THE UNIVERSITY OF CALGARY

STRUCTURAL AND PHYSIOLOGICAL RESPONSES OF *CHENOPODIUM RUBRUM* TO SALT STRESS.

by

Patricia G. Warne

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY

CALGARY, ALBERTA

DECEMBER, 1985

ISBN 0-315-30024-8

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Structural and Physiological Responses of Chenopodium rubrum to Salt Stress" in partial fulfillment of the requirements for the degree of Master of Science.

Dr. David M. Reid, Supervisor, Biology

Dr. C.C. Chinnappa, Biology

Dr. N.E. Henderson, Biology

Dr. T. Oliver, Geology

December 17, 1985

ABSTRACT

The effects of Na_2SO_4 and NaCl on some of the structural and physiological aspects of growth in Chenopodium rubrum was investigated. Plants exposed to both salts behaved like euhalophytes, adjusting osmotically by accumulating electrolytes from the growing solution rather than synthesizing organic osmotica like some other halophytes (glycohalophytes). The ions were presumably compartmented in the vacuoles of cells necessitating the accumulation of organic solutes to balance the osmotic potential in the cytoplasm. C. rubrum accumulated increasing amounts of glycinebetaine as the solute potentials of the growing media decreased, leading to the assumption that it may be involved in the osmotic adjustment of the cytoplasm. In all salt treatments, plant water potentials were below those of the growing solution and therefore turgor pressures were maintained in all cases. Plants grown in NaCl seemed to conserve osmotica (by limiting osmotic adjustment) while those exposed to Na_2SO_4 did not (i.e. shoot water potential was maintained parallel to the solute potential of the growing media).

Plants remained healthy in all salt treatments with the exception of those at the highest Na_2SO_4 concentration. Optimal growth occurred at -0.4 MPa and declined steadily below this point.

Increasing salinity did not significantly effect photosynthetic capacity of these plants except at -1.6 MPa Na_2SO_4. However stomatal resistance increased dramatically at all salinities, thus causing a decrease in transpiration rates. This led to an increase in water use efficiency (WUE) in all plants except those at the highest Na_2SO_4

concentration (-1.6 MPa). Values from carbon isotope analysis also indicated improved WUE with increasing salinities.

As salinity increased, there were changes in some of the morphological and anatomical features often associated with salinity stress. With increasing salt concentrations, decreases in leaf area, stomatal densities, total numbers of stomates per leaf, and stomatal sizes were measured. As well, leaf succulence was shown to increase, although NaCl had a greater effect on this parameter than did Na_2SO_4. Their possible roles in the adaptation to salinity are discussed in this thesis. Prostrate growth is a morphological feature known to occur in plants growing under natural saline conditions, however it could not be induced in laboratory experiments. This implies that one should never assume that conditions in the laboratory necessarily duplicate those in the natural environment.

ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant No. A-5727 to Dr. D.M. Reid.

I would like to express my sincere appreciation to Dr. David M. Reid for his advice and encouragement and the extreme patience he displayed throughout the duration of this thesis project.

Special thanks are also extended to Dr. Lorna Rollins and Dr. Robert Guy for their many helpful ideas and assistance on various aspects of this work.

As well, I would like to thank my friends and colleagues Ms. Ellen Macdonald, Mr. Kevin Watson, Mr. Anil Jayasekera, Dr. David Drakeford, Dr. Ilabanta Mukherjee, Mr. Luke DeWit and Big Steve and Shirley for making my time here most enjoyable. I also thank my mom and dad for having me. Eat your hearts out I'm going skiing!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Figures (continued)

LIST OF ABBREVIATIONS

C_3, C_4	Type of carbon metabolism in plants. Refers to the number of carbon atoms in the initial products of photosynthesis
C_a	ambient CO_2 concentration
C_i	intercellular space CO_2 concentration
DF	degrees of freedom
DW	dry weight
FW	fresh weight
HPLC	high performance (pressure) liquid chromatography
n	sample size
PAR	photosynthetically active radiation (400 to 700 nm)
P_n	net photosynthetic rate
R_s	stomatal resistance to CO_2 diffusion
RH	relative humidity
SE	standard error
WUE	water use efficiency (unit carbon fixed per unit water transpired)
$\delta^{13}C$	carbon isotope abundance parameter
Δ	change in some quantity
$^o/oo$	per mille (i.e. parts per thousand)
Ψ_w	water potential
Ψ_s	solute or osmotic potential
Ψ_p	pressure potential
Ψ_w^{leaf}	water potential of a leaf
Ψ_w^{env}	water potential of the environment
MPa	mega pascal (1 MPa=10 bars)

INTRODUCTION

EFFECTS OF SOIL SALINITY ON AGRICULTURE

Soil salinity is a common phenomenon and one of the basic features associated with arid and semi-arid regions of the world (Sen and Rajpurohit 1982). Until quite recently, the study of salinity and its effects upon plant growth has received limited attention in the literature. However, due to the loss in productivity of large tracts of agricultural land around the world, much more attention is being given to the problems associated with soil salinity. In Alberta many soils are naturally saline due to the marine origin of the parent material. The salts in these soils, derived from weathering of the soil and parent materials, are picked up in the ground water and carried to low lying areas where they collect creating saline seeps. The soluble salts that can be found in these soils consist primarily of various proportions of sodium, calcium, and magnesium cations and sulfate and chloride anions (Guy 1984). The increase in salinity in many areas can be attributed to agricultural practices such as irrigation and summer fallowing. The practice of summer fallowing for example, enhances seep development because it results in excess soil moisture which percolates below the crop root zone. The naturally occurring salts in the soil are dissolved in this water and are carried to discharge areas where evaporation at the soil surface occurs and causes a salt crust to build up. This crust of salt acts as a physical barrier to evaporation so more water remains and flows outward increasing the area affected by

the saline seep (Lilley 1982). In contrast, saline seeps rarely occur in native grassland areas because the actively growing vegetation uses all of the available moisture reducing the chance of precipitation percolating beyond the root zone. Irrigation practices also contribute to the accumulation of salt in soils and results when mineral salts dissolved in water are deposited at the surface of the soil through evapotranspiration. A major source of excess water in irrigated soils is seepage from canals, or from elevated water tables (Wainwright 1984), however poor management of irrigation water also contributes to this problem (i.e. over or under-irrigation).

It has been estimated that more than 50% of all irrigated land in the world has been damaged by secondary salinization and/or sodification and water logging (Zahran and Wahid 1982). In Alberta, approximately 40% of total yield has been lost on 175,000 acres of farm land due to poor irrigation practices (Rowan 1978). It has also been estimated that saline seeps are expanding at a rate of 10% or 50,000 acres per year (Lilley 1982). In terms of lost revenue, for 500,000 acres of land severely affected by salinity, the annual loss works out to be close to $25 million (Lilley 1982). If these lands are to be used to increase plant productivity, it is important to examine characteristics of plants which have evolved mechanisms to survive in saline environments (Jefferies 1980). In the future this information may be important in the modification of existing agriculturally important crop species. Alternatively salt tolerant plants that prove to have agricultural potential could be grown on non-productive saline soils. Utilization of these plants on saline soils may also be

considered a biological way for soil desalinization and reclamation (Zahran et al. 1982).

TOLERANCE OF PLANTS TO SALINITY

Tolerance to salinity varies greatly among plant species and has led to the division of species into two major physiological groups: glycophytes, which lack tolerance to salinity and do not normally survive in saline environments; and halophytes which includes any plant able to grow and complete its life cycle in the presence of high concentrations of salt (> 300mM) (Rains et al. 1980). Halophytes can further be divided into either euhalophytes ("true" halophytes) which take up and accumulate salts to effect osmotic adjustment, or glycohalophytes, plants which do not accumulate salt and who have to synthesize organic solutes (i.e. amino acids ,sugars etc.) to bring about osmotic adjustment (Guy 1984). Osmotic adjustment has been defined as a net increase in the quantity of osmotically active solutes (Turner and Jones 1980).

HALOPHYTIC ADAPTATION TO SALT STRESS

Generally plants growing in saline environments face two major problems: (1) obtaining sufficient water from soils of very negative osmotic potentials, and (2) dealing with high concentrations of potentially toxic ions (Rains et al. 1980). Plants cannot absorb water from the external medium unless the osmotic potential within the plant is lower than that of the external medium. In order to achieve a lower internal water potential a plant can respond in one of two ways. As mentioned previously, euhalophytes deal with the problem by

accumulating high internal concentrations of electrolytes to effect an osmotic adjustment with the concommitant maintenance of turgor and growth. On the other hand, plants (such as the glycohalophytes) which fail to absorb salt to a significant extent must build up high internal concentrations of organic solutes to effect osmotic adjustment if they are to survive in a saline environment. This is presumably a more expensive route metabolically.

Salt accumulation appears to be the superior mechanism and the most widespread adaptation to salinity stress in plants (Epstein 1980). The reasons for this are due to the fact that salt ions provide a readily available source of solutes which the plant can use to achieve lower internal water potentials. In addition to this, the plant does not have to expend great amounts of energy for transport of the ions to the shoot since they can be transported via the transpiration stream; unlike organic osmotica which would have to be transported via the phloem at great expense in terms of metabolic energy. Furthermore, because the plants are using solutes already available they need not use photosynthate or the energy required for synthesis of organic solutes (Epstein 1980).

With the accumulation of excess salts, euhalophytes must possess mechanisms to tolerate their potentially toxic effects (salt ions have the capability to disrupt cellular membranes i.e. organelles and plasma membranes, as well as causing the inactivation or activation of enzymes) (Levitt 1980). Several mechanisms have already been reported. For example, in certain desert shrubs, lower leaves are shed when salt accumulation builds up to critical levels. This is an effective means

of removing excess salt from the rest of the plant (Rains 1979). Another mechanism to decrease internal salt concentrations which has been well documented is by increasing the uptake of water into various tissue. This phenomenon is known as succulence (Jennings 1968, Levitt 1980). Cells, especially parenchyma, enlarge due to an increase in water content, supposedly diluting initial ion concentrations (Jennings 1968, 1976, Poljakoff-Mayber 1975). It should be pointed out however, that succulence occurs in plant groups that are not particularly salt tolerant (Queen 1974). Furthermore, in species like Salicornia europaea, where succulence, (induced by salt) develops well before maximum growth stimulation, dilution of high internal salt concentrations seems an unlikely role for succulence (Guy 1984). Other means of reducing internal levels of salinity are found in plants containing specialized structures called salt glands, or salt bladders. These structures collect salt from surrounding tissue and secrete it externally, where it can be washed away by rain (Queen 1974) (presumably this process involves large amounts of energy needed for ATP stimulated active transport). However, these glands are effectively external to the leaf in terms of water potential and therefore maintenance of suitable water potentials in the leaf would still depend on reasonably high solute concentrations in leaf cells (Caldwell 1974).

The question that must next be addressed is how such high internal concentrations of ions can possibly be tolerated by plant cells without the disruption of organelle and enzyme function. Many biochemical studies indicate that the metabolic processes of halophytes are not unusually tolerant to salt (Flowers et al. 1977, Maas et al. 1978,

Storey and Wyn Jones 1975). When enzymes extracted from salt tolerant plants were compared with those from non-salt tolerant plants, little difference was found in their response to increased levels of salt (Cavalieri and Huang 1977, Flowers et al. 1977, Ting and Osmond 1973). The fact that enzymatic reactions continue to occur in the cytoplasm despite the fact that ions are being accumulated by the plant suggests that the plant must somehow sequester the ions away from metabolic machinery in the cytoplasm. The generally accepted hypothesis is that the ions are compartmented within the cell. The structural organisation of plant cells are very well suited for this. Large membrane bound vacuoles (constituting over 80% of the cell volume) are believed to be the site for ion accumulation (Flowers 1972, 1972a, 1977, Levitt 1980, Rains 1979). If this is the case however, one can visualize an additional problem faced by the cell. The lower osmotic potential in the vacuole and cell walls would result in the dehydration of the cytoplasm. A number of organic solutes have been identified which could act as osmotically active agents in the cytoplasm to balance the osmotic pressure between vacuole and cytoplasm (Storey and Wyn Jones 1979). The synthesis of organic solutes for this purpose is metabolically expensive, however, only a small fraction of the total cell volume is involved (since most plant cells are highly vacuolated) (Epstein 1980). Substantial evidence exists that show that the organic substances identified apparently do not interfere with the activity of enzymes (Stewart and Lee 1974, Flowers et al. 1978, Pollard and Wyn Jones 1979, Paleg et al. 1981). Some of the substances found in both euhalophytes as well as glycohalophytes include methylated quaternary

ammonium compounds (Storey and Wyn Jones 1979), amino acids (Cavalieri and Huang 1979, 1981), polyhydric alcohols (mannitol, arabitol, glycerol) (Flowers et al. 1977) and reducing sugars (Jefferies 1980).

Of the quaternary ammonium compounds, glycinebetaine appears to be the most widely accumulated (Cavalieri and Huang 1981, Goas et al. 1982, Storey and Wyn Jones 1979, Stumpf and O'Leary 1985). Vegetative tissue, analyzed from a large number of halophytic species show glycinebetaine to be present in substantial quantities. Many species in the Chenopodiaceae, a family which includes some of the most salt tolerant species, contain significant quantities of this compound (Wyn Jones 1980). Proline is probably the most well known of the amino acids to be accumulated by plants (Stewart and Lee 1974, Cavalieri and Huang 1981) in response to salinity. In some plants it was found that proline accounted for as much as 30% of the total amino acid pool (Stewart and Lee 1974).

It would appear from the above discussion that halophytes should have little difficulty growing in saline environments since they have evolved mechanisms to overcome the adverse effects of salt. However, a simple visual comparison of plants from the same species growing in the absence of salt or under highly saline conditions does not substantiate this. Those grown at low solute potentials are clearly smaller than plants grown with either no salt or low salt concentrations (this inspite of osmotic adjustment). The basis for the decline of plant growth in response to saline conditions is still poorly understood (Papp et al. 1983). However, it has been suggested that the reduction in growth is caused by the diversion of photosynthate not into growth

but into synthesis of solutes for osmotic adjustment (Poljakoff-Mayber and Gale 1975), or that part of the energy derived by respiration may be shifted towards the maintenance of ion uptake mechanisms or to damage repair needs instead of to the usual cellular events (Penning De Vries 1975, Poljakoff-Mayber and Gale 1975). Others have attributed the depression of growth to the partial closure of stomates with the result that CO_2 assimilation rates are lowered (Papp et al. 1983).

That all plant growth ultimately depends on the carbon assimilated during photosynthesis is well established. Although increased stomatal aperture may be advantageous for photosynthesis, it can result in greatly increased water loss through transpiration. Thus, the primary dilemma facing not only plants growing in saline environments, but all terrestrial plants is that of getting as much CO_2 as possible into the plant, while retaining the maximum amount of water. This predicament becomes less problematic in situations where water is not limited (Nobel 1983). Furthermore, it should be noted that stomatal closure has a much more dramatic effect on transpiration than it does on CO_2 assimilation (Guy 1984). Several reports confirm that stomatal closure still occurs even when turgor pressure is maintained and there is no apparent damage to the basic photosynthetic machinery (Schwarz and Gale 1981). In addition to this, net photosynthesis of many halophytic species is not dramatically affected over a wide range of salt concentrations (Winter 1979). The combined effects of increased stomatal resistance and maintenance of photosynthetic assimilation rates ultimately results in higher water use efficiency in halophytes.

RESEARCH OBJECTIVES

From the preceeding discussion it is quite evident that the survival of halophytes is typically accompanied by various morphological, anatomical and physiological changes, some of which include: increased succulence (Longstreth and Nobel 1979, Jennings 1968); changes in the numbers and sizes of stomates (Poljakoff-Mayber 1975); decreased stomatal apertures which ultimately has effects on CO_2 assimilation rates and water use efficiency (unit carbon fixed per unit water transpired) (Downton et al. 1985); thickening of the cuticle; production of waxy layers on the epidermis (Sen et al. 1982); reduction in leaf area (St. Omer and Schlesinger 1980, Downton et al. 1985); reduced apical dominance (Poljakoff-Mayber 1975, Gale and Poljakoff-Mayber 1970); inhibition of differentiation; changes in diameter and numbers of xylem vessels (Sen and Rajpurohit 1982); compartmentation of ions (Yeo 1981, 1983); and production of organic solutes such as proline (Aspinall 1981, Cavalieri 1983), and glycinebetaine (Hanson and Wyse 1982, Storey and Wyn Jones 1975, 1977). It should be noted that the above information is somewhat fragmented since many different species are dealt with. Furthermore, these changes are often considered to be of adaptive value to plants living in saline environments, however such assumptions are often made in the absence of any confirmatory evidence.

The principal objective with which this research was concerned was to obtain data on some of the responses to salinity listed above for one particular plant species native to Alberta. I have also attempted to explain their possible adaptive significance based both on my own

work and the work of others. For my investigation I chose to work with a species from the Chenopodiaceae (Chenopodium rubrum L.) because many of the plants belonging to this family are well known for their salt tolerance, and I wanted to study a halophyte and, in particular one native to Alberta. Also, there are few detailed reports in the literature dealing with this species or any others native to Alberta.

In the majority of laboratory investigations dealing with salt tolerance in plants NaCl is the only salt utilized. However, the predominant salt found in saline soils of Alberta is Na_2SO_4 (Guy 1984, Lilley 1982). There have been very few reports comparing plant response to both salts. For this reason I have chosen to employ both salts for all of my experiments.

METHODS AND MATERIALS

GROWING CONDITIONS. In all experiments plants were grown in an Enconaire growth chamber or in the greenhouse. In the growth chamber, maximum temperature during the light period reached 24°C, this temperature dropped to 12°C during the night. The light source consisted of 16 Sylvania gro-lux lamps which provided an irradiance (PAR) of 313 $\mu E\ m^{-2}\ s^{-1}$ at plant height. This irradiance was increased to 483 $\mu E\ m^{-2}\ s^{-1}$ after the lamps were changed for the photosynthesis experiment. Total photoperiod lasted 16 hours. Humidity reached 46% during the day and rose to 75% during the night.

In the experiment performed in the greenhouse, irradiance ranged from 261 ± 10 $\mu E\ m^{-2}\ s^{-1}$ on overcast days to 1254 ± 20 $\mu E\ m^{-2}\ s^{-1}$ on sunny days. Relative humidities were variable, ranging from 30-40% during the day, and up to 70% at night. Daytime temperatures varied from approximately 23°C on overcast days up to 30-35°C on sunny days. At night this temperature dropped to around 14°C.

PLANT MATERIAL. Seeds of Chenopodium rubrum L. (randomly collected from plants grown in the greenhouse) were scarified and sown onto pots containing granite grit No. 2 (Imasco.). The seeds were originally collected from plants at a salt slough near Nanton, Alberta by Dr. Robert Guy, and were stored in a refrigerator at 5°C. Granite grit was chosen as the rooting media not only because of the ease of harvesting the roots at the end of experiments but also because it retains very little water and thus potential flooding problems could be avoided.

