THE ECOPHYSIOLOGICAL BASES OF SALT TOLERANCE IN *DISTICHLIS SPICATA*: BACKGROUND FOR DOMESTICATION

Gary L. Cunningham
Professor of Biology
New Mexico State University

Paul R. Kemp
Research Associate
New Mexico State University

Edith B. Allen
Post-Doctoral Fellow
New Mexico State University

Ann M. Cromer
Graduate Research Assistant
New Mexico State University

James F. Reynolds
Associate Professor of Botany
North Carolina State University

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ABSTRACT

Ditichilis spicata is a highly productive salt-tolerant grass species with considerable potential for domestication as a forage crop on saline soils. It is broadly distributed throughout North America in a variety of coastal and inland habitats. Effective efforts to domesticate D. spicata will require an ability to predict its primary production under a range of environmental and cultural practices to which it might be subjected. Ecotypic differences among populations from contrasting environments might make it necessary to match the physiological adaptations selected in particular natural environments with the environments and culture conditions under which it might be grown. Two coastal and two inland populations were evaluated for differential response to salinity level and temperature. Ecotypic variations with regard to growth, photosynthetic ability, biomass allocation and leaf chemical composition were found. Populations were also found to differ in their responses to the principal anion in the growth substrate. Modeling of photosynthetic response to growth salinity, temperature and light revealed the manner in which these variables influence productive capacity. Studies of the influence of mycorrhizae on growth and physiological function indicated that they are not essential to the ability of D. spicata to tolerate salinity.

Key words: Distichlis spicata, ecotype, photosynthesis, salt tolerance, mycorrhizae, temperature, salinity, photon fluence, sodium, chloride, sulfate, growth, biomass.

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INTRODUCTION

A number of rationales exist for the development of salt tolerant agricultural crops. The rationales would allow us to expand our agricultural land base into naturally salt affected areas that are prevalent in arid regions (Chapman 1960). The rationales would also allow us to again utilize previously irrigated areas whose salinities have been increased to the level where non-salt tolerant plants cannot grow and perhaps to increase productivity on lands where salt encroachment has significantly reduced plant productivity. The rationales would allow the use of water resources that are too saline for our present crops. These resources include both deposits of saline ground water and surface waters whose salinities have been detrimentally increased through use in irrigation.

Two approaches can be taken in the development of salt tolerant agricultural crops. One is the selection and breeding for enhanced salt tolerance in traditional crop plants. The other is the domestication of wild land species that already have salt tolerance. In the past these two approaches often have been thought of as distinct and to some extent mutually exclusive. The currently emerging, and more constructive view is that they represent two extremes along a continuum of approaches which should be pursued. Some significant advances toward the development of more salt tolerant crops are being achieved at various points along the continuum of possible approaches. At one end of the continuum, Epstein and his co-workers at the University of California at Davis have been involved in the selection of more salt tolerant genotypes of barley and wheat (Epstein et al. 1979 and Richards et al. 1982). At the other end,

the domestication of wild land species is being actively investigated by Somers and his colleagues at the University of Delaware (Somers 1979) and by the research group at the Environmental Research Laboratory at the University of Arizona (Glenn et al. 1982). At other points along the continuum, crosses between domesticated tomatoes and related salt tolerant wild species (Sacher et al. 1982) and cell selection with tissue cultures of alfalfa and rice (Rains et al. 1982) also show promise for the development of more salt tolerant agricultural crops.

The research reported here is aimed at gathering the information necessary for developing saltgrass (Distichlis spicata (L.) Greene) as a forage crop which would be tolerant to high levels of salinity. Along the continuum of approaches to the problem, this aim lies at one extreme. We hope, however, that our investigations will allow us to select genotypes that exhibit more desirable characteristics for specific environmental and cultural conditions. We also hope our investigations provide insights into characteristics that might be selected for, or introduced into, less tolerant species, thus contributing, as do other studies, along the whole spectrum of needed research.

The considerable potential for the utilization of saltgrass as a forage species has been pointed out by both Hansen et al. (1976) and Mudie (1974). Cattle are commonly grazed on native stands of saltgrass both in New Mexico (G. B. Donart, pers. comm.) and Utah (J. A. Ludwig, pers. comm.). Saltgrass is now being grazed by cattle following its introduction on the highly saline soils of the bed of the dry Lake Texcoco near Mexico City (Felger and Moto-Urbina 1982). Chemical analysis of saltgrass also indicates its value as a forage species. Saltgrass has protein, fiber and ash contents of 9.6 percent, 34.9

percent and 5.5 percent , respectively (Udell et al. 1969). These values compare favorably with those of the most important range forage species in south-central New Mexico (Hatch et al. 1968).

Although the potential value of saltgrass as a forage species has been alluded to by several authors, in each case a lack of knowledge concerning its physiological attributes and its variability has been pointed out as a factor limiting the effective evaluation of that potential (e.g. Hansen et al. 1976 and Mudie, 1974). Two varieties of D. spicata are currently recognized. Var. spicata is found predominately in coastal regions and in the northern United States into the Canadian inland regions. Var. stricta (Torr.) Beetle (D. stricta (Torr.) Rydb.) is more common in inland areas of the southwestern United States and Mexico (Correll and Johnston 1970). The wide occurrence of saltgrass in many geographic areas and in combination with a variety of other species indicates it has potential as a crop species under a variety of environmental conditions (Ungar 1974 and Nielson 1956). Saltgrass occurs in soils high in carbonates and bicarbonates (Tolstead 1942), high in chlorides (Ungar 1965) and high in sulfates (Flowers 1934 and Ungar 1970). The grass appears to have a broad range of salt tolerance, occurring in soils ranging from 0.03 percent to 5.6 percent total salts (Hunt and Durrell 1966 and Ungar et al. 1969). The pH range over which the species is found varies from 6.8 to 9.2 (Ungar 1974). It also grows well on a wide variety of soil textures, ranging from sand and gravel (Hunt and Durrell 1966) to clay (Ungar 1968).

This report summarizes our work to date on a comparative study of the environmental physiology of four populations of D. spicata from

environments of contrasting salinity and temperature. Much of what appears here already has been or soon will be published in peer reviewed journals. We are currently synthesizing these and other results into a predictive simulation model of primary production for this species.

The four populations that have been investigated are from a northern coastal habitat at Bodega Bay, California, a southern coastal habitat at Oceanside, California, a northern inland habitat in the San Luis Valley, Colorado and a southern inland habitat at Las Cruces, New Mexico. The contrasting temperature regimes of these habitats can be seen in Table 1. The two coastal habitats are obviously more moderate in temperature than are the inland habitats. Freezing temperatures do not occur in the coastal habitats, but they do occur in the inland habitats. The southern inland habitat experiences the highest summer temperatures and the northern coastal the lowest. The summer temperatures in the southern coastal and northern inland habitats are quite similar. Total soil salinity was greatest in the southern inland habitat and least in the southern coastal and northern inland habitats. Total soil salinity of the northern coastal habitat was somewhat greater than in the southern coastal and northern inland habitats but still much less than in the southern inland habitat (Table 2). Sodium was the major cation in the soils of all four habitats (Table 3). Calcium and magnesium were also important contributors to the cation concentrations of the inland habitats and particularly so in the southern inland habitat. Chloride was the most prevalent anion in the coastal habitats and was also a major contributor to salinity in the inland habitats. Sulfate was the major cation in the soils of the inland habitats.

TABLE 1. Temperature characteristics of the four habitats from which the experimental populations were taken.

Temperatures (°C)		Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
	Mean Annual	13	17	5	16
	Mean Max	13	16	1	13
Jan	Mean Min	4	9	-18	-2
	Mean	9	13	-8	5
	Mean Max	19	24	28	35
July	Mean Min	11	18	8	19
	Mean	15	21	18	27

TABLE 2. Electrical conductivity and major ion concentration in 100 ml H_2O/100 g soil extracts of soils from the habitats where sample populations were collected.

	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
Electrical Conductivity (mmhos)	7.4	3.8	3.9	14.8
Total Major Cation (meq/l)	73.9	58.5	42.6	194.5
Total Major Anion (meq/l)	74.5	57.6	43.4	191.1

TABLE 3. Major ion concentrations in 100 ml H_2O/100 g soil extracts of soils from the habitats where sample populations were collected.

