalfa</td><td>0.97</td></tr>
<tr><td>Red clover</td><td>0.79</td></tr>
</table>

Table 6.3: Regression equation and r^2 values for Figure 3.6. Regressions were completed with Sigmaplot 5.0 (SPSS Inc. 1999).

<table>
<tr><th>Measurement</th><th>r^2</th><th>Equation</th></tr>
<tr><td>Shoot</td><td>0.94</td><td rowspan="2">$f = \frac{a}{\left(1+e^{-\frac{(x-x_o)}{b}}\right)}$</td></tr>
<tr><td>Number of Stems</td><td>0.94</td></tr>
</table>