GROWTH SYSTEM. In most experiments 48 4" pots were suspended by their rims from lucite covers to a depth of 1 cm into plastic trays containing approximately 5 litres of growing solution. Each tray was fitted with a humidifier float whose function it was to maintain a constant level of solution in each tray. The levels were kept constant by the addition of deionized water which was gravity fed from a reservoir located above the trays. Solutions were continuously aerated (using aquarium air stones hooked up to an air manifold) to prevent anaerobic conditions and also to keep incoming deionised water mixed with the solutions. The nutrient solution chosen consisted of modified Hoagland's solution (Hoagland and Arnon 1950), made up to half strength for all ingredients (see Appendix 1). To this basal solution was added varying amounts of salt (either NaCl or anhydrous Na_2SO_4 A.C.S. certified). The amount of salt depending on the particular water potential desired. In most cases salt was added to the Hoagland's solution at 2 day intervals, (not exceeding -0.2 MPa every 2 days; see figure 1 for conversion of MPa to g salt/l solution). After maximum salt concentrations were reached, solutions were changed less frequently (once a week). Plants were maintained at the maximum salt concentrations for at least two weeks before harvesting.

MEASUREMENT OF STRUCTURAL PARAMETERS. The structural parameters described in this study include: (a) leaf succulence (leaf water content; leaf thickness) measured using several different techniques; (i) leaf disks of equal area (.385 cm^2) were taken using a cork borer and fresh and dry weights per unit area taken (FW/unit area - DW/unit area) (ii) free-hand cross sections of leaves were taken for

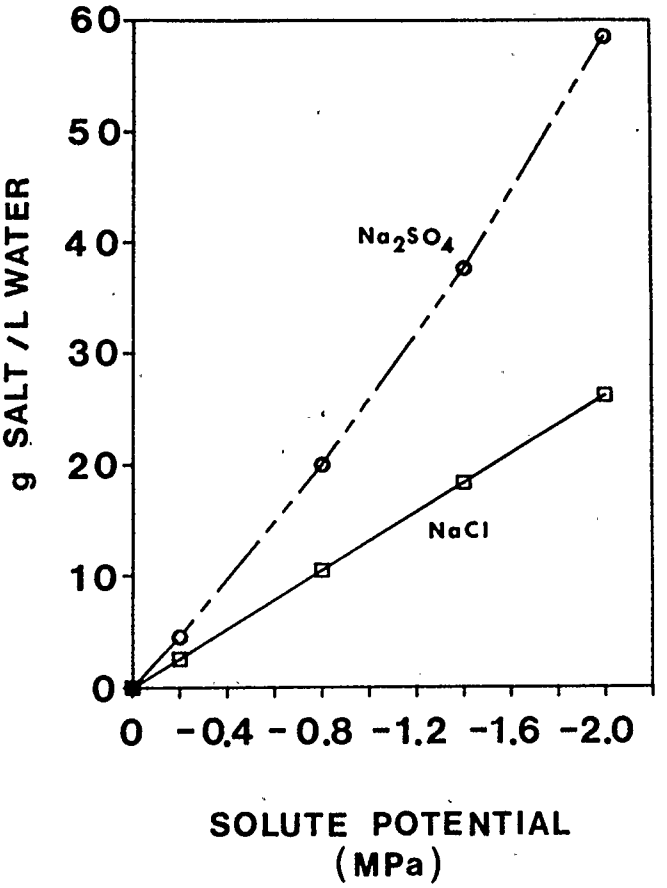

FIG. 1. Amount of NaCl or Na_2SO_4 needed to produce the corresponding solute potentials.

microscopic examination to measure leaf thickness. (iii) epidermal peels were observed under a microscope to determine numbers of cells per unit area (a decrease in numbers indicating that the cells were 'fatter' and therefore more succulent). (b) prostrate growth habit, determined by measuring plant width (at the widest point) vs. plant height. (c) changes in the numbers of stomata and epidermal cells, and in the sizes of stomates determined by employing a modification of the method used by Sampson (1961), in which clear rapidly drying nail polish (Max. Factor) is applied to leaf surfaces, allowed to dry and then peeled off and mounted on slides where they are then examined under a microscope. The microscope utilized in this case was a Reichert projecting microscope used at a magnification of 800X. (d) leaf areas determined by a leaf area meter (Delta-T devices Ltd. Cambridge, England), or by taking photocopies of leaves and tracing around them with an electronic planimeter. (e) changes in sizes and numbers of leaf cells determined by imbedding leaf cross sections in plastic and examining them under a microscope. Tissue was fixed in 3% glutaraldehyde (GAA) buffered at pH 6.8 with 0.05 M phosphate buffer. After dehydration, the leaf cross sections were embedded in LKB Historesin. Sections were cut with glass knives on an LKB (2218) Historange microtome. They were then mounted on slides and stained with periodic acid-Schiff's reaction (PAS) (Feder and O'Brien 1968) and counter stained with TBO (toluidine blue O).

GLYCINEBETAINE ANALYSIS.

PLANT MATERIAL. Growth conditions for Chenopodium rubrum were the same as those described previously. Analysis were performed on plant

material from two experiments; one carried out under greenhouse conditions and for comparison another was performed under growth chamber conditions. In both experiments NaCl and Na_2SO_4 were utilized. In the greenhouse experiment, treatments included a control (no salt), -0.4 MPa, -1.0 MPa, and -2.1 MPa. The treatments from the growth cabinet experiment consisted of a control, -0.8 MPa, -1.6 MPa, -2.0 MPa (Na_2SO_4 only) and -2.4 MPa. Plants were harvested after 50 days of growth (14 days after reaching maximum salt concentrations). Shoots were washed, blotted dry, weighed and then the leaves were stripped off and wrapped in tin foil which was then immersed in liquid N_2 ready for freeze drying. The dried tissue was then ground and stored in vials at 6°C. Analysis was performed on four plants from each treatment (greenhouse experiment only); only one randomly selected plant was analyzed from the growth chamber experiment. For information on the analysis of field specimens and the experimental conditions in which Salicornia europaea was used, refer to Guy (1984).

EXTRACTION. Approximately 100 mg of tissue was initially ground with 1.2 g acid washed silica sand and 1 to 2 ml of methanol: chloroform: water (12:5:3, v/v). To this was added 4.0 kBq [methyl-^{14}C]-betaine (Amersham). A further 6 ml of extractant was added for a total volume of 8 ml. This slurry was transferred to a 30 ml centrifuge tube with 2 X 2 ml water rinsing of the mortar and pestle, and was centrifuged at low speed for 5 minutes. After centrifugation, the aqeous layer was removed to another centrifuge tube containing 3 ml chloroform. This was thoroughly mixed and re-centrifuged. The aqueous layer was again

removed and reduced to a volume of approximately 1 ml under a stream of filtered air at 65°C.

ION EXCHANGE. Ion exchange purification was similar to that described by Hitz and Hansen (1980), consisting of a three-resin system. The extract was applied first to a 3 ml column containtng AG-1 (200-400 mesh, OH^- form). The effluent from this then flowed directly onto a 3 ml Bio-Rex 70 (200-400 mesh, H^+ form) column which in turn emptied onto a column containing 3 ml AG-50 W (200-400 mesh, H^+ form). A volume of 20 ml H_2O was used to wash the columns. The betaines (which became bound to the AG-50 W column), were then eluted with 10 ml of 4 M NH_4OH, dried down at 65 °C under a stream of air, and redissolved in 1-2 ml water. After millipore filtering the sample, aliquots were taken for counting on a Packard Minaxi Tri-carb 4000 series liquid scintillation counter prior to HPLC.

If high levels of betaine were likely to occur and no peak interference was expected, we did not utilize the AG-50 W column. Instead, the extract was applied to either a single mixed or layered bed column of AG-1 and Bio-Rex 70 and the effluent collected. In many cases this fraction could be directly filtered, counted and loaded onto the HPLC without further treatment. This afforded us a considerable saving in time.

LIQUID CHROMATOGRAPHY. High pressure (performance) liquid chromatography (HPLC) was performed using a Bio-Rad 250 X 4 mm Aminex HPX-87 C carbohydrate column, preceeded by a Micro-Guard Carbohydrate guard cartridge. Acetonitrile (25%) was used in the mobile phase and

was delivered by a Spectra-Physics SP 8700 solvent delivery system. The flow rate never exceeded 0.5 ml min^{-1} and was typically maintained at 0.3 ml min^{-1}. Column temperature was kept at 60°C. Betaine detection was achieved by using a Gilson Model HM Holochrome variable wavelength UV monitor (range 0.2, 192 nm). The effluent, in some cases (see Guy et al. 1984), also passed through a Berthold LB 503 HPLC Radioactivity Monitor (range 1K cpm, 10s time constant). For the analysis of C. rubrum specimens, effluent was collected on a Gilson Model 201 programmable fraction collector, and aliquots were then taken for counting. Samples were injected via a Rheodyne Model 7125 sample injector fitted with a 20 µl sample loop. Injection volumes ranged from 5-20 µl.

For some samples, the betaine content was verified using thin layer chromatograms (Bakerflex Silica Gel 1B2). They were developed according to Storey and Wyn Jones (1975).

WATER RELATIONS. Water and osmotic potential measurements were carried out using a Wescor HR-33T microvoltmeter (dew point mode) in combination with several C-52 type sample chambers (constructed by University Technical Services). Axial leaves from the fifth, sixth or seventh nodes (counted up from the bottom of the plant) were used in the determinations. Discs (.385 cm^2) from these leaves were cut out using a cork borer and placed into the C-53 sample dishes (1 disc per dish) for measurement of leaf water potentials. Equilibration times ranged from half an hour for salt free control plants to one and a half hours for plants grown at -1.0 to -2.0 MPa. After measurements were taken, the leaf discs were removed, wrapped in aluminum foil and frozen

in liquid N_2. Following this, they were allowed to thaw and were then returned to the C-52 chambers for measurement of leaf solute potentials (approximately 1/2 hour for equilibration). Leaf pressure potential was than calculated as the difference between Ψ_w^{leaf} and Ψ_s^{leaf}.

ASH WEIGHTS. Approximately 60 to 100 mg of freeze dried leaf tissue was loaded into pre-weighed acid-washed porcelain boats and combusted in a muffle furnace at 450°C for 21 hours. After cooling, the boats were reweighed and ash weights determined. Sample sized ranged from 3-4 plants per treatment.

GAS EXCHANGE ANALYSIS. The measurement of transpiration, CO_2 assimilation rates and related variables were made in a gas exchange cuvette as described by Guy (1984) with the following modifications: the cuvette was enlarged slightly to accommodate the larger Chenopodium rubrum leaves. As well, the entry port, originally designed for plants with long petioles or elongated leaves (i.e. grasses), had to be altered. This was accomplished by cutting a notch in the side wall of the cuvette and fitting a sliding door into it which could be removed while placing the leaf into the cuvette. Vacuum grease was then used to seal the small hole around the petiole. One disadvantage of this revised system was that only one leaf at a time could be measured.

The light source utilized for this study also differed considerably from that used by Guy. Instead of florescent tubes and a 300 watt sealed beam PAR lamp, I used the light from a slide projector. This was preferred over the other source(s) for the simple reason that the build up of heat was prevented.

The plant leaves were exposed to conditions of light intensity, temperature, humidity and CO_2 concentrations similar to those under which they were grown. Net photosynthesis rates and transpiration rates were allowed to reach a steady state before measurements were taken. This usually took approximately 30 minutes. The measurements taken while the leaf was enclosed in the cuvette included: cuvette input and output air temperature and relative humidities, (measured with 2 Vaisala RH and temperature probes); leaf temperature (measured by a copper-constantan thermocouple appressed to the lower leaf surface); flow rate of air into the cuvette; CO_2 concentration after it had flowed through the cuvette (measured with a Beckman infrared gas analyzer model 865, after the air had passed through an ice trap); and leaf areas (measured with a leaf area meter).

Calculations of variables were identical to those of Guy (1984), who used the formulations of Jarman (1974), Jarvis and Mansfield (1981), Nobel (1983), Nobel et al. (1978), for calculations of P_n and R_s and those of Caemmerer and Farquhar (1981) for the determination of C_i/C_a ratios.

CARBON ISOTOPE ANALYSIS. Carbon isotope analysis was performed by Dr. Robert Guy at the Carnegie Institution of Washington, Dept. Geophysics in Washington D.C. on plant material from an experiment performed under greenhouse conditions (covered previously). Each sample of leaf tissue (10 mg each) was combusted twice in quartz tubes (6 mm OD) at 850°C for 1 hour. The furnace was programmed to heat up to this temperature at 450°C per hour and to cool down from it at 40°C per hour. After the

removal of water vapour and non-condensable gas (eg. N_2) the CO_2 was analyzed on a Nuclide Triple Collector Mass Spectrometer. Linear regression lines were fitted to data for both NaCl and Na_2SO_4 salinity, and were also compared using a t-test (Zar 1974).

RESULTS

MORPHOLOGICAL AND ANATOMICAL ANALYSIS.

GROWTH. All plants growing in NaCl and Na_2SO_4 solutions (with the exception of those in solutions equal to or greater than -1.6 MPa Na_2SO_4) survived and showed no signs of necrosis or wilting throughout the duration of the experiments. The limit of tolerance for plants growing in NaCl was at approximately -3.0 MPa.

Optimal growth (by dry weight) was recorded for plants growing at -0.4 MPa. Plants grown in the absence of salt produced a lower amount of dry matter. Dry matter production was reduced significantly at media solute potentials below -0.4 MPa (fig. 2).

In another experiment growth was measured in terms of plant height. At -0.8 MPa, plants were slightly taller than controls (fig. 3), but this was reduced significantly at solute potentials lower than this. In figure 4, a comparison of plants grown in Na_2SO_4 is shown. Note the increase in size of plants at -0.4 MPa. Plants grown in NaCl showed a similar growth response, except that at higher NaCl concentrations plants appeared to be much healthier than those at high Na_2SO_4 concentrations.

SUCCULENCE. Another effect that increasing salt concentrations had on leaf tissue was an increase in succulence (initially measured in terms of water content per unit area) (fig. 5). Both salts showed substantial increases from 0 to -0.8 MPa, however NaCl had a much greater effect beyond this point. Free-hand cross sections of leaves were taken for microscopic examination in order to measure leaf thickness (another

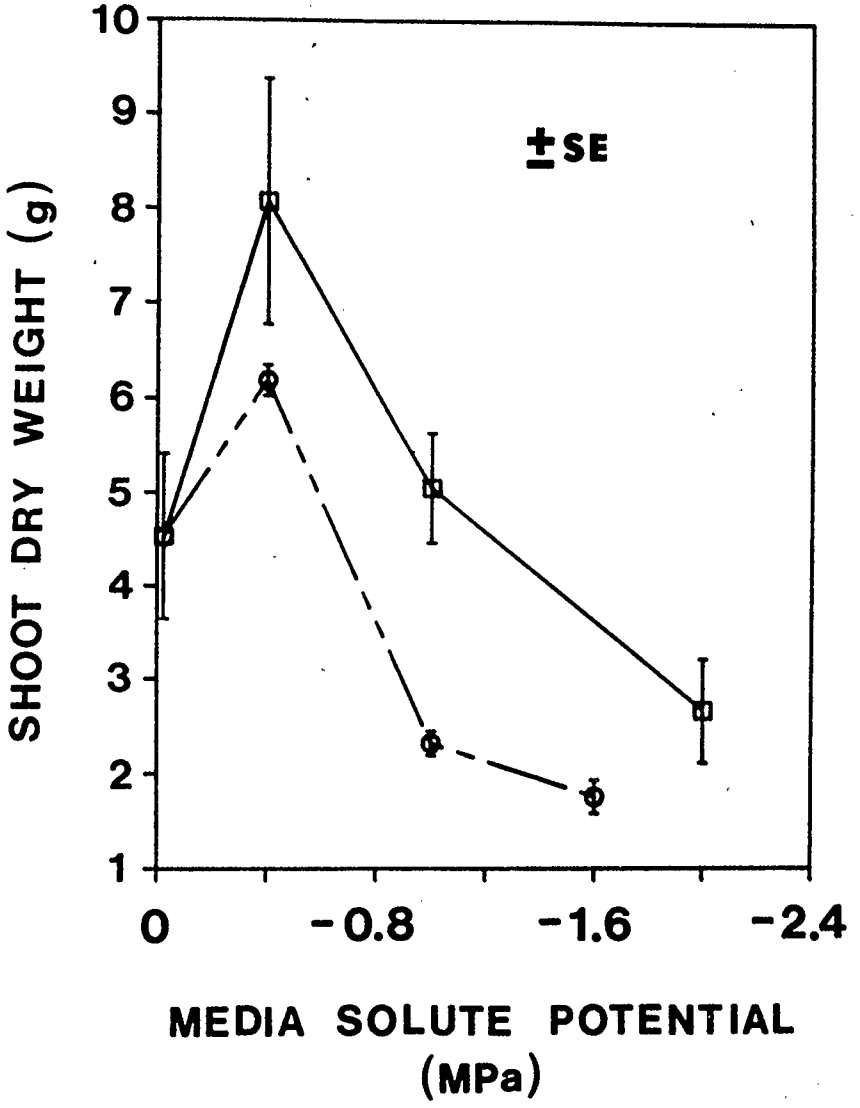

FIG. 2. Dry weights of shoots grown in NaCl (□), and Na_2SO_4 (o). Plants were left 14 days after final salt concentrations were reached before harvesting (45 days after germination). Data points represent the means ± SE of six plants per point.

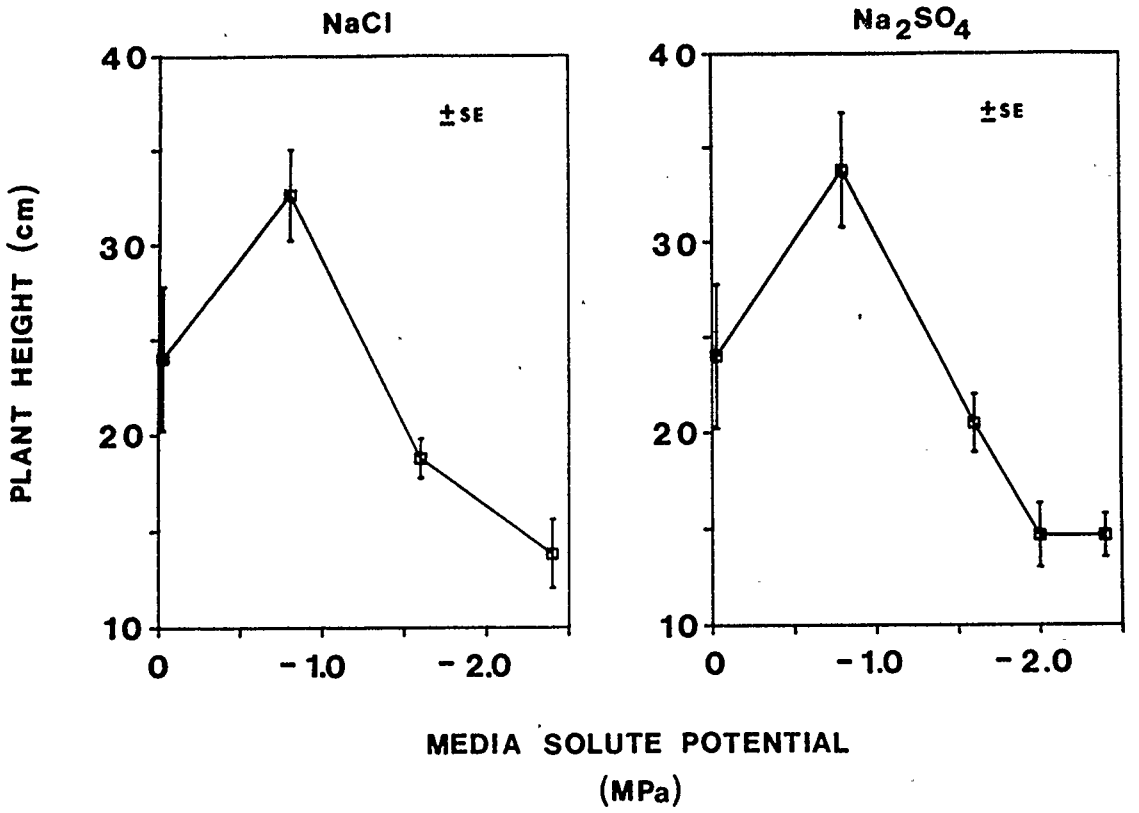

FIG. 3. Effect of decreasing media solute potentials on plant height. Data points represent the mean ± SE (six plants per point).