Ion (meq/l)	Northern Coastal	Southern Coastal	Northern Inland	Northern Inland
Na	65.37	38.11	40.00	142.58
Ca	1.41	9.84	.08	27.36
Mg	5.00	9.32	.10	18.61
K	2.14	.75	2.44	5.88
Cl	56.39	46.10	14.57	59.36
HCO_3	1.10	2.40	1.00	4.90
SO_4	17.00	9.12	27.80	126.80

CHAPTER 1

INTERPOPULATION COMPARISONS OF GROWTH RATE AND ITS PHYSIOLOGICAL DETERMINANTS

The occurrence of saltgrass over a wide range of geographic areas and environmental conditions poses an important question relative to its domestication. Are all the populations of the species capable of acclimation to these diverse conditions or has selection led to genetically diverse ecotypes that have different physiological responses to a set of environmental conditions? If the former is true, then provenance need not play a role in domestication efforts. If the latter is true, provenance will be an important consideration. However, genetic diversity within the species may allow greater opportunities for breeding and selection to provide well adapted cultivars for a variety of habitats.

To answer these questions, we conducted a comparative study of the four populations of saltgrass representing the range of its geographic and climatic distribution. The four populations were compared with respect to their growth, photosynthetic and respiratory responses to substrate salinity and temperature.

METHODS

Vegetative material was collected from four natural populations. The northern coastal population (NC) was collected at Bodega Bay, California. The southern coastal population (SC) was collected at Oceanside, California. The northern inland population (NI) was collected at Alamosa, Colorado. The southern inland population (SI) was collected

at Las Cruces, New Mexico. Sods (5 dm^2) were brought to the laboratory. Plants were washed free of sand and soil, and small pieces of rhizome with shoots and associated roots were established in plastic pots with styrofoam lids containing 2 l of deionized water with the following nutrient composition; 2mM KNO_3, 0.8mM $Ca(NO_3)$, 0.5mM $MgSO_4$, 0.3mM $NH_4H_2PO_4$, 0.04mM Fe-EDTA, micronutrients and with pH = 5.5. These hydroponic cultures were not aerated because preliminary experiments had shown growth was as good or better in nonaerated cultures as it was in aerated cultures. Solutions were replenished with deionized water regularly and completely replaced every two weeks. Salinity was varied by adding NaCl to the nutrient solution to yield three treatments: 0.1mM NaCl (nutrient solution), 250mM NaCl and 500mM NaCl. Plants were grown in controlled environment chambers under 16/8 d/n photoperiods and thermoperiods. Vapor density deficits were maintained at 18 $\pm$ 3g$H_2O m^{-3}$. High temperature treatments were 35°/25°C d/n and low temperature treatments were 25°/15°C d/n. Light was maintained at a quantum flux density of 1200 mol $m^{-2}s^{-1}$ (400-700nm, PPFD) corresponding to 344 cal$cm^{-2}d^{-1}$ and provided by a combination of fluorescent, incandescent and low pressure sodium lamps.

Growth measurements were made on plants which were initiated from a rhizome with only one shoot and associated roots. Relative growth rates (RGR) were calculated: $\ln W = RGR \cdot t + \ln W_0$ with W = dry weight of plants after 28 days, t = 28 days, W_0 = initial dry weight of plants.

Measurements of net gas exchange were made on leaves that had developed under the experimental conditions for four to six weeks. Carbon dioxide and water vapor exchange were measured using standard

Model 215B) and a dew point hygrometer (EG & G Cambridge Model 880-c1) in an open system similar to that described by Williams and Kemp (1978). The air source for the experiments was collected from outside the building and averaged 340 1 CO_2 l^{-1}. Temperature responses of net gas exchange (15 to 40°C) at 2100 mol $m^{-2}s^{-1}$ PPFD were measured on 6 to 10 of the most recently developed attached leaves of a plant. The leaves were sealed into an acrylic cuvette in which temperatures were regulated by circulating water through a water jacket. Leaf temperature was measured with a fine wire thermocouple attached to the bottom of the leaf with plastic putty. Irradiance was provided by a 300 W incandescent flood lamp filtered through 4 cm of acidified 0.06 M Fe_4 $(NH_4)_2$ $(SO_4)_2$. Vapor density differences between leaf and air in the cuvette were maintained at 18 ± 2 g H_2O m^{-3} from 20° to 35°C, 11 ± 2 g H_2O m^{-3} at 15°C and 30 ± 2 g H_2O m^{-3} at 40°C.

Root (nonphotosynthetic tissues, roots and rhizomes) and shoot (photosynthetic tissue, leaves and stems) dry mass measurements were made after 28 days of growth under the experimental conditions. Cation concentrations in tissues were determined using atomic absorption spectrometry of ammonium oxalate extractions of washed tissues. Chloride concentration in tissues were determined by ashing the tissue (three hours at 470°C) in the presence of CaO followed by solubilization in one percent HNO_3. Chloride concentration in the final extract solution was measured with a specific ion electrode.

Factorial analysis of variance was used to test for statistically significant differences among treatment and population means. If differences were found at the 0.05 level of probability a Student-Newman-

Keuls test was used for specific means comparisons (Zar 1974). Differences mentioned in the results section were significantly different at the 0.05 level of probability by this test.

RESULTS

At the lower treatment temperature (25°/15°C d/n) the northern coastal population had a significantly higher RGR than any of the other populations (Table 4). The higher RGR was apparent under all three levels of NaCl tested. The RGR of the northern coastal population was only slightly reduced at the highest salinity relative to the lowest salinity. The intermediate salinity had no detectable effect. The southern coastal population had a significantly higher RGR than the two inland populations at both the lowest and highest salinities, but its performance was quite comparable to the inland populations at 250 mM NaCl. The two inland populations appeared very similar in growth performance at all three salinities and were greatly limited by the low temperature.

At the higher treatment temperature the northern coastal population again had the highest RGR when salinity was low (Table 5). At the intermediate salinity both of the coastal populations had higher RGR than the inland populations. All populations seemed to perform about equally under the highest temperature and salinity. The inland populations were still somewhat limited by temperature even at 35°C since their RGRs did not show as dramatic a drop with increased salinity as did those of the coastal populations.

TABLE 4. RGR (d^{-1}) at low temperature (25°C day and 15°C night). The values given are the means and their standard errors for 8 to 12 samples.

Population	mM NaCl 0.1	200	500
NC	0.096 ± .006	0.089 ± .005	0.082 ± .006
SC	0.064 ± .003	0.049 ± .004	0.038 ± .004
NI	0.041 ± .005	0.043 ± .009	0.032 ± .005
SI	0.034 ± .004	0.038 ± .004	0.032 ± .003

TABLE 5. RGR (d^{-1}) at high temperature (35°C day and 25°C night). The values given are the means and their standard errors for 8 to 12 samples.

Population	mM NaCl 0.1	200	500
NC	0.105 ± .002	0.092 ± .006	0.079 ± .012
SC	0.090 ± .005	0.091 ± .003	0.064 ± .007
NI	0.089 ± .006	0.086 ± .004	0.065 ± .003
SI	0.080 ± .004	0.070 ± .007	0.069 ± .003

When grown at low salinity and low temperature the populations exhibited different net photosynthetic responses to temperature (Figure 1). The northern coastal population had substantially higher net photosynthesis rates in the temperature range from about 20°C to more than 35°C. The temperature response of the southern coastal population was essentially identical to that of the two inland populations under these growth conditions. Increasing the salinity at the lower growth temperature to 250 mM NaCl caused a depression in the rate of net photosynthesis for the northern coastal population (Figure 2). This salinity increase had no appreciable effect on the other populations and the net photosynthetic responses to temperature were, thus, indistinguishable among the populations under low temperature and moderate salinity growth conditions. At 500 mM NaCl and low growth temperature, the rates of net photosynthesis in the inland populations were depressed relative to the rates at 250 mM NaCl much more than they were depressed in the coastal populations (Figure 3). This depressed rate resulted in the coastal populations having higher net photosynthesis rates than the inland populations particularly above 25°C.

Growth at higher temperature increased the rate of net photosynthesis in all populations, particularly at low salinity (Figures 4, 5, and 6). The optimum temperature for net photosynthesis was higher for all populations when grown at higher temperature and low or moderate salinity. This shift in optimum temperature was most dramatic in the southern inland population at low salinity. The southern coastal population was unique in that net photosynthesis rates were as high at 500 mM NaCl as they were at 250 mM NaCl and no decline in net photosynthesis occurred even at 45°C in the 500 mM NaCl treatment.