FIG. 4. Effect of decreasing media solute potentials on growth of Chenopodium rubrum. Plants grown in NaCl showed a similar growth pattern.

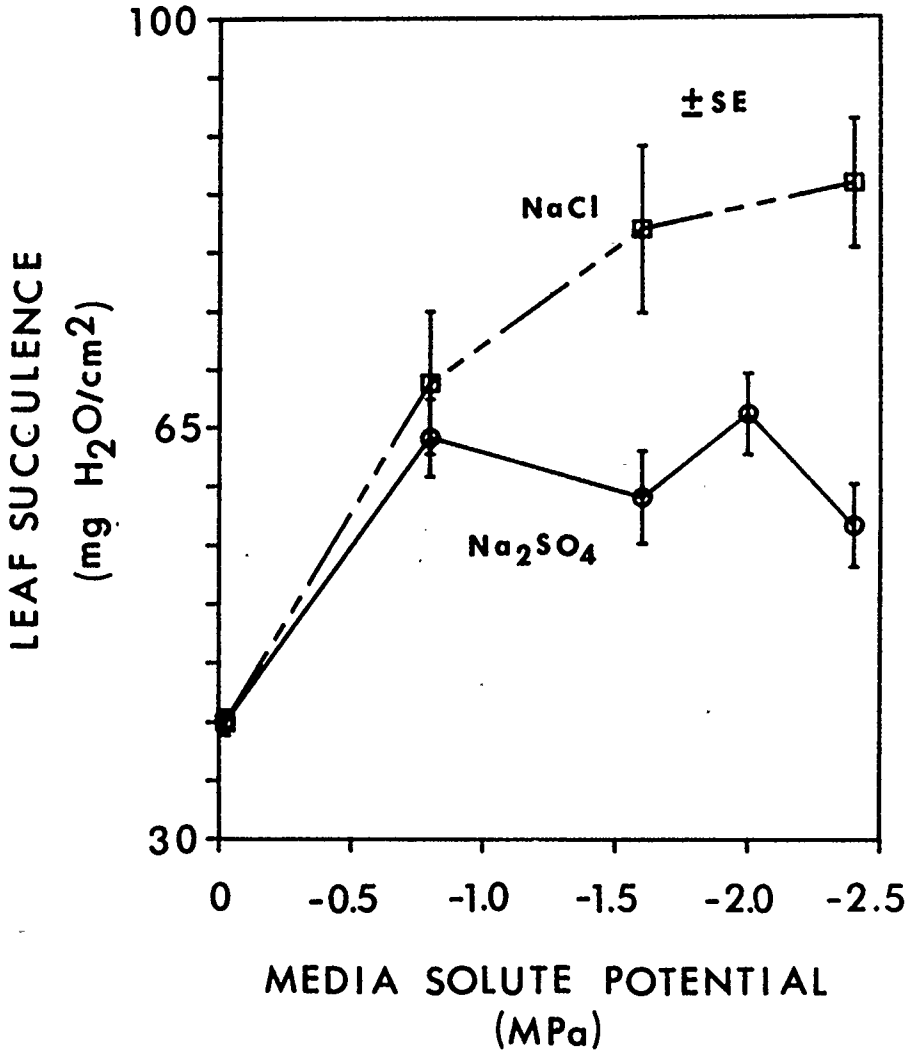

FIG. 5. Effects of NaCl or Na_2SO_4 on leaf succulence. Data points represent means ± SE (six plants per point).

indication of leaf succulence). However, obtaining good sections was very difficult and the results were highly variable even within the same treatment. Therefore cross-sections embedded in plastic were prepared in order to see more clearly the types of cells present as well as numbers and sizes of cells not seen as well in free-hand sections. Figure 6 generally shows that as media solute potentials decreased, leaf thickness increased, except in the -2.1 MPa Na_2SO_4 treatment which had values close to those found in the controls. Thickness ranged from about 630 µm in the controls to a maximum of about 990 µm in the -2.1 MPa NaCl treatment. It is quite obvious that treatment of plants with Na_2SO_4 did not have nearly as much of an effect on this parameter as NaCl did.

The increase in leaf thickness was due to an increase in both the palisade and spongy mesophyll layers (fig. 7), although the increase was not as substantial in Na_2SO_4 treatments (especially in the case of the spongy mesophyll where values remained relatively constant with increasing salinity). When this data was presented in terms of a percentage of total leaf thickness (fig. 8), one can see that with increasing salinity the amount of palisade increased relative to mesophyll (up until -1.0 MPa). Cells of the palisade layer increased both in length and diameter in treatments with NaCl (fig. 9). On the other hand, when plants were treated with Na_2SO_4, cells increased in length up to -1.0 MPa, while cell diameters remained relatively constant up to this point and then decreased significantly at -2.1 MPa (fig. 9B). Mean numbers of palisade cell layers also increased with increasing salt concentrations except in the case of the -2.1 MPa

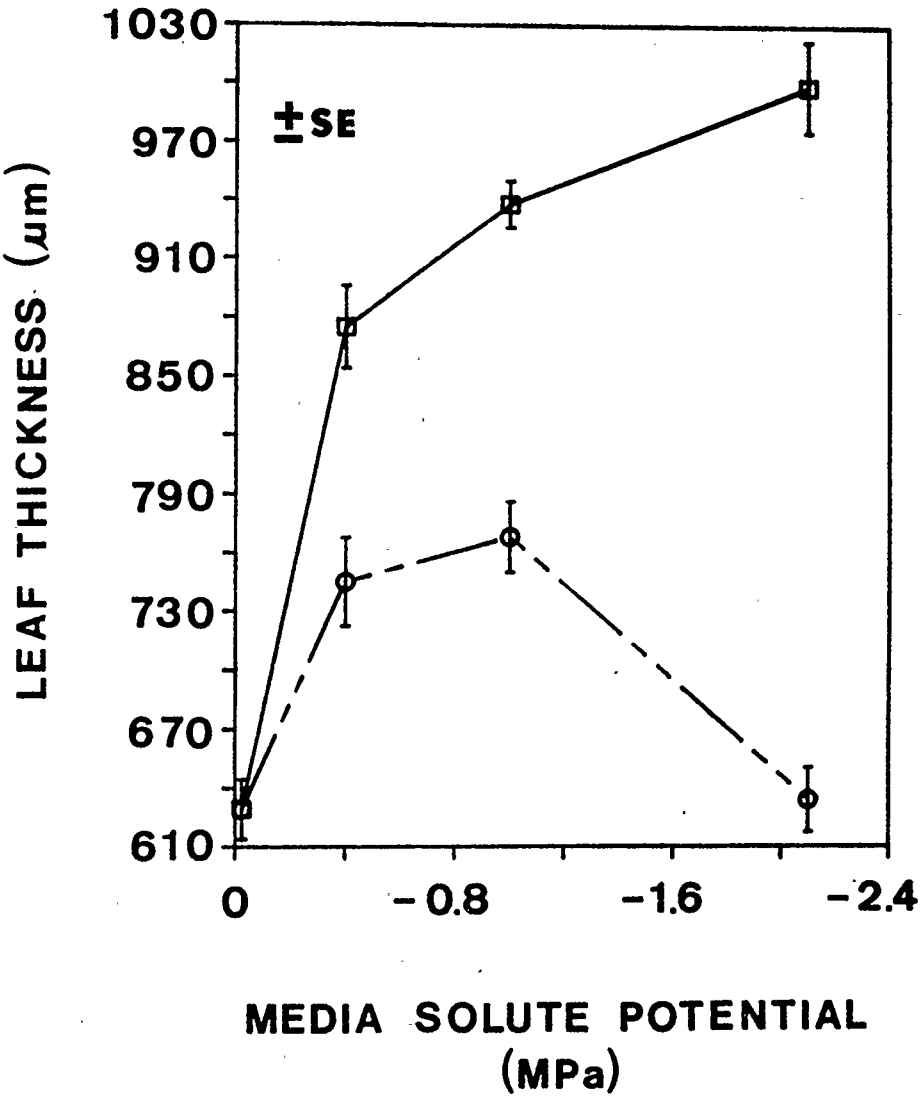

FIG. 6. Effect of increasing concentrations of NaCl □ , or Na_2SO_4 ○ on leaf thickness. Standard error bars are included and are based on at least six sections per treatment.

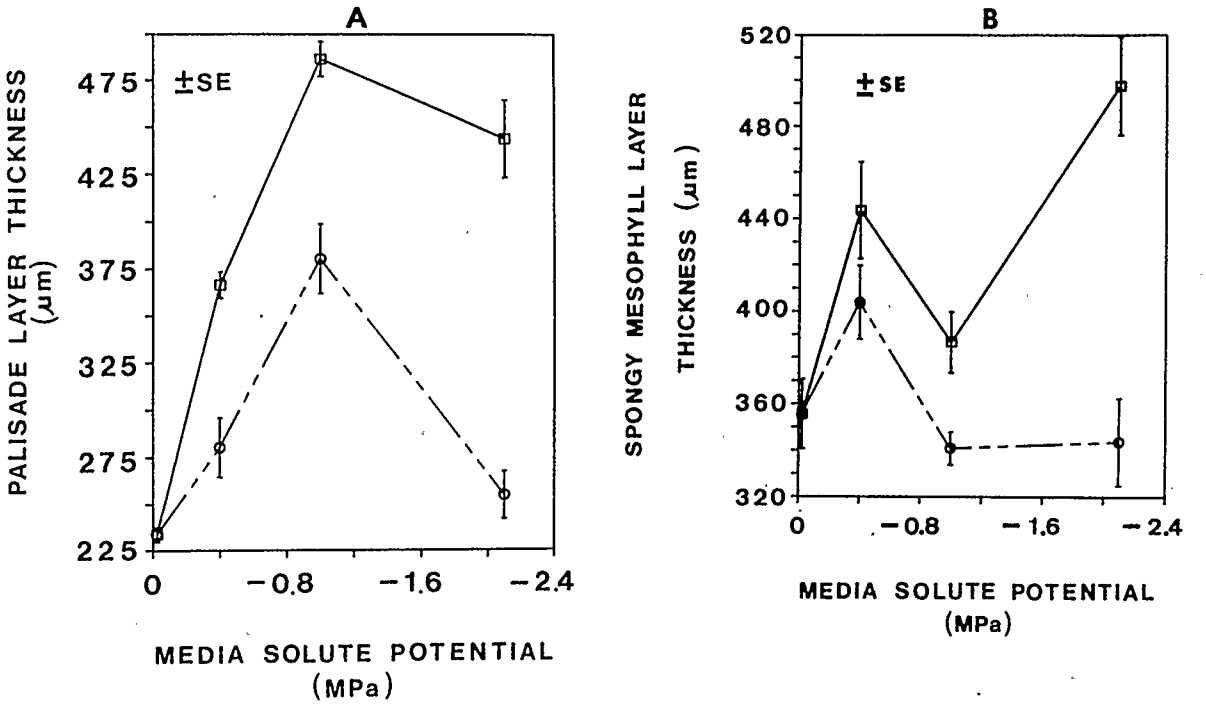

FIG. 7. Comparative effects of NaCl □ , or Na_2SO_4 O on the thickness of the two leaf cell layers. A. Palisade layer. B. Spongy mesophyll layer.

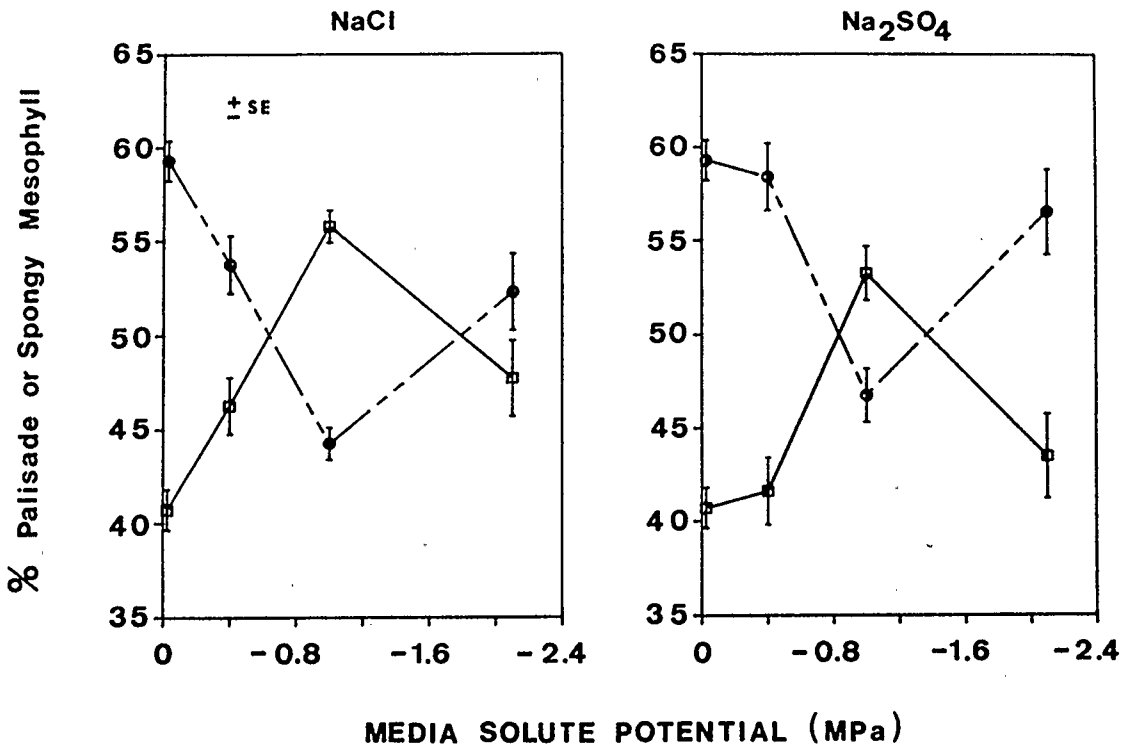

FIG. 8. Effect of increasing salinity on the relative proportions (as a percentage) of palisade or spongy mesophyll layers contributing to total leaf thickness. Data points represent means ± SE (n=10). Palisade layer □ ; Mesophyll layer ○ .

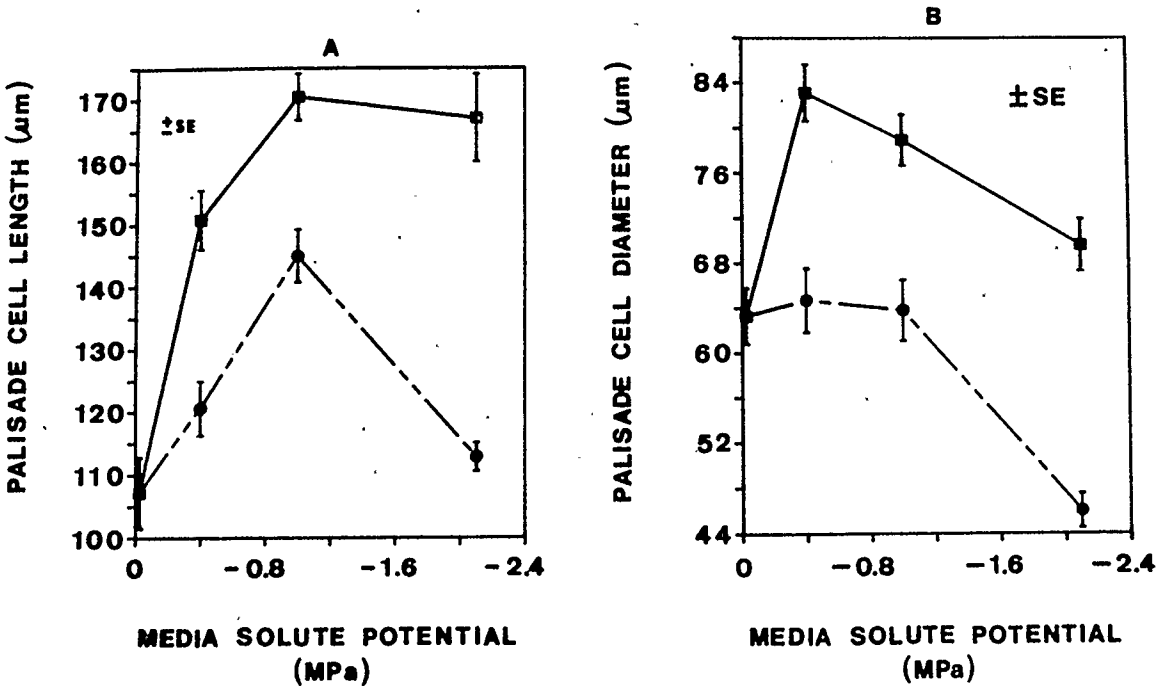

FIG. 9. Effect of decreasing external solute potentials on cells of the palisade layer. A. Palisade cell length. B. Palisade cell diameter. NaCl ■ ; Na_2SO_4 ● .

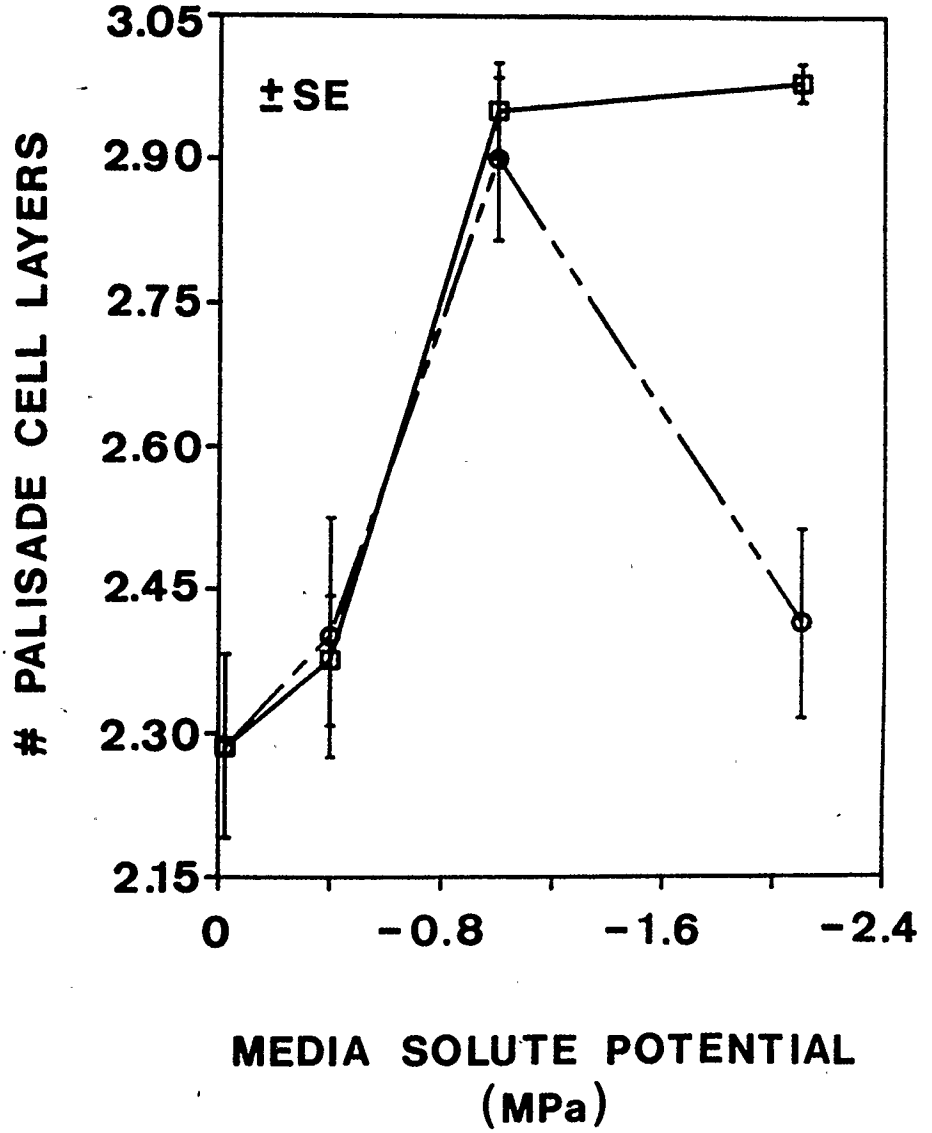

FIG. 10. Mean number of layers of palisade cells effected by increasing salinity. NaCl □ ; Na_2SO_4 ○ .

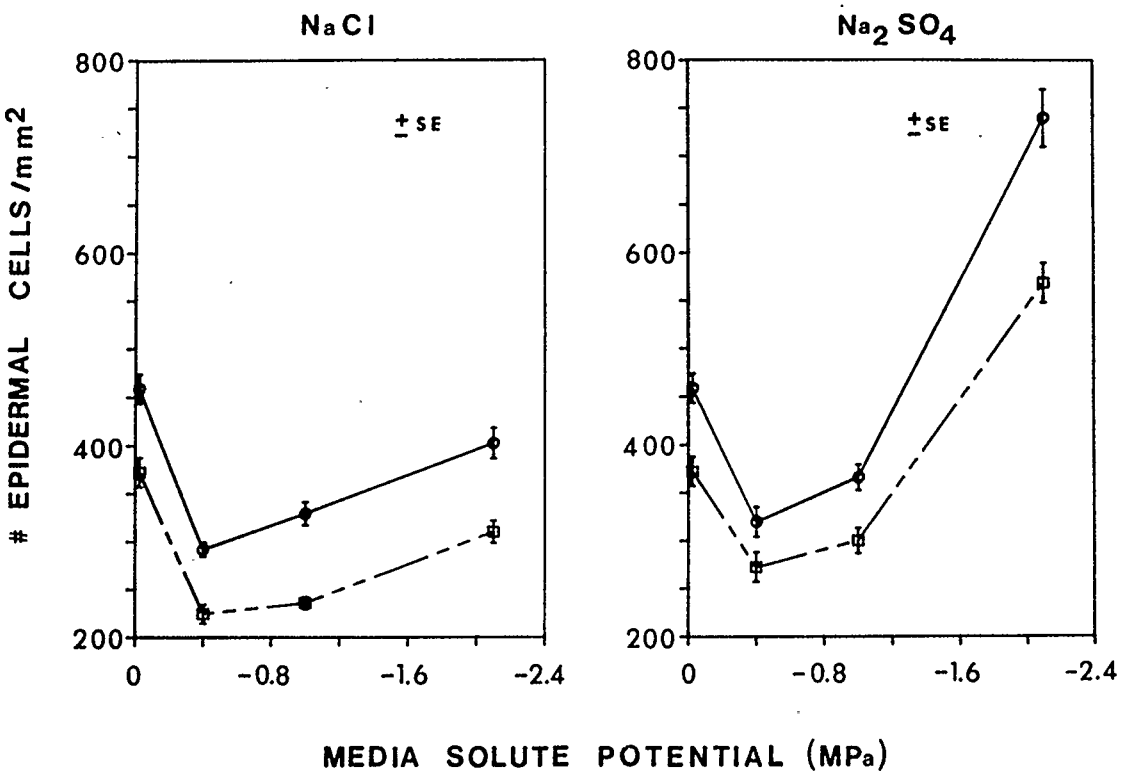

FIG. 11. Epidermal cell densities under different Na_2SO_4 or NaCl treatments. Data points represent means ± SE (12 slides per treatment). See the legend for fig. 12. Abaxial leaf surface O ; Adaxial leaf surface □ .

treatment (fig. 10). Similar data for mesophyll cells could not be obtained due to the fact that this cell layer was not as structurally uniform as the palisade layer.

Epidermal cells also showed an increase in succulence. Although actual sizes were not measured, the fact that numbers decreased per unit area indicated that cells were increasing in size (fig. 11). An exception to this was again, at the high Na_2SO_4 concentrations where a decrease in succulence was observed.

STOMATAL NUMBERS. In view of the fact that the growth of plants declined as salt concentrations increased, I decided that the effects of salt on stomatal numbers and sizes should be investigated since any changes would presumably have an effect on CO_2 uptake and hence the production of photosynthate needed for growth. As it turned out, when plants were exposed to increasing concentrations of both Na_2SO_4 and NaCl there was a definite change in stomatal densities. In figure 12 the number of stomates (on both abaxial and adaxial leaf surfaces) per mm^2 decreased from the controls, but increased again at higher salinities. This trend was especially evident in the Na_2SO_4 treatment where densities were actually higher at -2.1 MPa than in the controls. On the other hand, when data was presented on the basis of total leaf area, stomatal cell numbers decreased (fig. 13). In NaCl treatments the decrease in numbers occurred at -0.4 MPa whereas in Na_2SO_4 treatments, numbers were not significantly different between the controls and -0.4 MPa treatment. The reason for the difference in trends between figures 12 and 13 is that total leaf areas decreased with increasing salinity (fig. 14). Numbers of stomatal cells were always lower on the upper

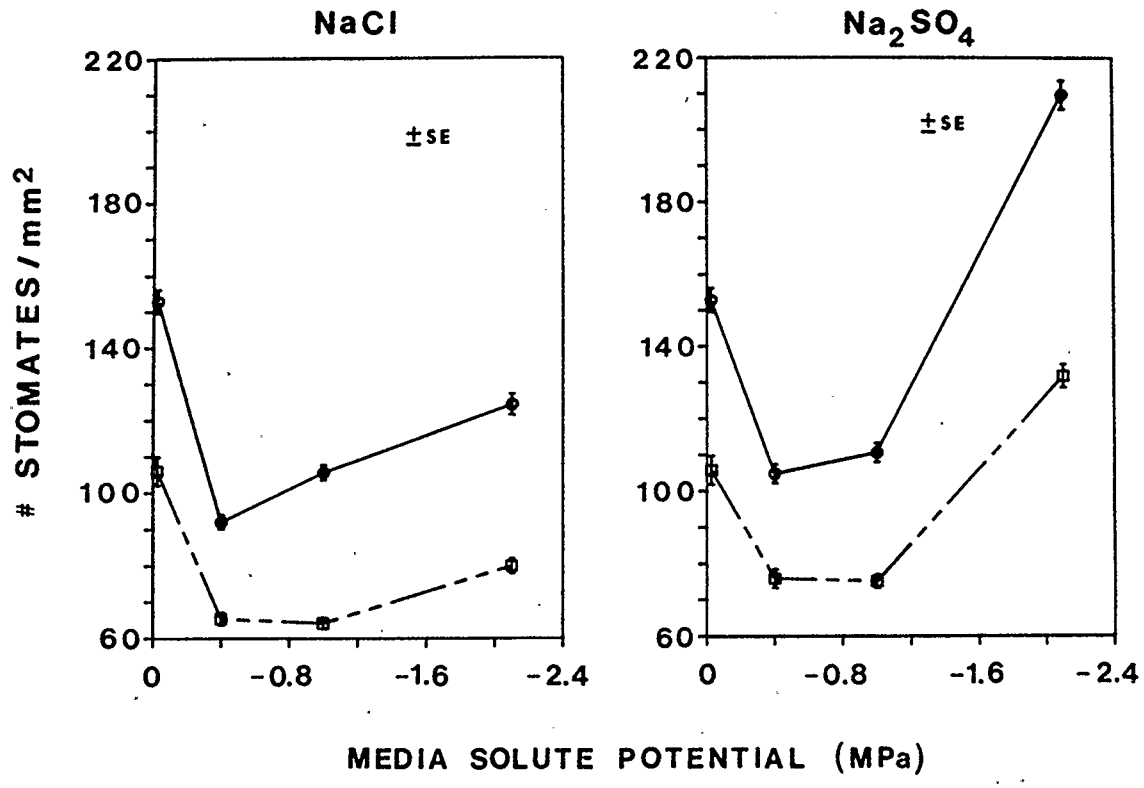

FIG. 12. Effects of varying concentrations of NaCl and Na_2SO_4 on stomatal densities. Plants were grown in the greenhouse under high irradiances and were 48 days old when harvested. Stomates were counted in five different areas on each leaf and then averaged. Each data point represents the mean and SE of 12 plants per point. Abaxial surface o ; Adaxial surface □ .

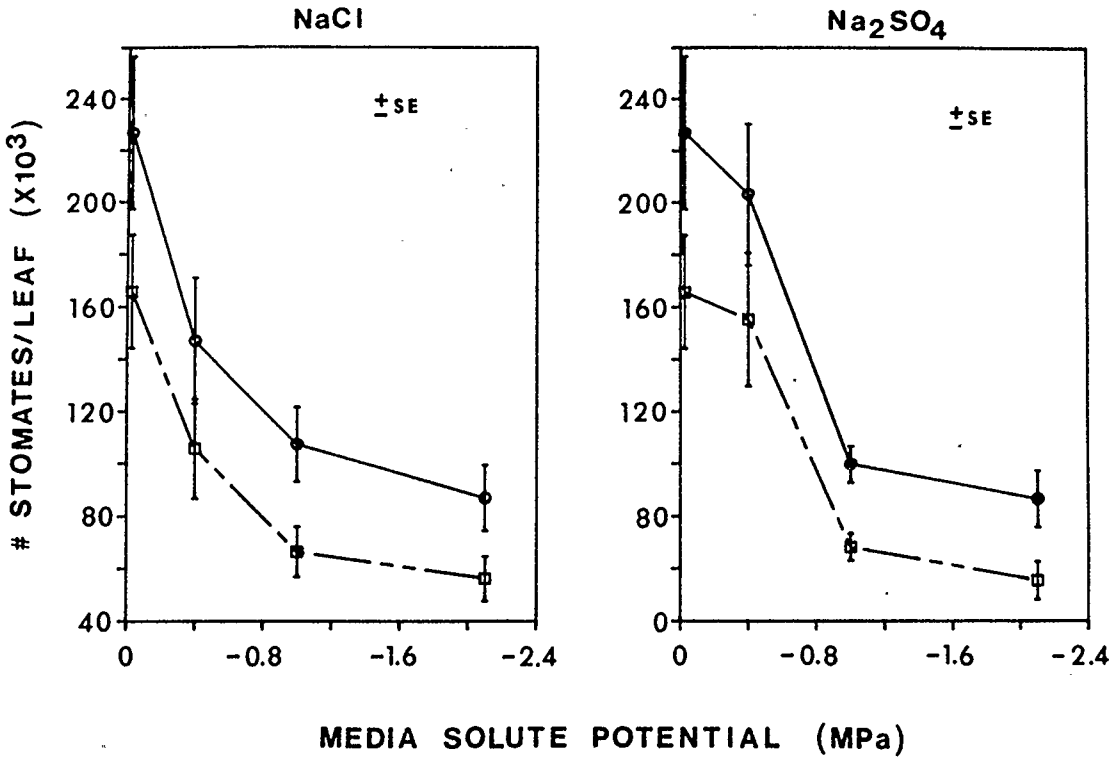

FIG. 13. Influence of increasing concentrations of NaCl and Na_2SO_4 on total numbers of stomates per leaf. Growth conditions and statistics were the same as in fig. 12. For more details see materials and methods section. Abaxial leaf surface O ; Adaxial leaf surface □ .

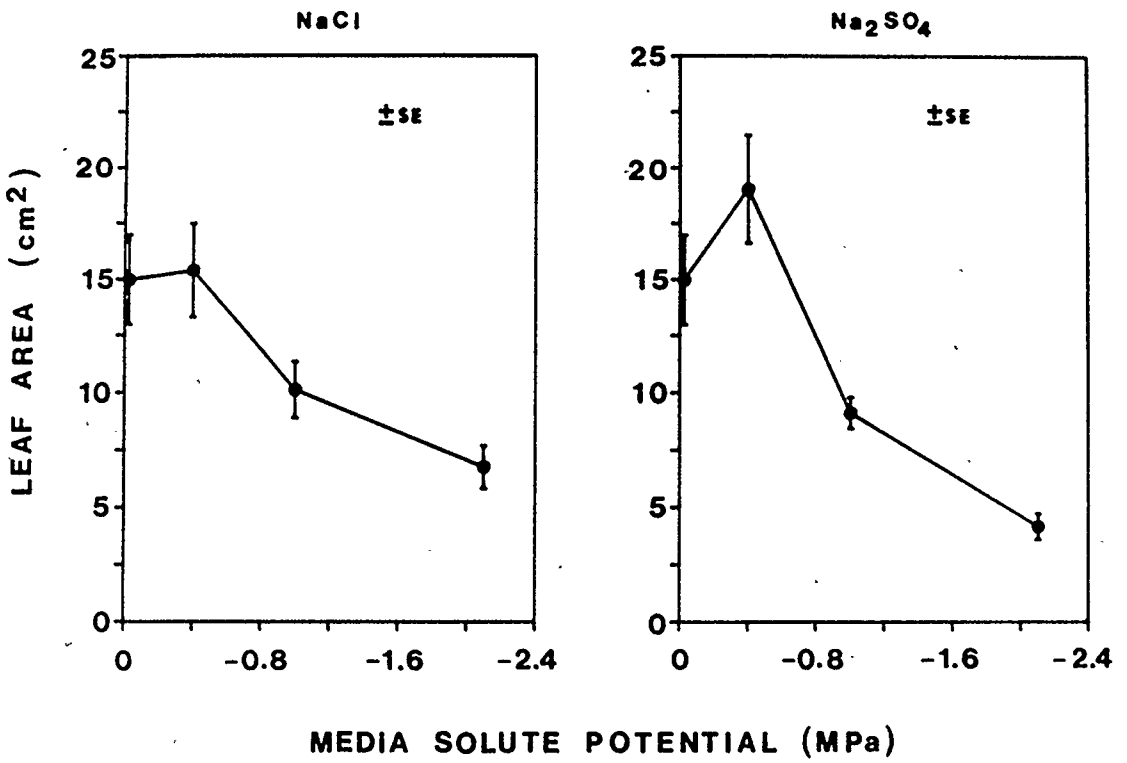

FIG. 14. Effect of different concentrations of NaCl and Na_2SO_4 on leaf areas. Compare with figures 12 and 13. Data points represent mean ± SE of 12 plants per point.

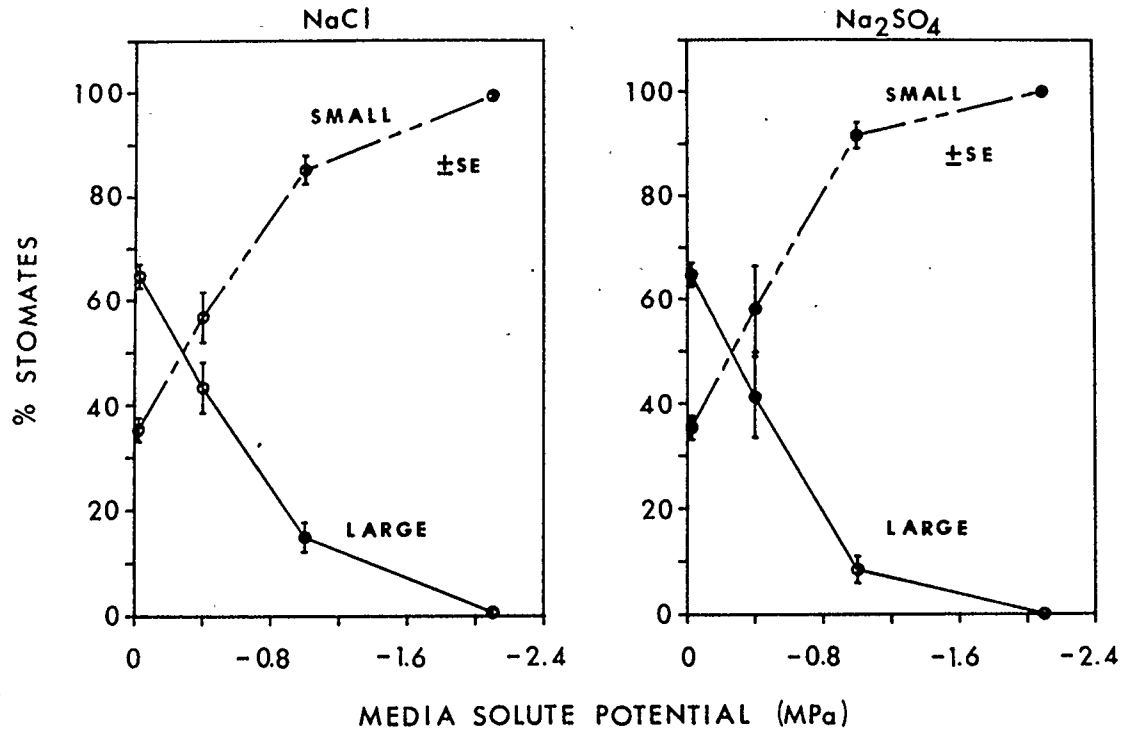

FIG. 15. Effect of decreasing media solute potentials on the size of stomates. Results are based on a % of the total numbers of stomates per mm^2 using the same epidermal peels as were used in figures 12 and 13. Each data point in this case represents the mean ± SE of six leaf epidermal peels per point. Large stomates (> 7mm in length) ; Small stomates (< 7 mm length).