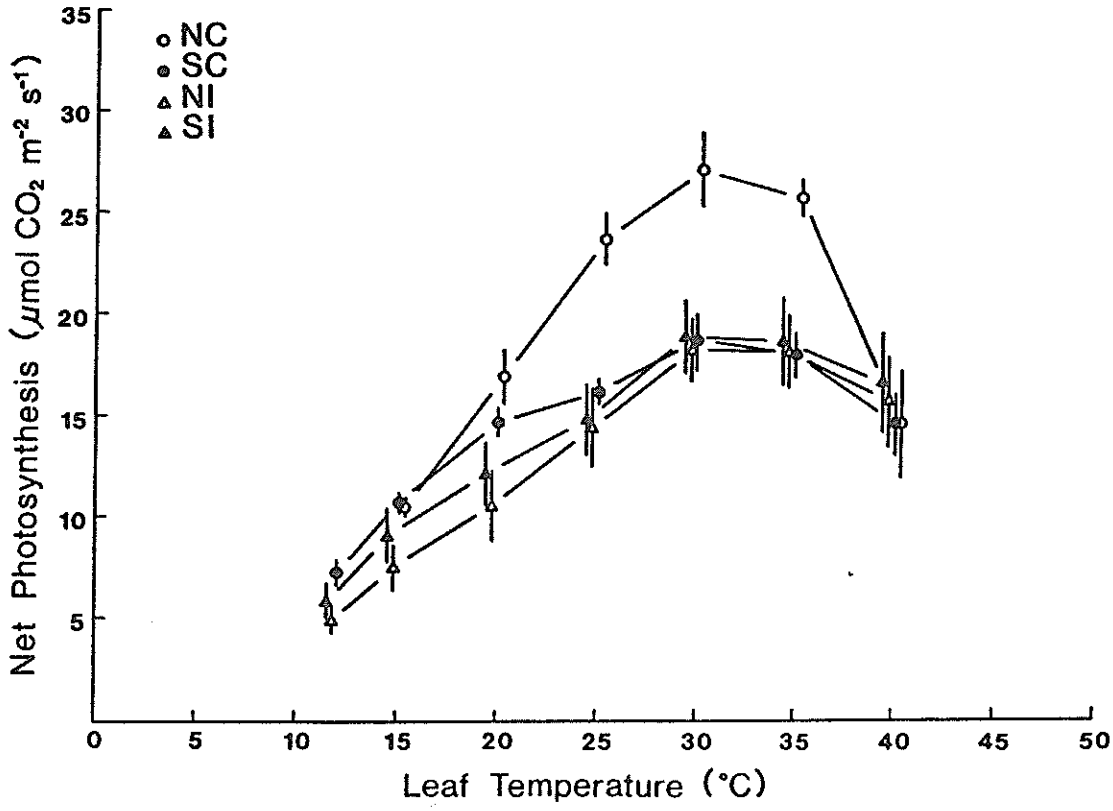

Figure 1. Response of net photosynthesis at a photon flux density of 2100 E $m^{-2}s^{-1}$ (PAR) to temperature for plants for the four populations (NC - northern coastal, SC - southern coastal, NI- northern inland, SI - southern inland) grown at 20°/15°C d/n temperature and 0.1 mM NaCl. The data points on this and subsequent figures are means of four replicates and the vertical bars indicate the standard error of that mean.

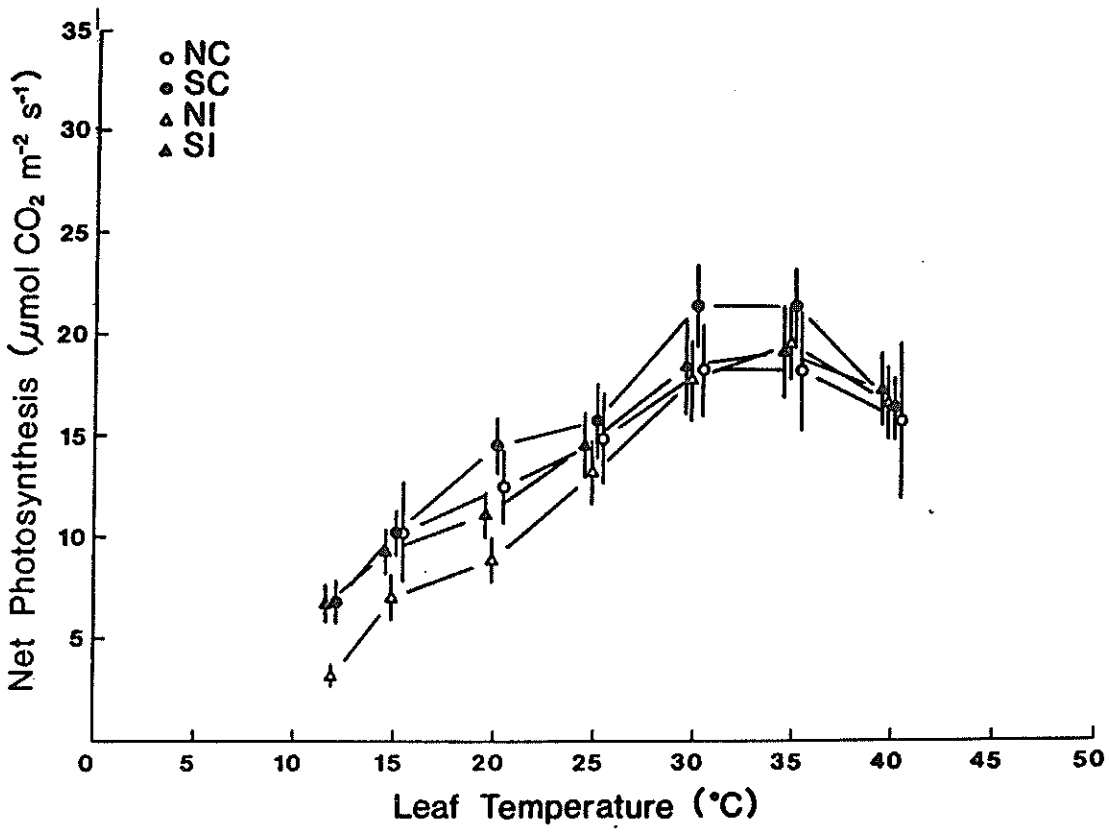

Figure 2. As in Fig. 1 for plants grown at 25°/15° C d/n temperature and 250 mM NaCl.

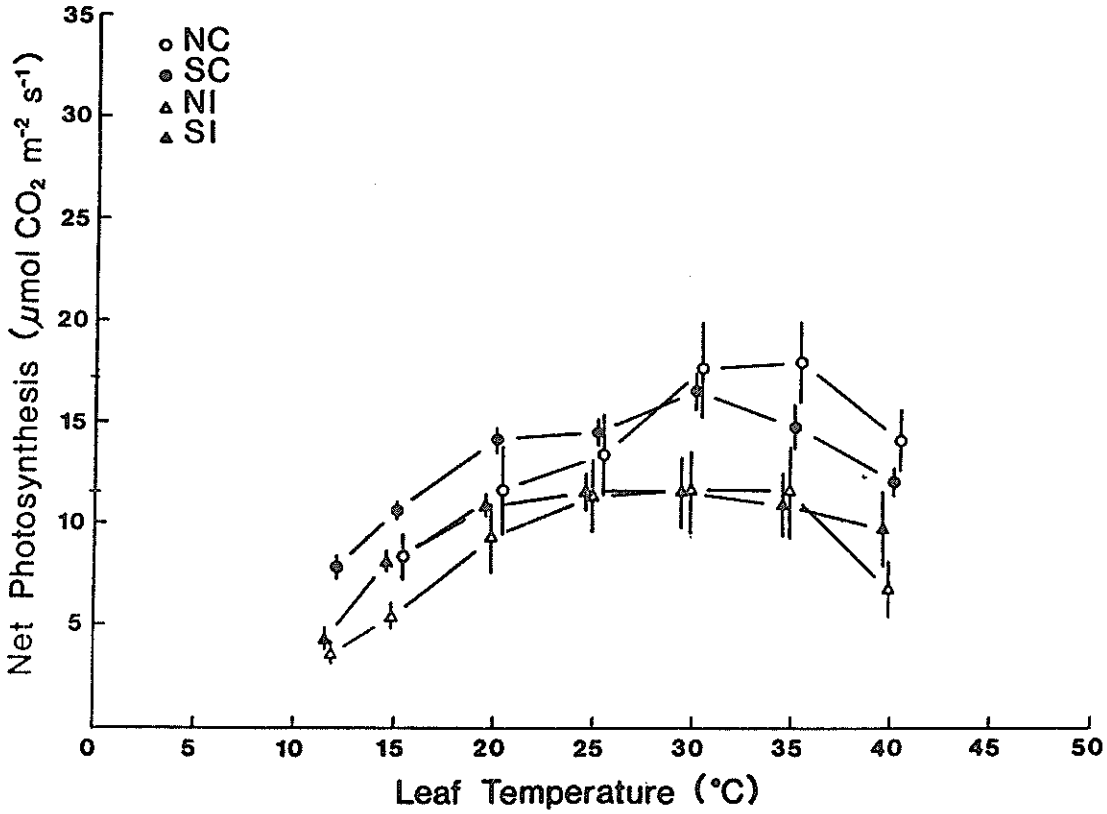

Figure 3. As in Fig. 1 for plants grown at 25°/15° C d/n temperature and 500 mM NaCl.

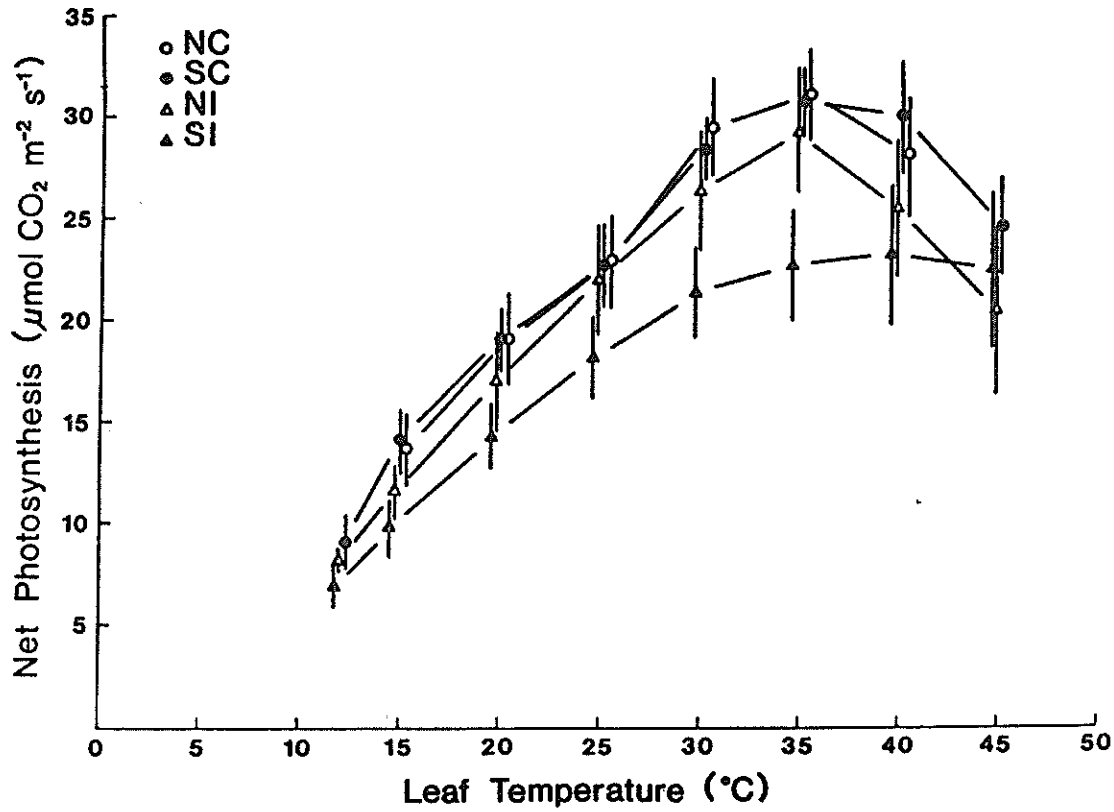

Figure 4. As in Fig. 1 for plants grown at 35°/25° C d/n temperature and 0.1 mM NaCl.

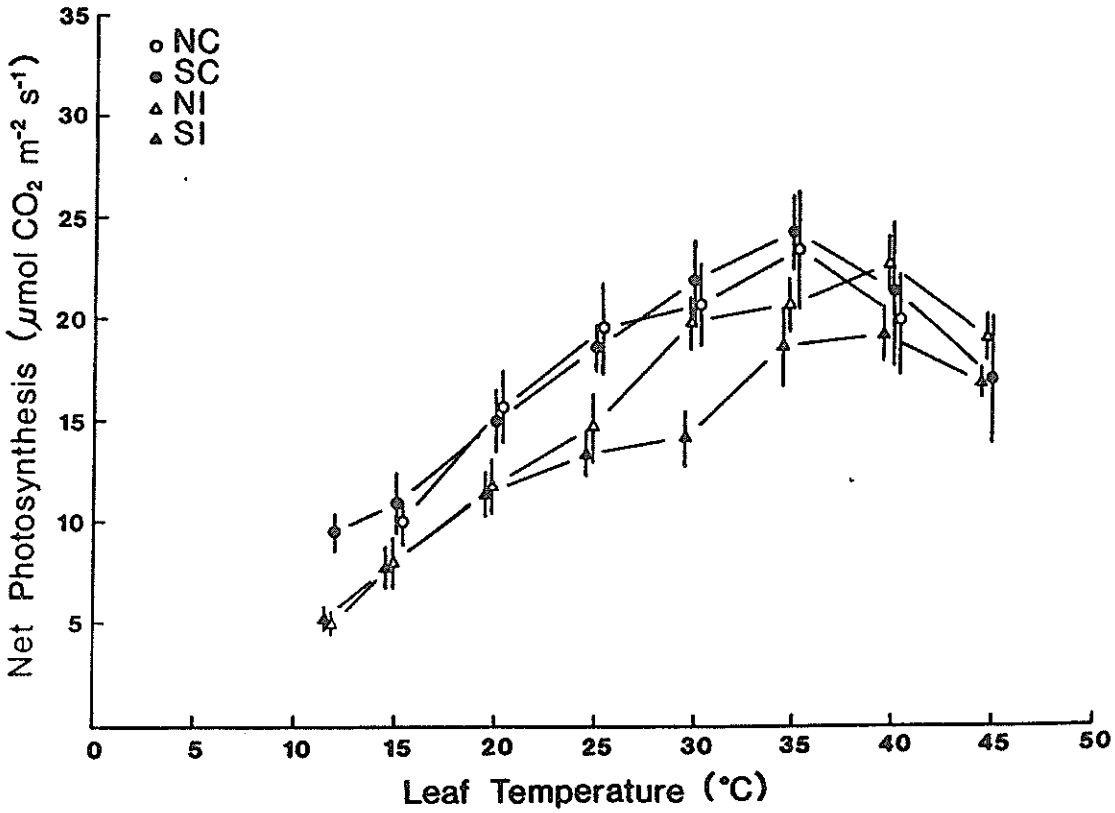

Figure 5. As in Fig. 1 for plants grown at 35°/25° C d/n temperature and 250 mM NaCl.