(adaxial) surface of the leaves. The preceeding data was from an experiment performed in the greenhouse (see materials and methods for more details), and although some of the growth conditions were significantly different than those in growth cabinet experiments, the results (not shown) were remarkably similar. A change in the size of stomates was also observed (fig. 15). Stomates were measured lengthwise using a Reichert projecting microscope usually at a magnification of 800X. They were classified into two catagories, large (> 7 mm) or small (< 7 mm). When the solute potential of the growing solutions was decreased it can be seen that the numbers of large stomates also decreased to around zero % at -2.1 MPa. On the other hand, there was a significant increase in the numbers of small stomates. This trend was almost identical for both salt treatments.

PROSTRATE GROWTH. Another parameter which was measured was prostrate growth habit. Although this phenomenon is known to occur in the natural environment under saline conditions, results from several experiments where this character was measured did not show much effect. Figure 16 shows the results from one such experiment performed under growth chamber conditions. At first glance it appears that at -2.0 MPa (NaCl) there is an increase in prostrate growth. However, the effect then decreased at -2.4 MPa. There appears to be little or no effect in the Na_2SO_4 treatments. Since the light intensity under natural conditions is significantly higher than that in the growth chamber, an experiment was initiated in the greenhouse to investigate whether or not light intensity in combination with salinity had any influence on this character. Results from that experiment (not shown) showed even less of

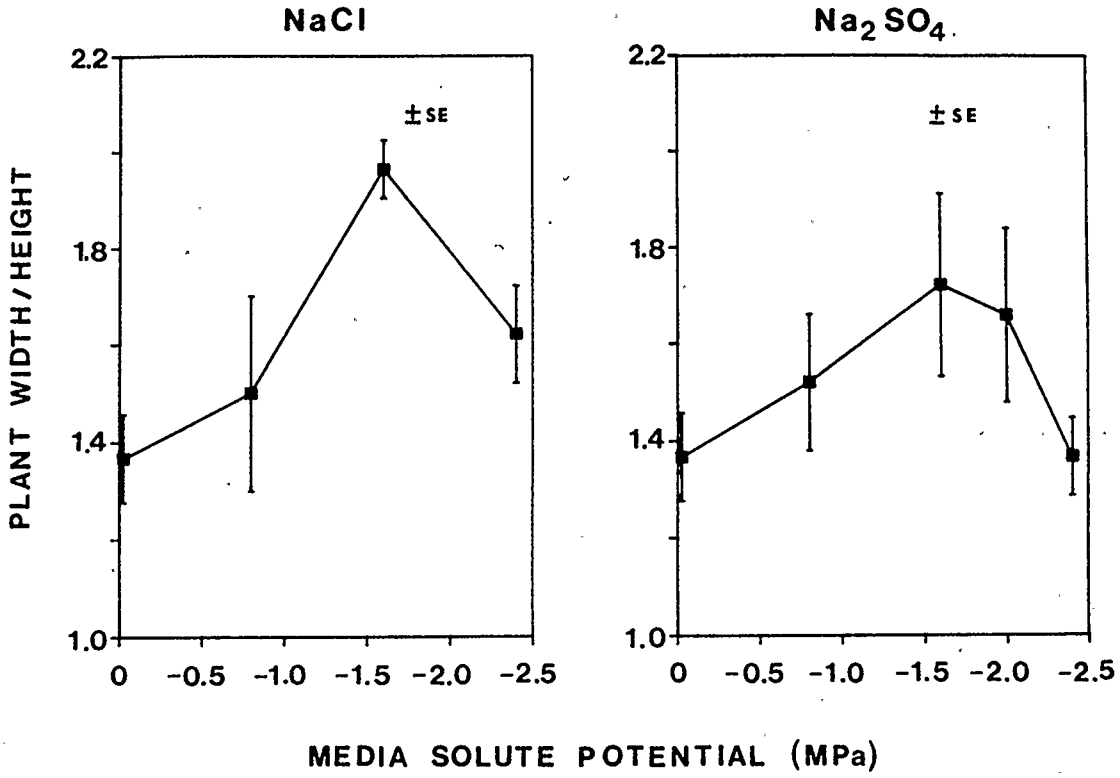

FIG. 16. Effect of increasing concentrations of NaCl and Na_2SO_4 on prostrate growth habit, measured by dividing the width of plants (at the widest point) by the height. Plants were grown in a growth chamber and were 45 days old when measured. Data points represent mean ± SE (from 3 to 6 plants per point).

an effect. Time constraints did not permit the pursuit of other lines of investigation to explore the causes of this particular phenomenon.

PHYSIOLOGICAL ANALYSIS

GLYCINEBETAINE ACCUMULATION. It is well known that many of the salt tolerant chenopods accumulate glycinebetaine in response to saline conditions. For this reason an investigation was initiated to see whether or not Chenopodium rubrum accumulated betaine as well.

The methods employed for the analysis of betaine were worked out by Dr. Robert Guy and myself. Many of the techniques traditionally used for the determination of betaine have major disadvantages. Thin-layer electrophoresis with scanning densitometry for example, is not particularly sensitive or convenient to use, and although pyrolysis-gas chromatography is an improvement over the latter technique, the probes which are required for it are quite expensive and need replacement frequently. These disadvantages prompted us to search for an alternative means of analysis. HPLC has been used successfully for the detection of betaine in the sugar beet and wine making industries (Vialle et al. 1981) and with some modification we have found this technique to be just as or more sensitive and specific than pyrolysis-gas chromatography. It can also be used on a wide variety of plant species and has the added advantage of providing information on other components such as carbohydrates.

We surveyed 27 species (collected from the field) using the above method and found that of 13 which qualified as betaine accumulators, (those accumulating significant levels), 8 were members of the Chenopodiaceae (Table 1, taken from Guy 1984). Chenopodium rubrum was

Table 1. Glycinebetaine contents of leaves of field collected plant specimens. Soil water potential (Ψ_w^{soil}) at the time of collection is given where measured, and estimated where not (NS = nonsaline, > ca. -3×10^2 kPa; MS = moderately saline, -10 to -3×10^2 kPa; VS = very saline, $< -10 \times 10^2$ kPa). Number of specimens checked (N) and plant type as halophyte (H), mesophyte (M), or somewhat salt-tolerant (T) is also present. Nomenclature is consistent with Scoggan (1978). 10^2 kPa = 1 bar, ND = non-detectable.

FAMILY	SPECIES	TYPE	N	μmol glycine-betaine $(g\ DW)^{-1}$	Ψ_w^{soil} ($\times 10^2$ kPa)
Chenopodiaceae	Atriplex nuttallii	H	1	151	VS
	A. patula	H	2	151,188	-1,-13
	Bassia hyssopifolia	H	1	180	VS
	Chenopodium glaucum	H	2	95,140	-22,-6
	C. rubrum	H	2	94,167	-10,-5
	Kochia scoparia	T	1	269	-11
	Salicornia europaea	H	10	141-296	-20 to -69
	Suaeda maritima	H	1	183	-19
Compositae	Aster laurentianus	H	1	63	-8
	Lactuca scariola	T	1	ND	NS
	Solidago gigantea	M	1	118	NS
	Sonchus arvensis	T	3	ND	0 to -13
Cyperaceae	Carex lasiocarpa	T	1	1	-4
	Scirpus maritimus	H	1	ND	-15
Gramineae	Agropyron smithii	T	1	119	-16
	Distichlis stricta	H	1	179	-26
	Hordeum jubatum	H	2	229,272	-8,-23
	Puccinellia nuttalliana	H	1	ND	-36
	Spartina gracilis	H	1	88	VS
Juncaceae	Juncus balticus	T	1	ND	-4

Table 1. (continued).

FAMILY	SPECIES	TYPE	N	µmol glycine-betaine (g DW)$^{-1}$	Ψ_w soil (x10^2 kPa)
Juncaginaceae	Triglochin maritima	H	2	ND	0,-21
Leguminosae	Glycyrrhiza lepidota	M	1	ND	MS
Polygonaceae	Polygonum ramosissimum	M	1	ND	-13
	Rumex occidentalis	T	1	ND	-3
Primulaceae	Glaux maritima	H	1	ND	VS
Ranunculaceae	Ranunculus cymbalaria	H	1	ND	VS
Rosaceae	Potentilla anserina	T	1	3	-4

among the species included. Betaine contents for both C. rubrum and Salicornia europaea grown in a range of NaCl or Na_2SO_4 (C. rubrum only) concentrations were also determined (see materials and methods for more details). Initially results were expressed on the basis of dry weight, however, this proved misleading since many euhalophytes take up salt to such an extent that it becomes a major part of the dry weight. S. europaea for example, had ash contents as high as 66% in plants grown at -6.3 MPa. When betaine results were presented on a dry weight basis, there was a trend towards lower betaine contents at the lower media solute potentials. However, when the same data was expressed in terms of μmol g organic matter^{-1} (subtracting the salt content) (figure 17, taken from Guy 1984) one can see that there is a definite increase in betaine contents from 3.9% in the absence of salt, to 7.7% in the presence of salt. Note that NaCl was the only salt utilized for the above experiment. The effects of both NaCl and Na_2SO_4 on betaine contents were investigated utilizing C. rubrum. Like S. europaea, this species also takes up significant amounts of salt, although not to the extent that Salicornia does. Ash contents for C. rubrum ranged from 14% in the absence of salt to about 40% at the lowest media solute potentials (fig. 18). When the betaine data for this species was plotted in terms of dry weight (fig. 19) there was still a definite increase from the controls. With values ranging from 82 μmol (no salt) to a maximum of 266 μmol at the highest Na_2SO_4 concentrations. However, the values were significantly higher when expressed in terms of μmol g organic matter^{-1} (fig. 20), i.e. 97 μmol to 433 μmol. The results presented here are from an experiment carried out under greenhouse

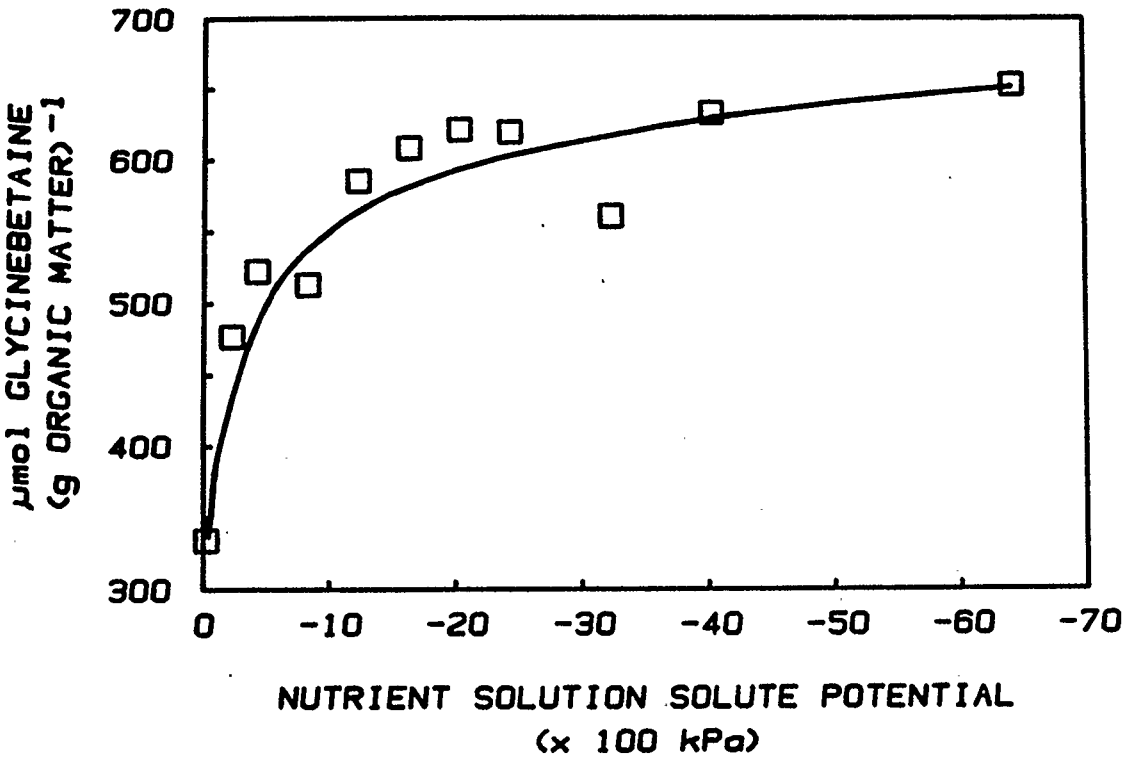

FIG. 17. Glycinebetaine content per g organic matter of Salicornia europaea at different levels of NaCl supplied to the nutrient solution.

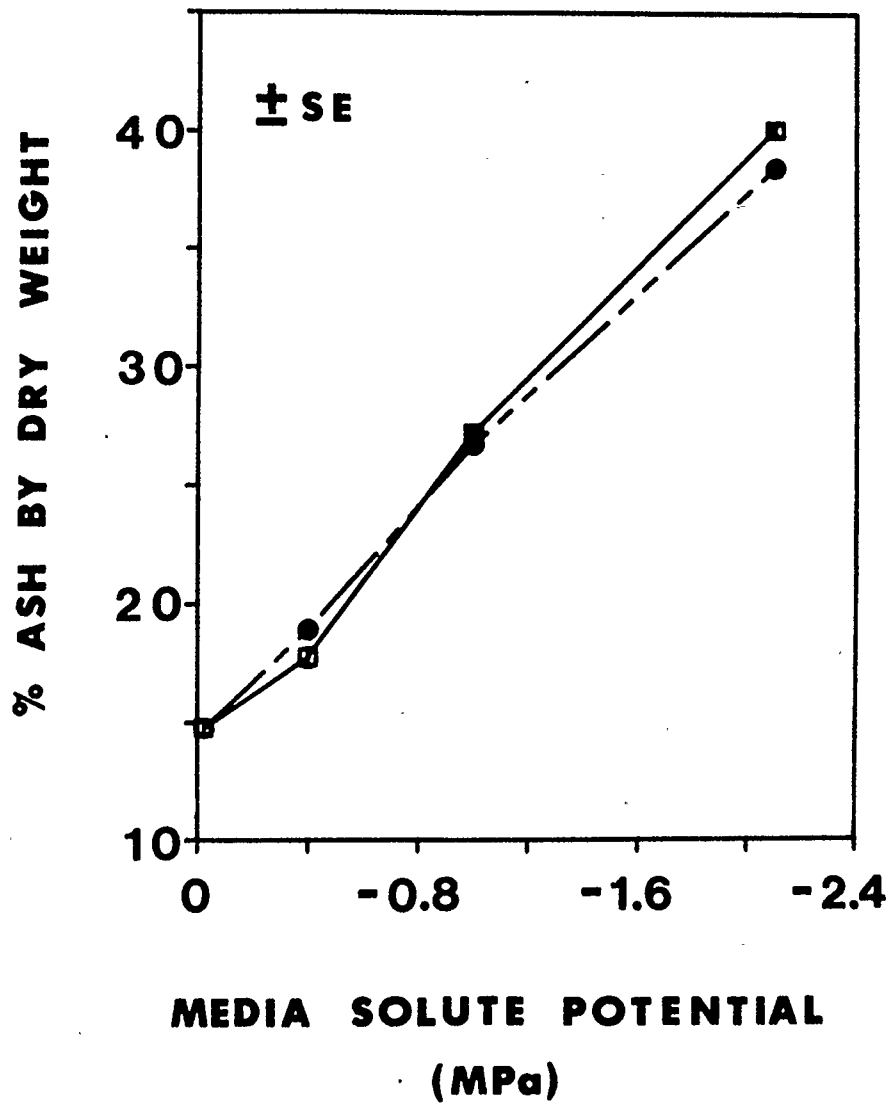

FIG. 18. Ash content of Chenopodium rubrum grown at different concentrations of NaCl and Na_2SO_4. Data points represent mean ± SE (three plants per point). Na_2SO_4 o ; NaCl □ .

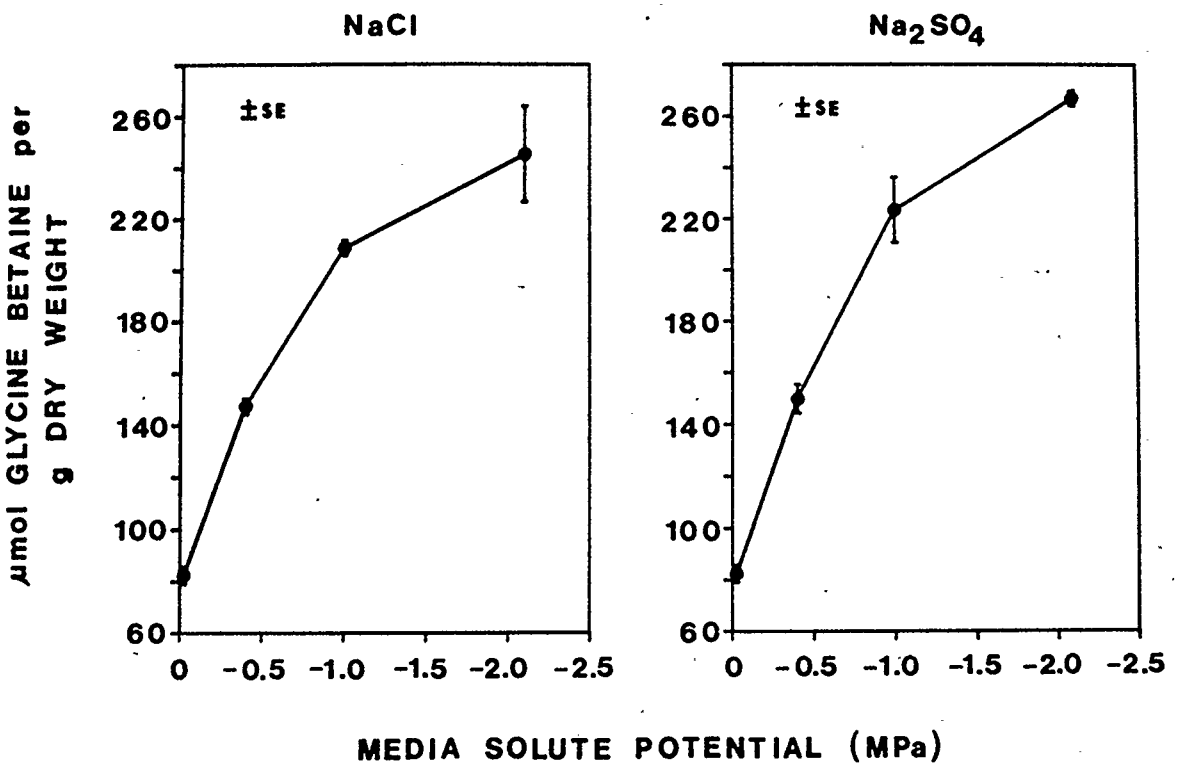

FIG. 19. Effect of different concentrations of NaCl or Na_2SO_4 on glycinebetaine contents in Chenopodium rubrum. Data are presented in terms of dry weight of tissue extracted. Plants were grown under green house conditions. Each point represents the mean ± SE (4 plants per point).