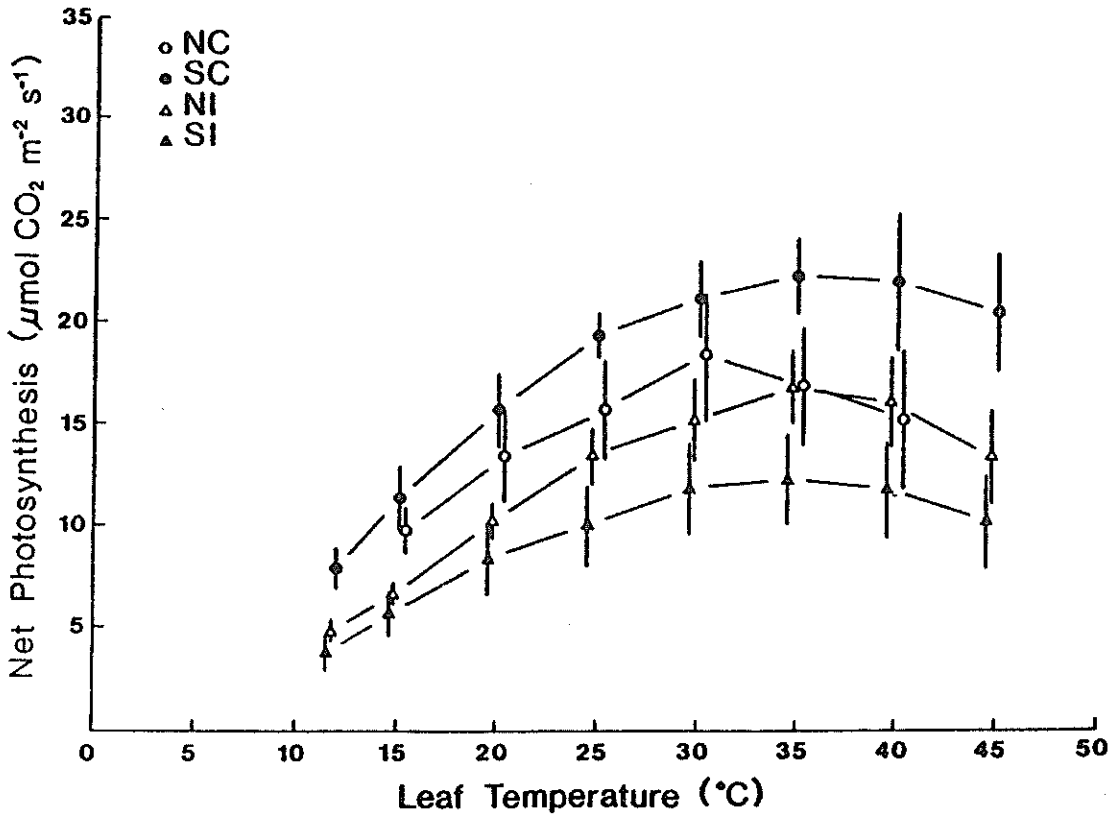

Figure 6. As in Fig. 1 for plants grown at 35°/25° C d/n temperature and 500 mM NaCl.

Dark respiration rates of leaves at 25°C for plants grown at 25°C day and 15°C night temperatures were quite different among the populations (Table 6). At low salinity the rate for each of the populations was different from the others, with the rates of the two coastal populations being most similar. The northern coastal population increased its respiration rate at 250 mM NaCl compared with the rate at 0.1 mM NaCl, but the rate returned to near the level at 0.1 mM NaCl in the 500 mM NaCl treatment. The southern coastal population showed a continual increase in dark respiration with increasing salinity. The inland populations maintained relatively high dark respiration rates at all three salinities. Leaf dark respiration rates are of course higher at 35°C for plants grown at the higher temperature (Table 7). There is, however, no consistant pattern or trend with either population or salinity.

Leaf tissue sodium concentration (Table 8) increased with increased substrate salinity and tended to be slightly higher in the warmer temperature treatments. There were, however, no consistant differences among populations that would indicate differences in their sodium retentions in leaves. Leaf tissue chloride concentrations (Table 9) were highly variable among individuals but tended to exhibit the same response pattern to growth salinity and temperature as did the sodium concentrations.

There was some indication that the northern inland population retained more chloride in its leaves than did the other populations. A final conclusion on this observation awaits confirmation because of the extremely high variance in the measurments. Leaf potassium concentrations (Table 10) showed a pattern opposite to the pattern

TABLE 6. Dark respiration of leaves at 25°C for plants grown at low temperature (25°C day 15°C night). The values given are the means and their standard errors for 4 samples.

Population	mM NaCl 0.1	250	500
NC	0.029 ± .003	0.066 ± .009	0.036 ± .004
SC	0.021 ± .033	0.048 ± .017	0.077 ± .011
NI	0.068 ± .005	0.059 ± .005	0.069 ± 0.12
SI	0.090 ± .015	0.057 ± .005	0.093 ± .020

TABLE 7. Dark respiration of leaves at 35°C for plants grown at high temperature (35°C day 25°C night). The values given are the means and their standard errors for 4 samples.

Population	mM NaCl 0.1	250	500
NC	0.126 ± .012	0.103 ± .005	0.103 ± .014
SC	0.091 ± .007	0.096 ± .004	0.119 ± .011
NI	0.127 ± .024	0.119 ± .017	0.137 ± .020

TABLE 8. Leaf Tissue Sodium Concentration (mg/g).

Treatment	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15°				
0.01 mM NaCl	1.57 ± 0.54	1.22 ± 0.33	0.75 ± 0.32	0.46 ± 0.21
250 mM NaCl	6.57 ± 5.36	5.36 ± 1.42	5.33 ± 0.78	5.56 ± 0.76
500 mM NaCl	9.74 ± 2.87	7.84 ± 2.71	7.58 ± 2.22	7.55 ± 1.55
35/25°				
0.01 mM NaCl	1.21 ± 0.39	1.93 ± 0.31	1.18 ± 0.31	0.80 ± 0.32
250 mM NaCl	8.51 ± 2.67	10.9 ± 1.42	10.9 ± 2.41	7.86 ± 2.02
500 mM NaCl	10.6 ± 2.62	12.7 ± 2.95	14.2 ± 3.23	9.70 ± 2.50

TABLE 9. Leaf Tissue Chloride Concentration (mg/g)

Treatment	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15°C				
0.01 mM NaCl	1.63 ± 0.74	2.22 ± 0.69	2.31 ± 0.72	1.12 ± 0.39
250 mM NaCl	9.46 ± 0.76	6.87 ± 1.49	6.19 ± 1.04	10.1 ± 1.06
500 mM NaCl	14.4 ± 3.17	9.16 ± 3.14	9.05 ± 3.93	11.7 ± 4.38
35/25°C				
0.01 mM NaCl	2.76 ± 0.63	1.77 ± 0.26	1.79 ± 0.58	1.94 ± 0.60
250 mM NaCl	17.2 ± 2.38	13.8 ± 2.68	15.4 ± 4.79	12.3 ± 2.73
500 mM NaCl	18.0 ± 6.05	19.7 ± 3.11	21.2 ± 4.51	16.0 ± 3.23

TABLE 10. Leaf Tissue Potassium Concentration (mg/g).

Treatment	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15°C				
0.01 mM NaCl	17.7 ± 4.72	18.1 ± 3.20	13.6 ± 1.61	12.2 ± 2.98
250 mM NaCl	15.4 ± 2.64	13.3 ± 2.68	11.4 ± 2.83	12.5 ± 1.75
500 mM NaCl	12.4 ± 1.88	12.4 ± 2.82	8.94 ± 1.06	9.72 ± 1.24
35/25°C				
0.01 mM NaCl	21.7 ± 4.79	21.7 ± 1.80	19.7 ± 2.80	21.2 ± 2.15
250 mM NaCl	15.9 ± 1.63	13.7 ± 0.82	13.5 ± 3.10	14.8 ± 1.81
500 mM NaCl	12.7 ± 1.36	10.5 ± 0.92	11.9 ± 2.28	16.0 ± 1.66

observed for sodium and chloride but again no differences among populations were detected.

Root tissue sodium concentrations (Table 11) were higher than concentrations in the leaf tissue but generally showed the same pattern of increase with growth salinity and temperature. No differences among the populations were evident. Chloride concentrations in the roots (Table 12) were also higher than in the leaves but generally showed the same pattern. The northern inland population again appeared different from the others in having lower root tissue chloride concentrations. This population appeared to retain more chloride in the roots and less in the leaves than did the other populations. Potassium concentrations in roots (Table 13) were much the same as in the leaves for all four populations.

Shoot/root ratios (Table 14) were influenced by the salinity of the nutrient solution. Southern coastal, northern inland and southern inland populations all allocated a greater proportion of their total drymass to nonphotosynthetic root tissue as sodium chloride concentration was increased. By contrast the northern coastal population exhibited a greater allocation to the photosynthetic shoots with increased salinity.

The chemical compositions of the leaves of plants from the four populations were investigated in a separate set of experiments. Potted plants were grown outdoors in a common garden at Las Cruces, New Mexico. Protein, lipid, hemicellulose, lignin, cellulose, organic acid and ash contents of plants irrigated with 250 mM NaCl nutrient solutions were compared with controls that received the same nutrient solutions in tap water. The results of this experiment are presented as differences

TABLE 11. Root Tissuye Sodium Concentration (mg/g)

Treatment	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15°C				
0.01 mM NaCl	2.31 ± 0.80	3.83 ± 0.69	2.62 ± 1.52	1.29 ± 0.44
250 mM NaCl	14.4 ± 1.35	20.1 ± 2.10	17.0 ± 4.39	17.1 ± 5.15
500 mM NaCl	26.3 ± 6.11	31.0 ± 8.98	29.6 ± 8.97	38.1 ± 7.02
35/25°C				
0.01 mM NaCl	4.65 ± 1.90	3.82 ± 0.37	3.36 ± 0.67	1.49 ± 0.27
250 mM NaCl	35.6 ± 4.52	36.1 ± 5.08	23.2 ± 6.44	29.8 ± 5.20
500 mM NaCl	41.4 ± 8.33	50.0 ± 7.14	43.9 ± 9.53	41.8 ± 6.63

TABLE 12. Root Tissue Chloride Concentration (mg/g).

Treatment	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15°C				
0.01 mM NaCl	1.24 ± 0.53	2.72 ± 0.41	3.20 ± 1.92	1.00 ± 0.20
250 mM NaCl	38.3 ± 9.21	23.1 ± 5.73	26.4 ± 5.44	30.8 ± 10.4
500 mM NaCl	57.9 ± 13.4	55.7 ± 7.41	46.0 ± 15.7	56.3 ± 5.57
35/25°C				
0.01 mM NaCl	1.78 ± 0.22	1.68 ± 0.88	2.82 ± 1.36	1.84 ± 0.36
250 mM NaCl	59.2 ± 7.49	50.8 ± 9.73	40.1 ± 12.7	53.2 ± 6.83
500 mM NaCl	75.9 ± 9.27	84.8 ± 11.5	72.8 ± 16.8	69.8 ± 19.7

TABLE 13. Root Tissue Potassium Concentration (mg/g).

Treatment	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15°C				
0.01 mM NaCl	20.3 ± 4.73	22.6 ± 3.07	18.3 ± 4.14	15.9 ± 6.28
250 mM NaCl	16.4 ± 5.56	13.2 ± 2.31	12.0 ± 1.87	18.8 ± 7.00
500 mM NaCl	13.1 ± 1.86	17.4 ± 1.27	11.3 ± 4.01	19.8 ± 4.68
35/25°C				
0.01 mM NaCl	25.2 ± 6.87	27.7 ± 4.86	25.0 ± 4.86	25.9 ± 4.01
250 mM NaCl	20.8 ± 4.10	20.6 ± 2.84	10.2 ± 4.34	21.6 ± 3.71
500 mM NaCl	19.0 ± 6.39	16.7 ± 2.94	11.8 ± 4.33	19.8 ± 4.68

TABLE 14. Shoot/Root ratios of *D. spicata* from the four populations grown for 28 days under the indicated temperature and salinities.

Growth Temperature (d/n °C)	Growth Salinity (mM NaCl)	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
25/15	0.01	7.95 ± 0.65	10.15 ± 0.74	11.00 ± 0.98	10.10 ± 0.74
	250	10.27 ± 1.09	6.63 ± 0.55	9.31 ± 0.68	9.38 ± 0.83
	500	8.67 ± 0.89	6.32 ± 0.60	7.08 ± 0.94	7.99 ± 0.89
35/15	0.01	7.26 ± 0.52	11.04 ± 1.01	11.58 ± 0.72	12.58 ± 0.78
	250	9.03 ± 0.90	9.87 ± 0.68	10.02 ± 0.67	7.78 ± 0.48
	500	9.16 ± 2.21	7.78 ± 0.66	6.48 ± 0.47	7.24 ± 0.41

between treatments and controls in percentage composition of each chemical constituent of the biomass (Table 15). Salinity caused an increase in protein content in all but the northern coastal population. Changes in other components were minor except for the decrease in hemicellulose in the southern coastal population and the decrease in cellulose in both of the southern populations.

A significant question that arises from the results (Table 15) concerns the extent to which the effects of growth substrate salinity and temperature on relative growth rates can be accounted for by effects on the rates of net photosynthesis. To investigate this question, relative growth rates (RGR) for each question and treatment were plotted as a function of net photosynthesis rates (Pnet) at the growth salinity and temperature (Figure 7). A linear regression was fit to these data using all 24 data points (Line A Figure 7). The relationship RGR = 0.0147 + 0.0027 Pnet (r^2 = 0.51) was obtained. Thus across all populations and treatments net photosynthesis accounted for only 51 percent of the variation in relative growth rates. Inspection of Figure 7 suggested that the data fell into three relatively distinct groups; some values lying on the overall regression line, some above it, and some below. Each of these three data groups were subjected separately to linear regression. Line B was obtained for the eight values above the overall regression line (RGR = 0.0595 + 0.0014 Pnet, r^2 = 0.79). Line C was obtained for the five values that were nearest the overall regression line (RGR = 0.0278 + 0.0023 Pnet, r^2 = 0.88). Line D was obtained for the 11 values below the overall regression line (RGR = -0.0027 + 0.0028

TABLE 15. Change in chemical composition of leaves when plants were grown with irrigation treatments of 250 mM NaCl irrigation treatments of 250 mM NaCl as compared with controls that received only tap water. Values are change in precent of total biomass composition. * indicates a significant difference between treatment and control. ($P < 0.05$).

	Northern Coastal	Southern Coastal	Northern Inland	Southern Inland
Protein	-0.7	+9.6*	+3.8*	+7.0*
Lipid	+1.7*	+1.7*	+1.5*	+1.9*
Hemicellulose	+0.8	-8.1*	-0.2	-1.8
Lignin	+1.1	+2.6*	-0.4	-4.8*
Organic Acid	+0.1	-2.6*	-0.5	-1.9*
Ash	-0.5	-0.7	-2.7*	+0.7

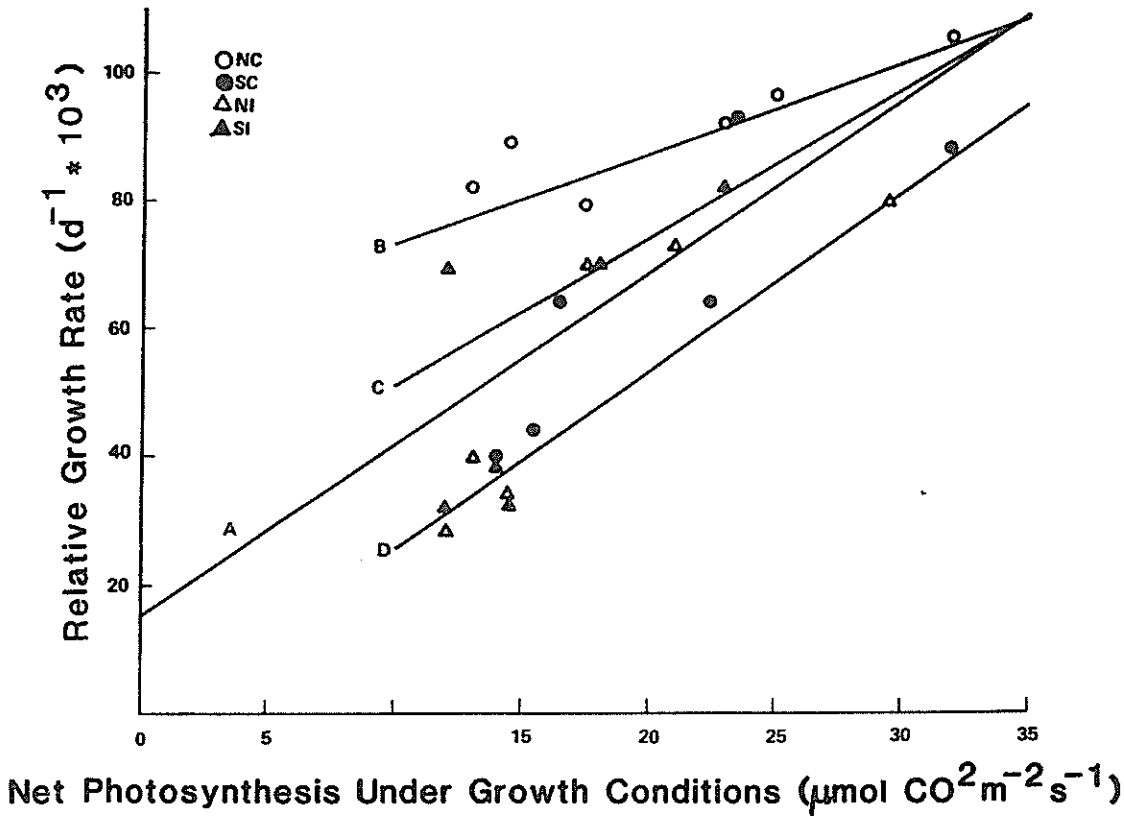

Figure 7. Regressions of relative growth rate as a function of net photosynthesis under growth conditions.