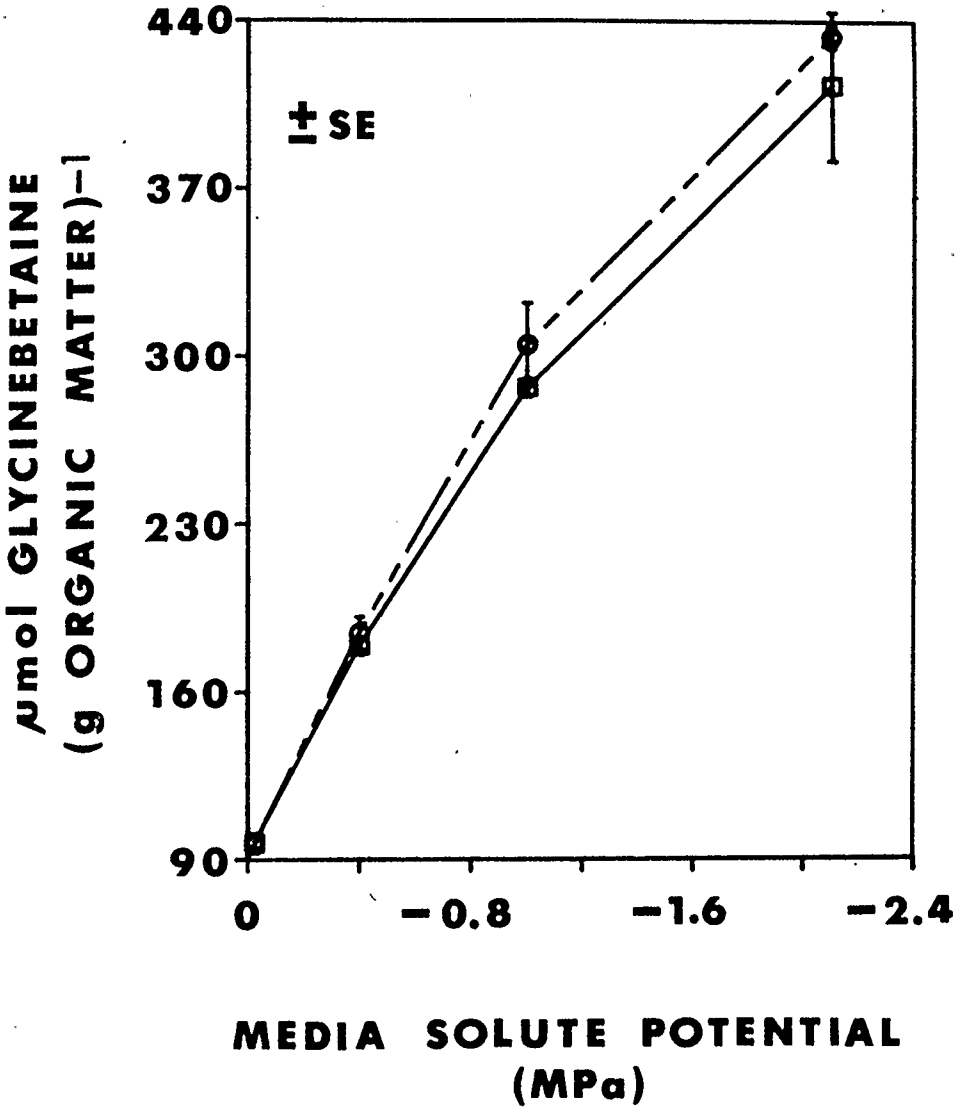

FIG. 20. Glycinebetaine content expressed in terms of g organic matter for Chenopodium rubrum at different media solute potentials. Plant tissue used was the same as that in fig. 19. NaCl □ ; Na_2SO_4 o .

conditions. As mentioned in the materials and methods section, tissue from a growth chamber experiment was also analyzed. The results from that experiment (not shown) were very similar to those presented above.

WATER RELATIONS. The above results confirm that C. rubrum accumulates both electrolytes and betaine. As mentioned in the introduction, the accumulation of these solutes is supposedly involved in the osmotic adjustment of plants in saline environments. In order to find out how well C. rubrum plants had adjusted osmotically to their saline environment, water, solute and pressure potentials of leaf tissue were measured. Results are presented in figure 21. Generally, as salt concentrations increased, both leaf Ψ_w and Ψ_s decreased and were maintained below the isosmotic limit (where $\Psi^{plant} = \Psi^{env}$). Ψ_p remained relatively constant in NaCl treatments, but increased slightly with increasing Na_2SO_4 concentrations. It is interesting to note that the leaf Ψ_w and Ψ_s in Na_2SO_4 treatments are lower than those in the NaCl treatments at the same media solute potentials (below -0.4 MPa). For example, at -1.6 MPa Na_2SO_4 the Ψ_w = -2.84 MPa, while plants grown at -2.0 MPa NaCl had Ψ_w of only -2.5 MPa. Superficially it would appear that plants grown under Na_2SO_4 conditions should have an advantage over those grown in NaCl since their Ψ_w are lower at the corresponding media solute potentials. However a visual inspection of these plants does not confirm this. Figure 22 shows a comparison of two typical plants grown at -2.0 MPa NaCl and -1.6 MPa Na_2SO_4. The plant grown in Na_2SO_4 is much smaller and its leaves are wilted and chlorotic, while the one grown under NaCl conditions looks quite healthy.

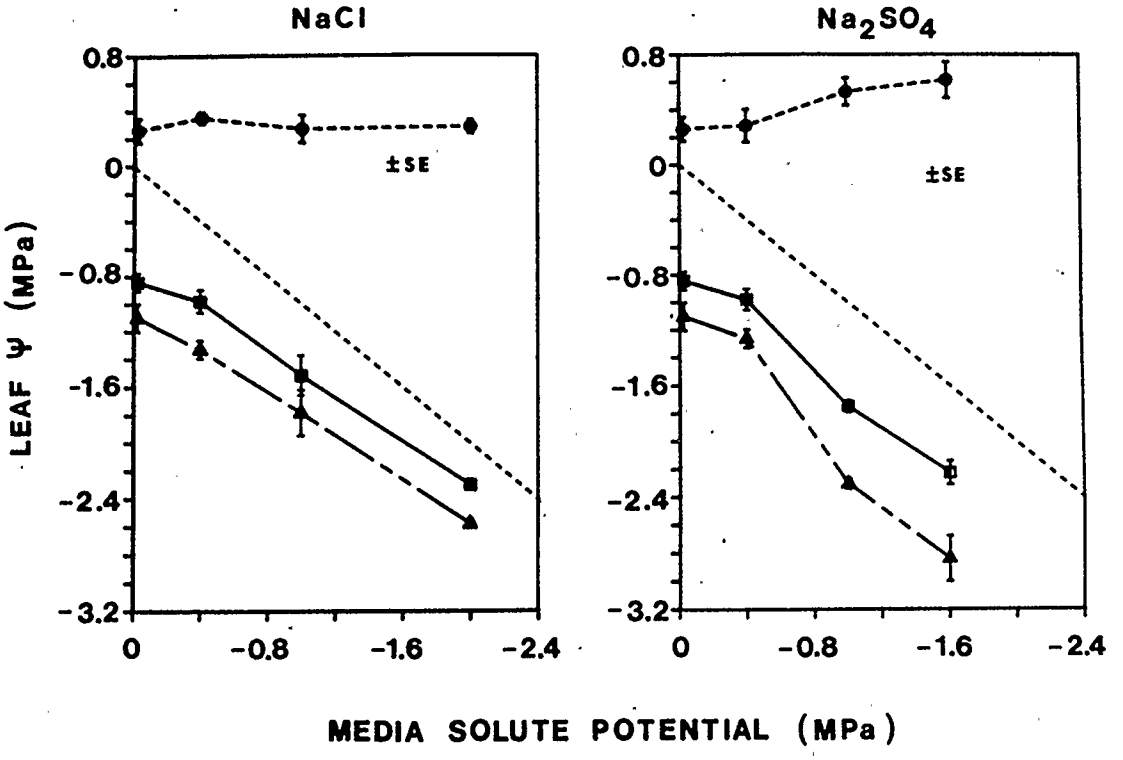

FIG. 21. Water relations of Chenopodium rubrum leaves from plants grown at increasing levels of NaCl or Na_2SO_4. Sample sizes ranged from three to five with each data point representing the mean ± SE. Note that for the NaCl treatment, media solute potentials went down to -2.0 MPa, not -1.6 MPa like in the Na_2SO_4 treatment. Dashed lines represent equimolar concentrations in leaf tissue water and the external solution ($\Psi^{plant} = \Psi^{env}$). o , Ψ_p; □ , Ψ_w; Δ , Ψ_s.

FIG. 22. Comparative effects of the highest concentrations of NaCl and Na_2SO_4 on growth of C. rubrum plants.

PHOTOSYNTHETIC CO_2-ASSIMILATION AND RELATED VARIABLES. Although previous results showed changes in stomatal anatomy, one parameter which could not be measured but which was very important to this research was stomatal aperture obtained by measuring the stomatal resistance (R_s) of leaves. Measurements of R_s were attempted using a stomatal diffusion porometer, however the results were extremely variable. It was therefore decided that a gas exchange system would be employed to measure R_s. Another advantage of using this system was that other important parameters such as net photosynthetic rate (P_n), and water use efficiency (WUE) could also be measured.

Under steady-state conditions, net photosynthetic rates for C. rubrum leaves grown without salt averaged about 6 μg CO_2 cm^{-2} min^{-1} (fig. 23). This rate decreased slightly at -0.4 MPa (for both Na_2SO_4 and NaCl) but increased again at -1.0 MPa and -2.0 MPa (NaCl) to a level similar to the control plants. At -1.6 MPa Na_2SO_4 however, the photosynthetic rate plunged to about 2.5 μg CO_2 cm^{-2} min^{-1}. The plants grown in this particular treatment looked very unhealthy, and were much smaller than plants in the other treatments (even those at -2.0 MPa NaCl).

At all salt concentrations there was a significant increase in stomatal resistances (fig. 24A). They were approximately 3X higher than the controls. In a few species it has been shown that an increase in stomatal resistance can lead to a concommitant decrease in the rate of CO_2 assimilation (Winter 1979). However, in C. rubrum at least, this does not appear to be the case since P_n remained relatively constant at all salt concentrations except at -1.6 MPa Na_2SO_4. Transpiration rates

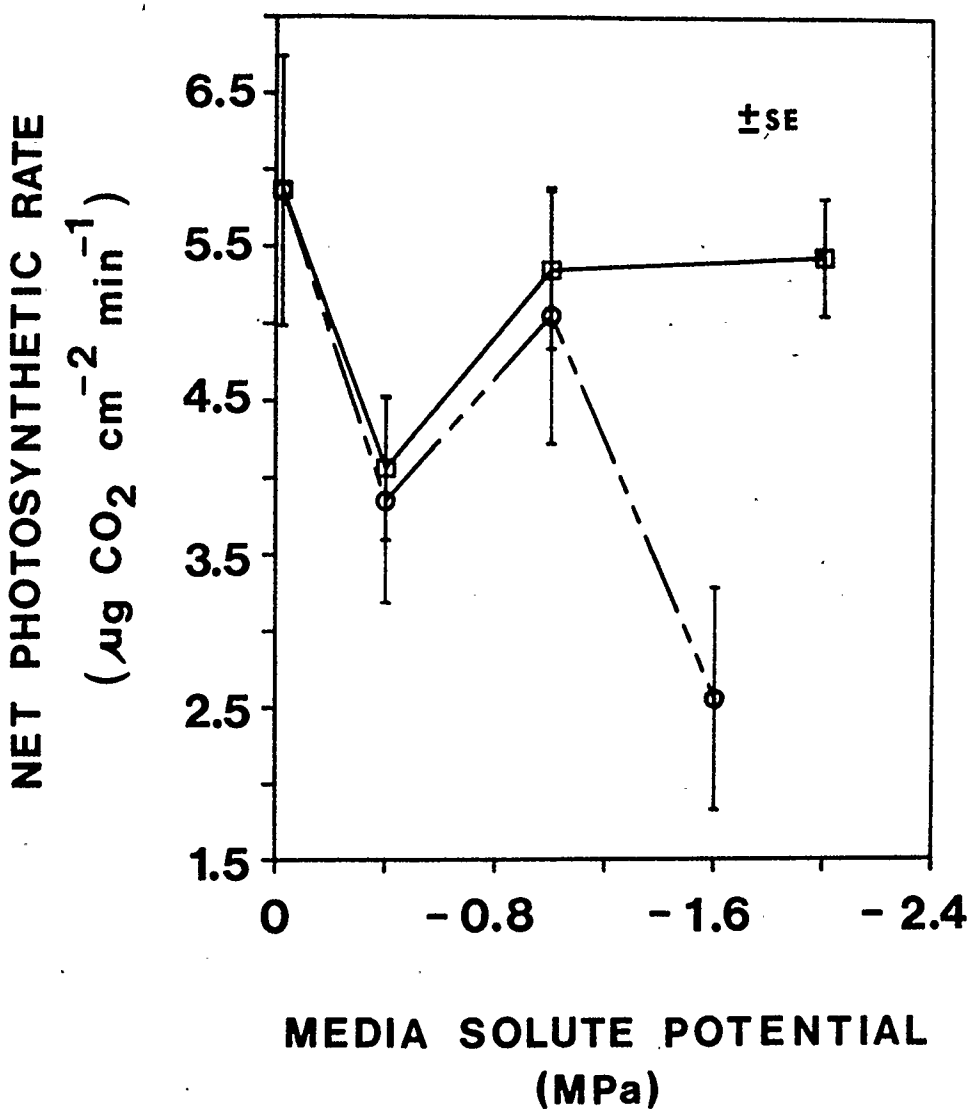

FIG. 23. Net photosynthetic rates of leaves of C. rubrum plants at increasing salinities under steady-state conditions. Data points represent the mean ± SE (six plants per point). NaCl □ ; Na_2SO_4 ○ .

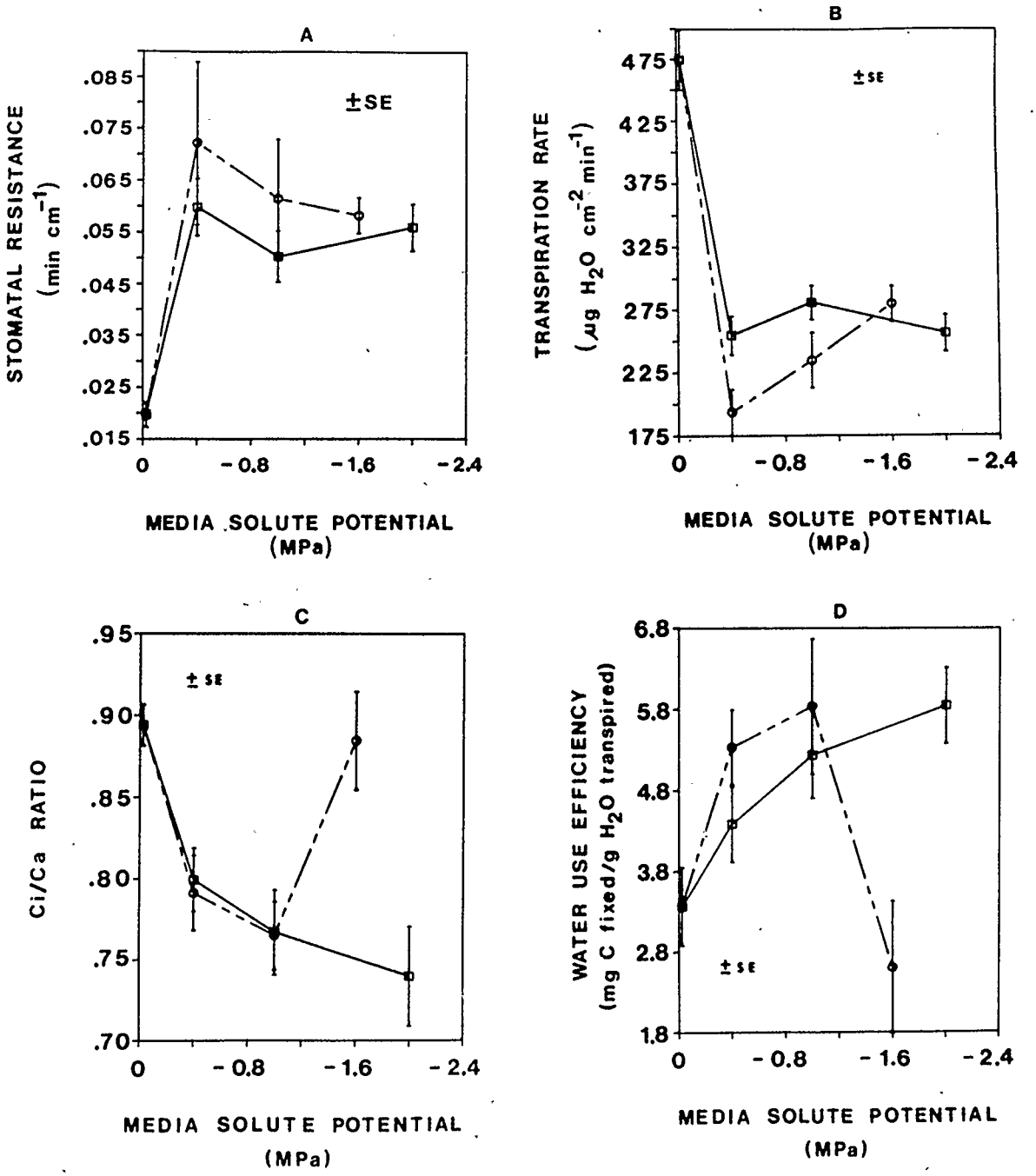

FIG. 24. Effects of increasing salt concentrations on various gas exchange parameters under steady-state conditions (same plants as in fig. 23); A. Stomatal resistance. B. Transpiration rate. C. Intercellular to ambient CO_2 ratio (C_i/C_a). D. Instantaneous water use efficiency. NaCl □ ; Na_2SO_4 O .

are also shown (fig 24B). In the control plants rates averaged about 470 μg H_2O cm^{-2} min^{-1}, but decreased by approximately 1/2 when plants were exposed to salt.

The calculated C_i/C_a ratio also showed a reduction for all salt treatments except -1.6 MPa Na_2SO_4 (fig. 24C). The fact that the internal CO_2 concentration in plants from this particular treatment did not decrease, as well as the decline in P_n indicates that photosynthesis was somehow impaired.

Instantaneous water use efficiencies for these plants are shown in figure 24D. With increasing salinity there appears to be an increase in WUE (with the exception again of the -1.6 MPa Na_2SO_4 treatment). The above preliminary results compare quite well with those of Guy (1984).

CARBON ISOTOPE ANALYSIS. Stable isotopes occur in two different forms: ^{12}C and ^{13}C. Although they both react similarily during chemical reactions, the rates at which they react differ considerably due to differences in their atomic weights.

In order to measure the differences in isotopic composition, the $^{13}C/^{12}C$ ratio of plant material is compared with the ratio of a standard. The difference, known as the relative ^{13}C content (designated by δ) is measured in parts per thousand, or per mil ($^{o}/oo$). So if, for example, a sample of plant material is found to have a $^{13}C/^{12}C$ ratio which is less than that of the standard by 20 per mil, it is said to have a $\delta^{13}C$ value of -20 $^{o}/oo$.

During the process of photosynthesis, plants discriminate against the heavier of the two isotopes (^{13}C) and thus fix more ^{12}C (Bender 1971). This is referred to as isotopic fractionation. The amount of

discrimination is dependent on the mode of carbon fixation, i.e. C_3 plants tend to discriminate against ^{13}C to a greater extent then those possessing the C_4 pathway (Willmer and Firth 1980). The result is that all plants contain less ^{13}C relative to the atmosphere.

Atmospheric CO_2 has a $\delta^{13}C$ value of approximately -7.8 °/oo (Keeling et al. 1979), therefore the $\delta^{13}C$ values of plants are subsequently more negative, i.e. C_3 species normally have $\delta^{13}C$ values ranging from -36 to -22 °/oo while values for C_4 plants range from -16 to -9 °/oo (Bender 1971, Smith and Epstein 1971, Troughton 1971). A less negative number indicates that a sample is richer in ^{13}C (O'Leary 1981).

Water use efficiency is usually determined by gas exchange techniques, however, only instantaneous WUE can be obtained using these methods. On the other hand, stable isotope data ($\delta^{13}C$) yields WUE over the life time of a plant. The $\delta^{13}C$ values can be correlated with increased WUE in plants due to the fact that they are both related to the ratio of C_i (internal CO_2 concentration) to C_a (ambient CO_2 concentration). In the majority of salt tolerant plants, a reduction in stomatal aperture usually leads to improved WUE. When the stomatal aperture is reduced, the supply of CO_2 to the sites of carboxylation is restricted causing $\delta^{13}C$ values to become more positive, approaching that of the ambient air. In other words, when the internal CO_2 concentration is high (i.e. when stomates are open) the cells have an opportunity to discriminate between ^{12}C and ^{13}C, and a large fractionation is observed. However, when the CO_2 concentration is low (during stomatal closure) cells discriminate between the two isotopes

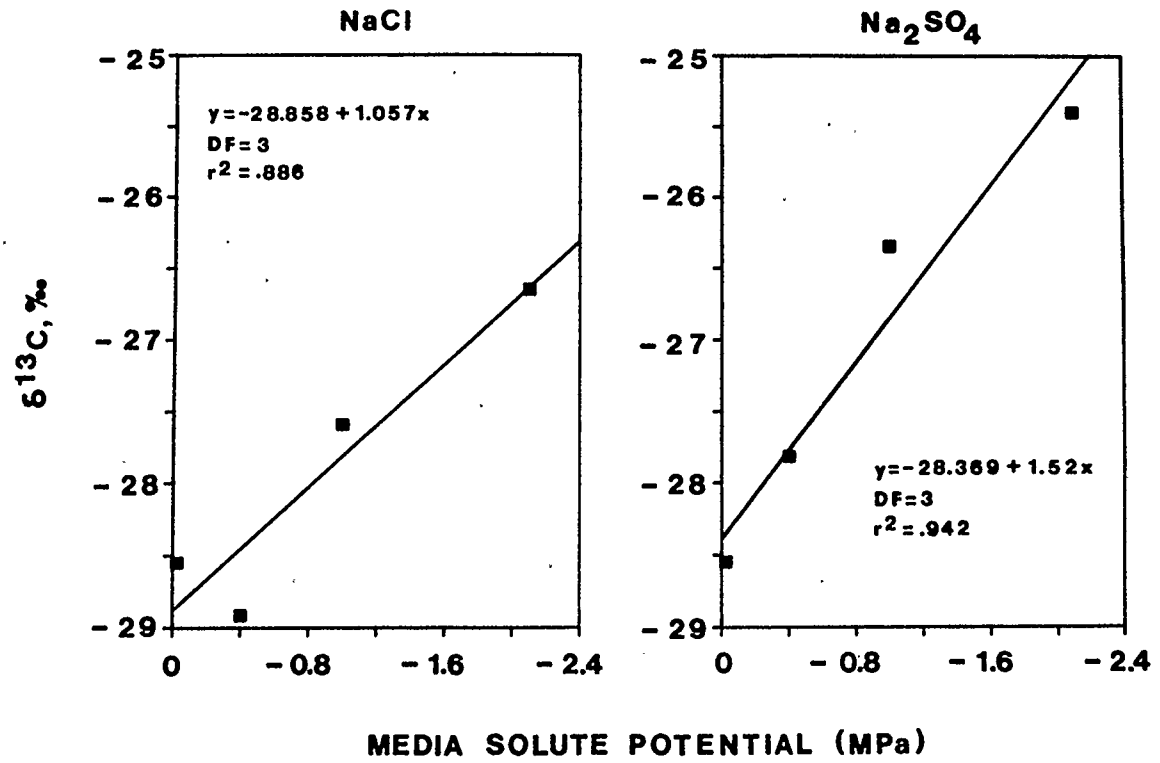

FIG. 25. Effect of increasing concentrations of NaCl or Na_2SO_4 on the isotopic composition of leaves of C. rubrum. Analysis was performed on single pooled samples from six plants per treatment.

to a lesser degree, thus the more positive $\delta^{13}C$ values.

The results of isotope analysis on tissue from C. rubrum are presented in figure 25. The values were well within the range found in C_3 plants by other workers (Willmer and Firth 1980). Generally, less negative $\delta^{13}C$ values were obtained with increasing salt concentrations, although the values for plants grown in NaCl appeared to be slightly more negative than those grown in Na_2SO_4. Linear regression lines were fitted to both sets of data, however when they were compared (t-test) no significant difference was found in their slopes at the $P<.05$ level.

DISCUSSION

Chenopodium rubrum is one of several commonly occurring plant species that can be found growing on saline sloughs in Alberta. However, this species has received almost no attention in the literature with respect to its salt tolerance. The major purpose of this research was therefore to provide background information characterizing some of the growth and physiological aspects of its adaptation to salinity. It was mentioned previously that in almost all of the research being done in this area the major salt utilized is sodium chloride, however, the predominant salt occurring in soils of Alberta is sodium sulfate. Since this is the type of salt C. rubrum is exposed to under natural conditions it was the obvious choice for my experiments, however, I have also included NaCl in my investigations so that a comparison could be made between my research and the research of others.

From the introduction we know that if plants are to survive in saline environments thay must not only be able to obtain water from soils of very low water potentials, but also must somehow withstand the potentially toxic effects of high concentrations of salt ions. Salt tolerant plants (or halophytes) have evolved several mechanisms to deal with these problems. Euhalophytes (or "true" halophytes) accumulate electrolytes in order to provide the necessary internal solute potentials needed for water absorption. In contrast, the glycohalophytes must synthesize organic osmotica for the same purpose. The logical first step regarding my research was therefore to ascertain

which one of the two strategies C. rubrum employed to bring about osmotic adjustment and thus water absorption. The generally acknowledged idea that "true" halophytes adjust osmotically by uptake of ions (Greenway and Munns 1980) appears to hold for C. rubrum (the role of betaine in osmotic adjustment will be discussed later). This conclusion is based on the fact that ash contents increased almost linearly with decreasing media solute potentials (increasing salt concentrations) (fig. 18). No attempts were made at this time to analyze tissue to find out which electrolytes were involved in the osmotic adjustment. Ion accumulation is considered to be the more energy efficient of the two forms of osmotic adjustment for reasons outlined in the introduction.

In this study, leaf succulence increased with the presence of NaCl, and to a lesser extent Na_2SO_4, in the rooting solution (fig. 5). This is a well known phenomenon in many halophytes exposed to increasing salinity (Handley and Jennings 1977, Jennings 1968, 1976). It has been suggested that the increase in succulence in higher plants is brought about by high internal salt concentrations in leaf cells following an increase in the concentration of electrolytes in the external medium (Handley and Jennings 1977). The increased concentration of salt inside the cells is thought to produce a greater water potential gradient between the plant and the external environment causing an increase in turgor pressure. Larger cells are the result, although it should be noted that an increase in ion concentration and its subsequent effect on internal osmotic pressure does not always lead to increased succulence within the cell in other species (Jennings

1976).

An interesting result in the present study was that NaCl had a much greater influence on the creation of succulence than did Na_2SO_4. Similar trends in different species have been observed by other workers (Poljakoff-Mayber 1975). Future investigations should be initiated to determine the extent of succulence under field conditions since it is known (personal observation) that this species exhibits extensive succulence under natural conditions (where Na_2SO_4 predominates). This result confirms the view that it is not always valid to assume that conditions created in the laboratory simulate those of the natural environment.

Leaf succulence in C. rubrum was measured as an increase in the water content per unit area and an increase in leaf thickness (figures 5 and 6). The increase in leaf thickness was due in part to the development of larger cells in the palisade layer (i.e. both cell lengths and cell diameters increased) (fig. 9). The number of cell layers in this tissue also increased with increasing salinity (except at the highest Na_2SO_4 concentration) (fig. 10). Although the size of individual cells from the spongy mesophyll could not be measured accurately, an estimate of the thickness of this layer was made. An increase was observed in NaCl treatments, however, there was not much change in the Na_2SO_4 treatments (fig. 8). Epidermal cells also increased in size (i.e. numbers of cells decreased per unit area, fig. 11).

It has been suggested that succulence develops because salt in some way inhibits cell division and stimulates cell extension in

halophytes (Abdulrahman and Williams 1981), with the result that cell numbers decrease but their sizes increase. Future studies could investigate the possible role of plant growth regulators in the control of the induction of succulence. I know of two other situations in which it is possible to experimentally manipulate leaf thickness. Application of an inhibitor of gibberellic acid (GA) biosynthesis (AMO-1618) promoted leaf thickness (Crozier et al. 1973) suggesting that this parameter might be naturally controlled by alterations in the level of endogenous hormones. Another situation involving leaf thickness was examined by McLaren and Smith (1978) who showed that both light quantity and quality contributed to the observed change in leaf thickness. From the above, it would appear that plants are rather plastic in their ability to alter leaf thickness and perhaps this in some way confers upon them an advantage in a diverse range of habitats.

What role succulence plays in the adaptation to salinity has not yet been clearly explained. Some researchers consider it to be beneficial to plants growing in saline conditions because the increase in water content has a diluting effect on the high concentration of ions in the cells (Jennings 1968). This mechanism could possibly operate at low salinities, however, at high salt concentrations the amount of succulence needed to dilute the vast amounts of internal ions would have to be huge.

It has now been firmly established that organisms like halophylic bacteria contain highly modified enzymes which show an obligate requirement for salt, however, the evidence for such modifications in halophytic higher plants does not indicate that adaptations of this

nature have occurred (Flowers et al. 1977), although osmotically shocked plants have been known to produce novel polypeptides (Singh et al. 1985). Unfortunately, the function of these peptides is still unknown. A commonly held view is that ions accumulated for osmotic adjustment are compartmented away from the cytoplasm in the vacuoles which comprise some 80-90% of the mature leaf cell volume (Hajibagheri et al. 1984, Harvey et al. 1981, Hess et al. 1975, Yeo 1981). A relatively small amount of organic solutes would then be required to balance the osmotic potential in the cytoplasm (Greenway and Munns 1983). Since there were reports in the literature that various members of the Chenopodiaceae accumulated glycinebetaine for this purpose (Storey and Wyn Jones 1975, 1977, Stumf 1984), it was decided that an analysis of C. rubrum tissue should be undertaken. In conjunction with a fellow graduate student a new method to quantify betaine was established. The results from field plants presented in Table 1 (taken from Guy 1984) shows that field grown C. rubrum plants contain substantial quantities of betaine when exposed to salinity. An analysis of laboratory grown plants under various salt concentrations followed. A linear relationship between betaine content and the water potential of the shoot (Ψ_w^{shoot}) is often pointed to as evidence for its role in osmotic adjustment. It is also known that, unlike NaCl or Na_2SO_4, glycinebetaine is compatible with cytoplasmic enzymes at levels as high as 1000 mol m^{-3} (Pollard and Wyn Jones 1979).

C. rubrum plants grown hydroponically in a range of NaCl or Na_2SO_4 salinities had betaine contents which rose fairly linearly with decreasing media solute potentials (figures 19 and 20). Salicornia

europaea (another halophytic species from the Chenopodiaceae) was included for the purpose of comparison. Unlike C. rubrum however, results had to be corrected for salt in order to show an increase in betaine content with increasing salinities (fig. 17). The increase in this case was not linear. On the other hand it has been pointed out that a linear response should not be expected in species that exhibit pronounced succulence (like Salicornia europaea) where there is little or no information on the relative proportions of cell wall to cytoplasmic to vacuolar material or knowledge of cytoplasmic to vacuolar volumes at increasing salinities (Guy et al. 1984). It is also possible that osmotica may move back and forth between the cytoplasm and the vacuole depending on the level of stress encountered (Leigh et al. 1981). If this were the case, one would expect to get only an approximate correlation between betaine accumulation and water potential of the shoot.

Whatever the case, the levels of osmotica were sufficient enough to maintain the osmotic potential of tissues lower than that of the external solution (fig. 21), so that water could be taken up by the plants. Trends for both salts were similar in as much as both Ψ_w and Ψ_s were below the isosmotic limit (dashed line in fig. 21). However, plants grown in NaCl seemed to conserve osmotica (by limiting osmotic adjustment) more so than those grown in Na_2SO_4 (i.e. the difference between Ψ_w^{shoot} and Ψ_w^{env} of plants grown in NaCl was not as great as that for plants growing in Na_2SO_4). The conservation of osmotica is one means by which plants can presumably save energy in saline environments. There was also a trend towards higher Ψ_p as Na_2SO_4

concentrations increased unlike NaCl plants where Ψ_p stayed relatively constant. This could possibly be due to the fact that the Na_2SO_4 plants were not as succulent as those grown in NaCl (i.e. since cells were not expanding like they would during the development of succulence there may be a build up of pressure). The above may partly explain why plants grown at high concentrations of Na_2SO_4 were not as healthy as those in NaCl treatments.

Despite sufficient accumulation of solutes to provide a favorable water potntial gradient for water uptake and turgor maintenance, a reduction in growth still occurred in plants at higher salt concentrations. In contrast, plant growth was stimulated slighly at low salinities. The growth pattern of Chenopodium rubrum associated with decreasing external solute potentials is quite similar to that reported by other researchers for a number of halophytic species (Gale et al. 1970, Neales and Sharkey 1981, St. Omer and Schlesinger 1980). The optimal solute potential for growth occurred between -0.4 MPa and -0.8 MPa. Growth decreased significantly below this point (figures 2 and 3). Plants remained healthy in all treatments with the exception of the -1.6 MPa Na_2SO_4 treatment. This was surprising since C. rubrum plants in the field have regularly been found growing in soils with solute potentials well below -1.6 MPa and they appeared to be quite healthy. The leaves of hydroponically grown plants exposed to solute potentials of -1.6 MPa or greater showed signs of both chlorosis (loss of chlorophyll production), and necrosis (patches of dead cells or tissues), and were substantially smaller than even those plants exposed to -2.0 MPa NaCl. Again, this emphasizes the fact that experiments

carried out under laboratory conditions do not necessarily mimic conditions found in the natural environment. The rooting material used in all of the experiments for example, was totally different from the soil in which plants would normally be found. Soils surrounding salt sloughs consist primarily of clay and are probably anaerobic. It has also been pointed out that the roots of halophytic plants growing under natural conditions are never exposed to uniform concentrations of salt (Waisel 1985). Other possible conditions differing from those in the laboratory include the amount of light plants were exposed to (much lower in growth chamber experiments), relative humidity, and ion contents.

Several reasons for the decline in growth of halophytes with increasing salinities have been suggested. The diversion of photosynthate into the production of osmotica at first glance seems unlikely in the case of C. rubrum since inorganic ions are presumably the principle osmotic solutes in this species and ion uptake is supposedly a cheaper source of osmotica. However, researchers have suggested that growth limitation may be due to inadequate osmotic regulation in expanding cells (Clipson et al. 1985, Greenway and Munns 1983). Osmotic regulation is simply the metabolic processes resulting in changes in internal solute content leading to the maintenance of turgor. This could be due to an inadequate respiratory system to provide energy for active transport or to an insufficient number of carriers required for the rapid rate of ion uptake required in growing cells (i.e. the rate of ion accumulation into the vacuoles of growing cells may be inadequate to sustain turgor and therefore cell

expansion).

The partial closure of stomates, which interferes with CO_2 diffusion into the leaf and thus reduces photosynthesis, has also been suggested as a reason for growth reduction. In the present study, stomatal resistance (R_s) increased significantly in all of the salt treatments (fig. 24A). However, the net photosynthetic rate was not significantly affected (except at -1.6 MPa Na_2SO_4) (fig. 23). Similar results for other halophytes have been reported (Winter 1979). It has been suggested that the increase in succulence, due to exposure to salinity, could lower the resistance to CO_2 uptake thereby maintaining photosynthetic rates by increasing the amount of internal leaf surface area for CO_2 exchange (Longstreth and Nobel 1979). The increase in R_s also resulted in a reduction in transpirational water loss in all of the salt treatments (fig. 24B). The combined effects of reduced transpiration with the maintenance of P_n thus resulted in a higher water use efficiency (WUE) for all plants except those grown at -1.6 MPa Na_2SO_4. The increase in WUE was further substantiated by results obtained from carbon isotope analysis, whereby more positive $\delta^{13}C$ values indicate an increase in the "assimilation-averaged" WUE (see results section for an explaination). One can immediately see that there is an obvious discrepancy with this data however. The $\delta^{13}C$ values appear to indicate that WUE increases in all of the salt treatments, but from the gas exchange analysis we can see that this is not the case for plants at -1.6 MPa Na_2SO_4 (fig. 24D). A drastic decline in P_n and the increase in C_i/C_a ratio indicate that CO_2 assimilation has been in some way seriously impaired (figures 23 and 24C). The explanation for

this discrepancy is simple. The $\delta^{13}C$ values provide information about stomatal closure and thus indirectly about WUE over a period of time, not instantaneously as in gas exchange measurements. Salts were stepped up to their final concentrations over a period of weeks. Therefore, plants which were ultimately exposed to -1.6 MPa Na_2SO_4 were also exposed to lower "less destructive" concentrations for a period of time and this is reflected in the $\delta^{13}C$ values obtained.

Although the above preliminary results show that photosynthetic capacity is relatively unaltered by salinity, it should be emphasized that measurements were based on photosynthetic rates per unit leaf area. However, that salinity can induce modifications in leaf area is a well known phenomenon (Gale and Poljakoff-Mayber 1970, Kaplan and Gale 1972, Wignarajah et al. 1975). A change in leaf area will alter the total plant photosynthetic capacity regardless of the fact that P_n per unit area remains constant. In one study (Papp et al. 1983), NaCl salinity was shown to inhibit leaf extension growth in sugar beet (a moderatly salt tolerant chenopod) and presumably this could account for the reduction in growth due to the fact that the surface area for photosynthetic CO_2-assimilation was reduced. Greenway (1968), found that low concentrations of salt did not substantially affect the net photosynthetic assimilation rates in Atriplex nummurlaria, however, as a consequence of increased cell expansion, leaf areas of plants increased. The result was that overall growth of these plants was stimulated. Similar results were obtained in the present study with C. rubrum (fig. 4).