Pnet, $r^2 = 0.96$). All of the data points for the northern coastal population were included in group B. The lower slope of this line indicates that the northern coastal population did not gain as much in relative growth rate per unit increase in net photosynthesis as did the other poulations. It did, however, maintain a greater relative growth rate at any given value of net photosynthesis.

The data presented suggest two reasons why this might be the case. The northern coastal population tended to allocate more biomass to leaf production as salinity increased. That is, those conditions that decreased the rate of net photosynthesis per unit leaf area also increased the relative production of new leaf material, thus compensating to some extent for its reduced efficiency. Increased salinity caused the other three populations to increase their leaf protein contents. Thus the new leaf material they produced under high salinity was more expensive in terms of energy and carbon than was the leaf tissue produced by the northern coastal population. The higher rates of allocation to energetically less expensive leaves allowed the northern coastal population to maintain higher relative growth rates with increased salinity than could the other populations.

DISCUSSION

With the above results in mind we can now return to the question posed. Do the populations of saltgrass exhibit ecotypic variation across the geographic range of the species? The answer is yes. When grown in common environments, the four populations showed differences in the responses of relative growth rate, net photosynthesis, and dark respiration to salinity and temperature. This conclusion has some

important implications with regard to domestication of this species as a salt tolerant forage crop. The genome of the plant material used to establish a crop should be selected with careful regard for the match between its physiological characteristics and the environmental and cultural conditions to which it will be subjected. From the results presented here it might seem that this selection would be a simple task. The northern coastal population was clearly superior to all other populations in relative growth rate at the lower growth temperature under all salinities. At the higher growth temperature its relative growth rate was as great or greater than that of the other populations at all salinities tested. The net photosynthesis measurements, however, indicated that the southern coastal population might be the genotype of choice if growth temperatures exceed about 35°C under highly saline conditions.

Can the inland populations be dismissed as unsuitable for exploitation? The results presented here would certainly suggest this to be the case. Soil samples from the native habitats of these populations indicate that while the southern inland population grows in soils with approximately the same Cl^- levels as the coastal populations (about 60 meq/l) the SO_4^- levels are about 12 times as great (about 10 meq/l coastal, about 127 meq/l southern inland.) This suggests that further study might reveal greater SO_4^- tolerance in the southern inland population. We also know that the inland populations exhibit a photoperiodically indiced dormancy. This could be an important characteristic under some environmental conditions.

Each of the populations of saltgrass may possess certain genetically determined physiological characteristics which might be valuable in a particular environment. It is, therefore, important that we view these genetically diverse poulations as a composite resource of genetic material which might be combined in various ways to provide cultivars giving maximum yields in environments of diverse climate and soil or water chemistry characteristics. In the development of our traditional agricultural crops we have often lost the natural genetic resource for the breeding of new cultivars. This is of course the consequence of the fact that we began using these species as crops long before the development of modern scientific agriculture. The development of new crops must go hand in hand with the preservation and study of the native populations. We should be developing a knowledge of how the native populations function in the background of the physical and biological stresses to which they are subjected. This will allow us to select and breed for the appropriate stress resistances in the agricultural context. These types of studies could be very important in identifying those populations which should be protected. In the case of halophytes, such as saltgrass, the need is imperative because many of the sites, which might be developed for agriculture, could harbor genetic resources which we will need in the not too distant future.

CHAPTER 2

PHOTOSYNTHETIC RESPONSES OF SALTGRASS (*DISTICHLIS SPICATA*) TO IRRADIANCE, TEMPERATURE AND SALINITY GROWTH TREATMENTS: A MODELING SYNTHESIS

In general, the physiological and morphological responses of salt tolerant C_4 plants to salinity, and the interactions of salinity with other environmental variables, are poorly understood. Kemp and Cunningham (1981, Chapter 1) examined some of the photosynthetic responses of *D. spicata* to various irradiance, temperature and salinity growth environments. High salinity decreased net photosynthesis under all temperature and irradiance conditions evaluated. These decreases were associated with increased stomatal resistance in all cases and with increased residual (i.e., mesophyll) resistance only when plants were grown at low temperature and/or low irradiance. Photon-use efficiencies were quite variable across the numerous treatments but in general the lowest values were associated with the highest salinity treatments. These results led to the tenative conclusion that the principal effects of salinity on net photosynthesis in this species were through effects on stomatal resistance and photon-use efficiency. Very little could be concluded regarding the interactions of temperature and irradiance with salinity in determining net photosynthetic responses.

The objective of the present study is to examine in greater detail the effects of growth treatment conditions of salinity, temperature and irradiance on relevant partial processes of net photosynthesis in *D. spicata*. We do this by (1) developing a mathematical model of net CO_2 exchange, (2) estimating values for the model parameters using photon

fluence rate response data obtained from plants grown in different environmental conditions of salinity, temperature and irradiance, and (3) examining the pattern of variation in the fitted model parameters using cluster, principal component and variance analyses.

METHODS AND MATERIALS

Experimental Data

Distichlis spicata plants were collected from the shore line at Bodego Bay, California, U.S.A. and maintained in hydroponic culture as described by Kemp and Cunningham (1981) (see Chapter 1). Plants were grown in controlled environmental chambers under one of 12 different treatment conditions (Table 16). There were three salinity treatments consisting of 0.1 mM NaCl (nutrient solution only), 250 mM NaCl and 500 mM NaCl. Two temperature treatments were used: (1) a high temperature of 35/25 C d/n, and (2) a low temperature of 25/15 C d/n. Growth irradiance was maintained at two levels. Low irradiance was obtained by a combination of fluorescent and incandescent lamps producing a photon fluence rate of 600 ± 100 mols m^{-2} s^{-1} (400-700 nm, measured with a Lambda Li-185 quantum sensor), which corresponded to an irradiance of 140 ± 20 W m^{-2} (400-1300 nm, measured with a Lambda Li-185 pyranometer sensor). The high irradiance treatment was provided by a combination of fluorescent, incandescent, and low pressure sodium lamps producing a photon fluence rate of 1200 ± 150 mol m^{-2} s^{-1} (400-700 nm), which corresponded to an irradiance of 270 ± 30 W m^{-2} (400-1300 nm). Thus, there was some difference in radiation quality as well as quantity. All growth treatments had the same 16/8 h d/n photoperiod and thermoperiod and 18 ± 3 g m^{-3} vapor density deficit.

Measurements of net gas-exchange rates were made on attached leaves that had developed under the experimental growth treatments for four to six weeks. The open gas-exchange measurement system used has been described by Kemp and Cunningham (1981 Chapter 1). Responses of net photosynthesis to photon fluence rate were measured at leaf temperatures equal to the respective growth-treatment daytime air temperatures and at the respective growth salinities. Gas exchange measurements were replicated on four plants from each of the 12 growth-treatment conditions (2 temperatures x 2 irradiances x 3 salinities).

Model Development

Thornley (1976) summarizes various models that have been used to describe leaf photosynthesis as a function of irradiance and CO_2 concentration. A familiar model is the rectangular hyperbola:

$$Pg = \frac{I\ Pg_{max}}{I + Pg_{max}} \qquad (1)$$

where Pg is gross photosynthesis (mg CO_2 $m^{-2}s^{-1}$), I is photon fluence rate of photosynthetically active radiation (PAR, 400 to 700 nm) (mol $m^{-2}s^{-1}$), is the initial slope of the Pg:I curve (i.e., incident photon-use efficiency at limiting I [mg CO_2 mol photon^{-1}], and Pg_{max} is the maximum rate of Pg at saturating I (I). If at saturating photon fluence Pg_{max} is limited by internal leaf CO_2 concentration, C_i (mg CO_2 m^{-3}), then we can write (following Watson et al. [1978]):

$$Pg_{max} = Ci/r_m \qquad (2)$$

where r_m is mesophyll resistance (s m^{-1}). From the diffusion for net CO_2

flux between the external atmosphere and the chloroplasts, C_i is given by (Jones and Slatyer 1972 and Nobel 1974):