A reduction in growth of plants which inhabit arid or semi-arid

saline regions can not necessarily be considered a disadvantage since a large vegetative body with a large leaf area, although beneficial for photosynthesis, also presents a considerable area for transpirational water loss. It has been demonstrated that reductions in leaf area can lead to substantial savings in transpiration at a given R_s (Smith and Geller 1980). The decrease in leaf size may be an evolutionary alternative to stomatal closure (since effects on transpiration would be significantly greater than effects on CO_2 uptake). Cuticular transpiration in the leaves of some plants can be another source of significant water loss (Levitt 1980). An increase in succulence may be an advantage in this case since the reduction in leaf suface area would result in substantial decreases in water loss.

A significant decrease in the numbers and sizes of stomates was also observed with increasing salinity (figures 12 and 13). Similar decreases in stomatal density (nos. stomates /unit area) have been observed by other workers (St. Omer and Schlesinger 1980). It has generally been assumed that the increase in stomatal resistance (R_s) is due to a reduction in stomatal aperture. However, when R_s is measured (i.e. by gas exchange methods or porometry), stomatal apertures are not directly measured. Therefore, the fact that both sizes and numbers of stomates decreased in the present study may possibly explain the increase in R_s. This could be considered a useful adaptation for plants in saline environments since water loss could be substantially reduced. It might also explain why there was no substantial reduction in P_n with increasing salinity (since apertures would remain open). Furthermore, a saving in energy by the plants might be realized since they would not

have to expend any energy on the opening and closing of their stomates. As far as the -1.6 MPa Na_2SO_4 treated plants are concerned, perhaps the reason they did not survive very well is because the stomatal densities of these plants were too high (fig. 12) resulting in excessive transpiration. The consequence of this may have been a loss of turgor leading to stomatal closure which ultimately affected CO_2 assimilation.

Another growth character which I had initially intended to investigate was prostrate growth. As mentioned previously, this particular growth habit is known to occur in C. rubrum plants under natural saline conditions, however, I was unsuccessful in my attempts to induce prostrate growth under laboratory conditions. Perhaps photoperiod or light quality are in some way involved. There is some evidence that phytochrome may be responsible for a plant's ability to detect shading from neighboring plants which induces them to grow taller. In saline areas, the density of plants is decreased (due to the stressful nature of the environment) therefore, less shading is expected. It is also possible that this growth habit is simply a result of wind blowing plants over. In any case, more field studies would be useful.

LITERATURE CITED

Abdulrahman, F.S., and G.J. Williams III. 1981. Temperature and salinity regulation of growth and gas exchange of Salicornia fruticosa(L.) L.. Oecologia 48:346-352.

Aspinall, D., and L.G. Paleg. 1981. Proline accumulation: physiological aspects. In The Physiology and Biochemistry of Drought Resistance in Plants. Edited by L.G. Paleg, and D. Aspinall. Academic Press, Sydney. pp. 205-241.

Bender, M.M. 1971. Variations in the $^{13}C/^{12}C$ ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation. Phytochemistry 10:1239-1244.

Caemmerer, S. von, and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376-387.

Caldwell, M.M. 1974. Physiology of desert halophytes. In Ecology of Halophytes. Edited by R.J. Reimold and W.H. Queen. Academic Press, New York. pp. 355-378.

Cavalieri, A.J. 1983. Proline and glycinebetaine accumulation by Spartina alternifolia Loisel. in response to NaCl and nitrogen in a controlled environment. Oecologia 57:20-24.

-----, and A.H.C. Huang. 1981. Accumulation of proline and glycinebetaine in Spartina alterniflora Loisel. in response to NaCl and nitrogen in the marsh. Oecologia 49:224-228.

-----, and -----. 1979. Evaluation of proline accumulation in the adaptation of diverse species of marsh halophytes to the saline environment. Am. J. Bot. 66:307-312.

-----, and -----. 1977. Effect of NaCl on the in vitro activity of malate dehydrogenase in salt marsh halophytes of the U.S. Physiol. Plant. 41:79-84.

Clipson, N.J.W., A.D. Tomos, T.J. Flowers, and R.G. Wyn Jones. 1985. Salt tolerance in the halophyte Suaeda maritima L. Dum. The maintenance of turgor pressure and water-potential gradients in plants growing at different salinities. Planta 165:392-396.

Downton, W.J., W.J.R. Grant, and S.P. Robinson. 1985. Photosynthetic and stomatal responses of spinach leaves to salt stress. Plant Physiol. 78: 85-88.

Epstein, E. 1980. Responses of plants to saline environments. In Genetic engineering of osmoregulation. Impact on plant productivity for food, chemicals, and energy. Edited by D.W. Rains, R.C. Valentine,

and A. Hollaender. Plenum Press, New York. pp. 7-21.

Flowers, T.J. 1972. The effect of sodium chloride on enzyme activities from four halophyte species of Chenopodiaceae. Phytochemistry 11:1881-1886.

-----. 1972a. Salt tolerance in Suaeda maritima L. Dum. The effect of sodium chloride on growth, respiration, and soluble enzymes in a comparative study with Pisum sativum L. J. Exp. Bot. 23:310-321.

-----, J.L. Hall, and M.E. Ward. 1978. Salt tolerance in the halophyte Suaeda maritima (L.) Dum.: Properties of malic enzyme and PEP carboxylase. Ann. Bot. 42:1065.

-----, P.F. Troke, and A.R. Yeo. 1977. The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol. 28:89-121.

Gale, J., R. Naaman, and A. Poljakoff-Mayber. 1970. Growth of Atriplex halimus L. in sodium chloride salinated culture solutions as affected by the relative humidity of the air. Aust. J. Biol. Sci. 23:987-952.

-----, and A. Poljakoff-Mayber. 1970. Interrelationships between growth and photosynthesis of salt bush (Atriplex lalimus L.) grown in saline media. Aust. J. Biol. Sci. 23:937-945.

Goas, G., M. Goas, and F. Larher. 1982. Accumulation of free proline and glycinebetaine in Aster tripolium subjected to a saline shock: A kinetic study related to light period. Physiol. Plant. 55:393.

Greenway, H. 1968. Growth stimulation by high chloride concentrations in halophytes. Israel J. Bot. 17:169-177.

-----, and R. Munns. 1983. Interactions between growth, uptake of Cl^- and Na^+, and water relations of plants in saline environments. II. Highly vacuolated cells. Plant Cell Environ. 6:575-589.

-----, and -----. 1980. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31:149-190.

Guy, R.D. 1984. Ecophysiology of inland halophytes: Strategies for osmotic adjustment, water-use, and photosynthetic carbon assimilation PhD. Thesis, Biology Department, University of Calgary. Calgary, Alberta, Canada.

-----, P.G. Warne, and D.M. Reid. 1984. Glycinebetaine content of halophytes: Improved analysis by liquid chromatography and interpretations of results. Physiol. Plant. 61:195-202.

Hajibagheri, M.A., J.L. Hall, and T.J. Flowers. 1984. Stereological analysis of leaf cells of the halophyte Suaeda maritima (L.) Dum. J. Exp. Bot. 35:1547-1557.

Handley, J.F., and D.H. Jennings. 1977. The effect of ions on growth and leaf succulence of Atriplex hortensis var. cupreata. Ann. Bot. 41:1109-1112.

Hanson, A.D., and R. Wyse. 1982. Biosynthesis, translocation and accumulation of betaine in sugar beet and its progenitors in relation to salinity. Plant Physiol. 70:1191.

Harvey, D.M.R., T.J. Flowers, and J.L. Hall. 1976. Localization of chloride in leaf cells of the halophyte Suaeda maritima by silver precipitation. New Phytol. 77:319-323.

-----, J.L. Hall, T.J. Flowers, and B. Kent. 1981. Quantitative ion localization within Suaeda maritima leaf mesophyll cells. Planta 151:555-560.

Hess, W.M., D.J.Hansen, and D.J. Weber. 1975. Light and electron microscopic localization of chloride ions in cells of Salicornia pacifica var. utahensis. Can. J. Bot. 53:1176-1187.

Hitz, W.D., and A.D. Hanson. 1980. Determination of glycine betaine by pyrolysis gas chromatography in cereals and grasses. Phytochemistry 19:2371-2374.

Hoagland, D.R., and D.I. Arnon. 1950. The water culture method for growing plants without soil. Circ. Univ. Calif. Agric. Exp. Sta. #347, 32pp.

Jarman, P.D. 1974. The diffusion of carbon dioxide and water vapour through stomata. J. Exp. Bot. 25:927-936.

Jarvis, P.G., and T.A. Mansfield. 1981. Stomatal Physiology. Cambridge University Press, Cambridge. 295 pp.

Jefferies, R.L. 1980. The role of organic solutes in osmoregulation in halophytic higher plants. In Genetic engineering of osmoregulation. Impact on plant productivity for food, chemicals, and energy. Edited by D.W. Rains, R.C. Valentine, and A. Hollaender. Plenum Press, New York. pp. 135-154.

Jennings, D.H. 1976. The effects of sodium chloride on higher plants. Biol. Rev. 51:453-486.

-----. 1968. Halophytes, succulence and sodium in plants. A unified theory. New Phytol. 67:899-911.

Kaplan, A., and J. Gale. 1972. Effect of sodium chloride salinity on the water balance of Atriplex halimus. Aust. J. Biol. Sci. 25:895-903.

Keeling, C.D., W.G. Mook, and P.D. Trans. 1979. Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. Nature 277:121-123.

Leigh, R.A., N. Ahmad, and R.G. Wyn Jones. 1981. Assessment of glycine betaine and proline compartmentation by analysis of isolated beet vacuoles. Planta 153:34-41.

Levitt, J. 1980. Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses. 2nd Edition. Academic Press, New York. 607 pp.

Lilley, J. 1982. Dryland salinity in Alberta. EC482-17/1B 13. Edmonton: Environment Council of Alberta.

Longstreth, D. J., and P.S. Nobel. 1979. Salinity effects on leaf anatomy. Consequences for photosynthesis. Plant Physiol. 63:700-703.

Maas, E.V., and R.H. Nieman. 1978. Physiology of plant tolerance to salinity, In Crop tolerance to suboptimal land conditions. Edited by G.A. Jung. Am. Soc. Agron. Special Publication #32. pp. 277-299.

Neales, T.F., and P.J. Sharkey. 1981. Effect of salinity on growth and on mineral and organic constituents of the halophyte Disphyma australe (Soland.). J.M. Black. Aust. J. Plant Physiol. 8:165-179.

Nobel, P.S. 1983. Leaves and fluxes. In Biophysical plant physiology and ecology. Edited by P.S. Nobel. W.H. Freeman and Co., San Francisco. pp. 387-460.

-----, D.J. Longstreth, and T.L. Hartsock. 1978. Effect of water stress on the temperature optima of net CO_2 exchange for two desert species. Physiol. Plant. 44:97-101.

O'Leary, M.H. 1981. Carbon isotope fractionation in plants. Phytochemistry. 20:553-567.

Paleg, L.G., T.J. Douglas, A. van Daal, and D.B. Keech. 1981. Proline, betaine and other organic solutes protect enzymes against heat inactivation. Aust. J. Plant Physiol. 8:107-114.

Papp, J.C., M.C. Ball, and N. Terry. 1983. A comparative study of the effects of NaCl salinity on respiration, photosynthesis, and leaf extension growth in Beta vulgaris L. (sugar beet). Plant Cell Environ. 6:675-677.

Penning De Vries, F.W.T. 1975. The cost of maintenance respiration in plant cells. Ann. Bot. 39:77-92.

Poljakoff-Mayber, A. 1975. Morphological and anatomical changes in plants as a response to salinity stress. In Plants in saline environments. Edited by A. Poljakoff-Mayber and J. Gale. Springer-Verlag, New York. pp. 97-117.

-----, and J. Gale. 1975. Plants in saline environments. Ecological Studies 15. Springer-Verlag, New York.

Pollard, A. and R.G. Wyn Jones. 1979. Enzyme activities in concentrated solutions of glycinebetaine and other solutes. Planta 144:291.

Queen, W.H. 1974. Physiology of coastal halophytes. In Ecology of Halophytes. Edited by R.J. Reimold, and W.H. Queen. Academic Press, New York. pp. 345-353.

Rains, D.W. 1979. Salt tolerance of plants: Strategies of biological system. In The biosaline concept. Edited by A. Hollaender. Plenum Press, London. pp. 47-67.

-----, T.P. Croughan, and S.J. Stavarek. 1980. Selection of salt-tolerant plants using tissue culture. In Genetic engineering of osmoregulation. Edited by D.W. Rains, R.C. Valentine, and A. Hollaender. Plenum Press, New York. pp. 279-292.

Rowan, M.K. 1978. Alberta report blames poor irrigation for salt buildup. The Globe and Mail, Toronto, Ontario, Canada. August 23, pp. 37.

Sampson, J. 1961. A method of replicating dry or moist surfaces for examination by light microscopy. Nature 191:932-933.

Schwarz, M., and J. Gale. 1984. Growth response to salinity at high levels of carbon dioxide. J. Exp. Bot. 35:193-196.

-----, and -----. 1981. Maintenance respiration and carbon balance of plants at low levels of sodium chloride salinity. J. Exp. Bot. 32:933-941.

Sen, D.N., and K.S. Rajpurohit. 1982. Ecological and ecophysiological problems. In Tasks for vegetation science. Vol. 2. Edited by D.N. Sen, and K.S. Rajpurohit. Dr. W. Junk Publishers, The Hague, pp. 109-110.

Singh, N.K., A.K. Handa, P.M. Hasegawa, and R.A. Bressan. 1985. Proteins associated with adaptation of cultured tobacco cells to NaCl. Plant Physiol. 79:126-137.

Smith, B.N., and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiol. 47:380-384.

Smith, W.K., and G.N. Geller. 1980. Leaf and environment parameters influencing transpiration: Theory and field measurements. Oecologia 46:308-313.

St. Omer, L., and W.H. Schlesinger. 1980. Field and greenhouse investigations of the effect of increasing salt stress on the anatomy of Jaumea carnosa (Asteraceae), a salt marsh species. Am. J. Bot. 67:1455-1465.

Stewart, G.R., and J.A. Lee. 1974. The role of proline accumulation in halophytes. Planta 120:279-289.

Storey, R., and R.G. Wyn Jones. 1979. Responses of Atriplex spongiosa and Suaeda monoica to salinity. Plant Physiol. 63:156-162.

-----, and -----. 1977. Quaternary ammonium compounds in plants in relation to salt resistance. Phytochemistry. 16:447-453.

-----, and -----. 1975. Betaine and choline levels in plants and their relationship to NaCl stress. Plant Sci. Lett. 4:161-168.

Stumpf, D.K. 1984. Quantitation and purification of quaternary ammonium compounds from halophyte tissue. Plant Physiol. 75:273-274.

-----, and J.W. O'Leary. 1985. The distribution of Na^+, K^+, and glycinebetaine in Salicornia bigelovii. J. Exp. Bot. 86:550-555.

Ting, I.P., and C.D. Osmond. 1973. Photosynthetic phosphoenol pyruvate carboxylases. Characteristics of alloenzymes for leaves of C_3 and C_4 plants. Plant Physiol. 51:439-447.

Troughton, J.H. 1971. Aspects of the evolution of the photosynthetic carboxylation reaction in plants. In Photosynthesis and photorespiration. Edited by M.B. Hatch, C.B. Osmond, and R.O. Slatyer. John Wiley and Sons, New York. pp. 124-129.

Turner, N.C., and M.M. Jones. 1980. Turgor maintenance by osmotic adjustment - a review and evaluation. In Adaptations of plants to water and high temperature stress. Edited by N.C. Turner, and P.S. Kramer. Wiley Interscience, New York. pp. 87-103.

Vialle, J., M. Kolosky, and J.L. Rocca. 1981. Determination of betaine in sugar and wine by liquid chromatography. J. Chromtogr. 204: 429-435.

Wainwright, S.J., 1984. Adaptations of plants to flooding with salt water. In Flooding and plant growth. Edited by T.T. Kozlowski. Academic Press, New York. pp. 295-343.

Waisel, Y., 1985. The stimulating effects of NaCl on root growth of Rhodes grass (Chloris gayana). Physiol. Plant. 64: 519-522

Wignarajah, K., D.H. Jennings, and J.F. Handley. 1975. The effect of salinity on the growth of Phaseolus vulgaris I. Anatomical changes of the first trifoliate leaf. Ann. Bot. 39:1029-1038.

Willmer, C.M., and P. Firth. 1980. Carbon isotope discrimination of epidermal tissue and mesophyll tissue from the leaves of various plants. J. Exp. Bot. 31:1-5.

Winter, K. 1979. Photosynthetic and water relationships of higher plants in a saline environment. In Ecological processes in coastal environments. Edited by R.L. Jefferies, and A.J. Davy. Blackwell Scientific Publications, Oxford. pp. 297-320.

Wyn Jones, R.G. 1980. An assessment of quaternary ammonium and related compounds as osmotic effectors in crop plants. In Genetic engineering of osmoregulation. Impact on plant productivity for food, chemicals and energy. Edited by D.W. Rains, R.C. Valentine, and A. Hollaender. Plenum Press, New York. pp. 155-170.

Yeo, A.R. 1983. Salinity resistance: Physiologies and prices. Physiol. Plant. 58:214-222.

-----. 1981. Salt tolerance in the halophyte Suaeda maritima L. Dum.: Intracellular compartmentation of ions. J. Exp. Bot. 32: 487-497.

Zahran, M.A., and A.A. Abdel Wahid. 1982. Halophytes and human welfar. In Tasks for vegetation science. Vol. 2. Edited by D.N. Sen, and K.S. Rajpurohit. Dr. W. Junk Publishers, The Hague. pp. 235-257.

Zar, J.H. 1974. Biostatistical anlysis. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

APPENDIX 1

VERSION OF HOAGLAND'S SOLUTION USED (HOAGLAND AND ARNON 1950)

Stock solutions (Each solution is made up separately using deionised water)

	For each litre use x g
1. $Ca(NO_3)_2 4H_2O$	236.2
2. KNO_3	101.1
3. $MgSO_4 7H_2O$	246.5
4. KH_2PO_4	136.1
5. FeEDTA (9% Fe)	57.8

6. Micronutrients	For each litre use x mg
H_3BO_3	2,860
$MnCl_2$	1,810
$ZnCl_2$	110
$CuCl_2 2H_2O$	50
$NaMoO_4 2H_2O$	250

To make a half strength working solution add x mls of stock solution to 500 mls of deionised water and bring up to 1 litre

	x
1. $Ca(NO_3)_2 4H_2O$	2.5
2. KNO_3	2.5
3. $MgSO_4 7H_2O$	1
4. KH_2PO_4	0.5
5. FeEDTA (9% Fe)	0.5
6. Micronutrients	0.5