$$C_i = C_a - P_n r_d \tag{3}$$

where C_a is the ambient CO_2 concentration (mg CO_2 m^{-3}), P_n is net photosynthesis, and r_d is the leaf diffusive resistance (s m^{-1}) to CO_2 flux. Leaf diffusive resistance can be partitioned into a boundary layer (r_a) and stomatal (r_s) resistance (Gaastra 1959 and Jarvis 1971), i.e.,

$$r_d = r_a + r_s \tag{4}$$

Substituting equations 2-4 into equation 1, and setting $P_g = P_n + R_d$, net photosynthesis (P_n) is given by:

$$P_n = \frac{I\,[C_a - P_n\,(r_a + r_s)]}{Ir_m + [C_a - P_n\,(r_a + r_s)]} - R_d \tag{5}$$

where R_d is dark respiration (mg CO_2 $m^{-2}s^{-1}$). Equation 4 is a nonrectangular hyperbola that, when rewritten in quadratic form, has as the solution for P_n:

$$P_n = \frac{b - \sqrt{b^2 - 4ac}}{2a} \tag{6}$$

where

$$a = r_a + r_s$$

$$b = I\,(r_m + r_a + r_s) + C_a - R_d\,(r_a + r_s)$$

$$c = IC_a - R_d\,(Ir_m + C_a)$$

The maximum rate of net photosynthesis at saturating photon fluence rate, i.e., $P_n(I\ )$, is:

$$P_{nmax} = \frac{C_a - R_d r_m}{r_a + r_s + r_m} \qquad (7)$$

The relationship of stomatal resistance to photon fluence rate over the various treatments was examined using the following model (Lommen et al. 1971):

$$r_s = (1 + K/I)\ r_{smin} \qquad (8)$$

where K (mol $m^{-2}s^{-1}$) is the value of I at which r_s equals twice its minimum value and r_{smin} (s m^{-1}) is the minimum (asymptotic) value of r_s.

Equations 6 and 8 were fitted to the photosynthetic photon fluence rate response curves obtained for the four individual plants grown in each of the 12 pretreatment conditions, giving a total of 48 cases. The response curves were obtained at leaf temperatures corresponding to the respective growth temperatures, i.e., 25°C and 35°C and at the growth treatment salinity (Table 16). Component leaf resistances to CO_2 flux, i.e., stomatal (r_s) and boundary layer (r_a) resistances, were calculated from the CO_2 and water vapor flux measurements and leaf area measurements in the manner described by Longstreth and Strain (1977). A nonlinear least squares fitting procedure (SAS NLIN, Helwig and Council 1979) was used to obtain estimates of the parameters , r_m and R_d of equation 6 and K and r_{smin} of equation 8.

Statistical Analyses

A 48 cases x 6 variables (, r_m, R_d, P_{max}, n, r_{smin}, and K) primary data matrix was formed from the results of the nonlinear fitting

procedure. Seven cases were dropped from the analyses due to failure to meet the convergence criterion of the fitting algorithm. The resulting 41 x 6 primary data matrix was subjected to both cluster and principal component analyses to (1): identify clusters or groups of treatments with similar photosynthetic responses, (2) determine the experimental growth conditions important in the separation of these groups, and (3) examine the variation in model parameters across treatments. Examples of the potential usefulness of multivariate statistical models in physiological research can be found in Van Andel and Nelissen (1973), Kowal et al. (1976) and Ceulemans et al. (1980).

Cluster analysis was performed using relative Eucliden (standardized) distance (Orloci 1974) and the average linkage (UPGMA) option of the SAHN clustering model (Lance and Williams 1967 and Sneath and Sokal 1973). The cluster analysis was employed as a preliminary trend-seeking approach to identify major clusters of treatments based on their simlarity (as measured by the six gas exchange variables); the average value for each variable in a treatment was used.

To examine the relationships between these clusters, the primary data matrix was converted to standardized principal components (Blackith and Reyment 1971). This analysis was performed on the standardized variable correlation matrix, and the first three latent roots of this matrix were used to obtain projections of the 41 cases onto the component axes. This provides a reduction of the original multidimensional space and the correlations of the original variables with each axis can be determined. The computational details of this method are given by Seal (1964), and the theoretical aspects are discussed by Gower (1966).

The effects of salinity, temperature and irradiance on the least squares estimates of the model parameters from equation 6 and 8, and the derived quantity P_{nmax} in equation 7, were evaluated for significant effects and interactions using an analysis of variance (Zar 1974).

RESULTS

The three-parameter photosynthesis model, equation 6, provided excellent fits to the gas exchange data over all treatments. Representative photon fluence rate response curves are shown in Figure 8, where the model was fitted to individual cases from treatments D, E, and F (Table 16).

The results of the cluster analysis are summarized in Figure 9 in the form of a phenogram. At a Euclidean distance of 1.5, two major treatment groups are identifiable (1 and 2, Figure 9). Treatments in group 1 are mainly those of low growth irradiance (140 W m^{-2}) (71 percent of the cases), 35°C temperature (71 percent) and either 0.1 or 250 mM NaCl (86 percent). Group 2 consists mainly of high growth irradiance (270 W m^{-2}) (80 percent), 25C temperature (80 percent) and 500 mM NaCl (60 percent) treatments. Hence, it appears that some degree of separation of photosynthetic responses has occurred over the range of treatments.

Results of the principal components analysis are presented in Figure 10. Projections of the 41 cases within the space of the first two principal components show a general separation of the two groups delimited in the cluster analysis. Each case is identified with its treatment letter A-L (Table 16). Generally, scores on the first principal axis are positive for cluster group 1 (uncircled letters) and

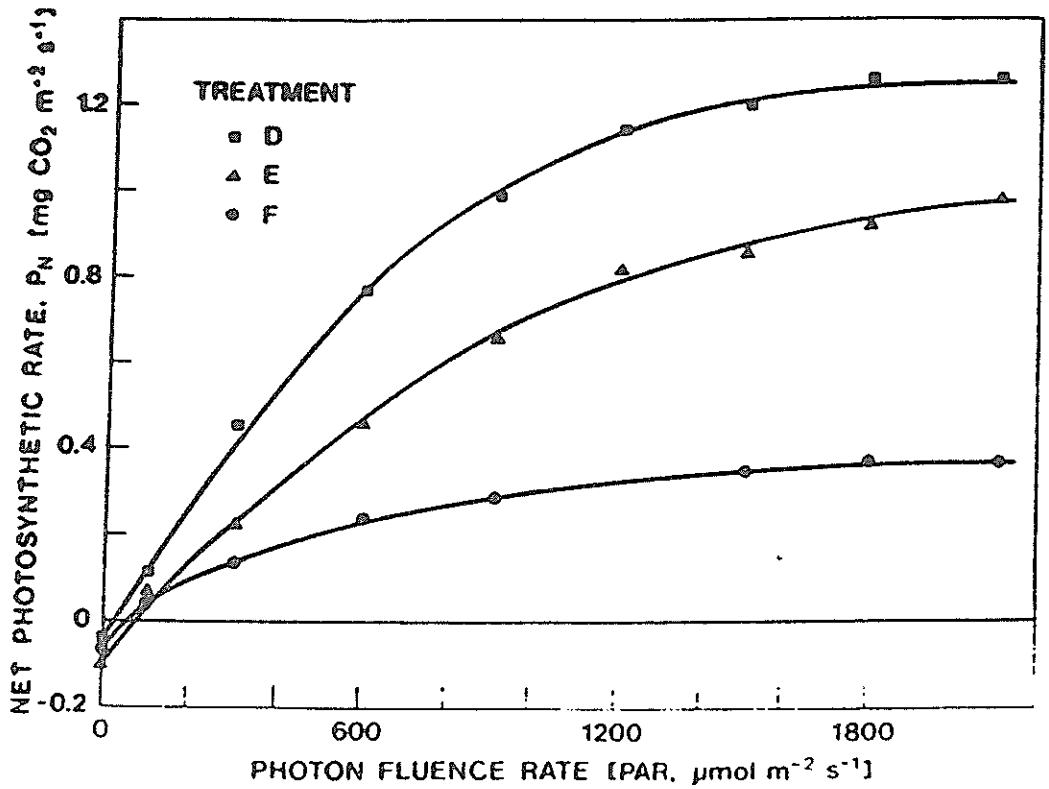

Figure 8. Photon fluence response curves (eqn 6) fitted through measured data for three individual cases representing a growth temperature of 25 C, irradiance of 270 W m^{-2}, and 3 salinity levels: 0.1 mM Na Cl (curve D), 250 mM NaCl (curve E) and 500 mM NaCl (curve F).

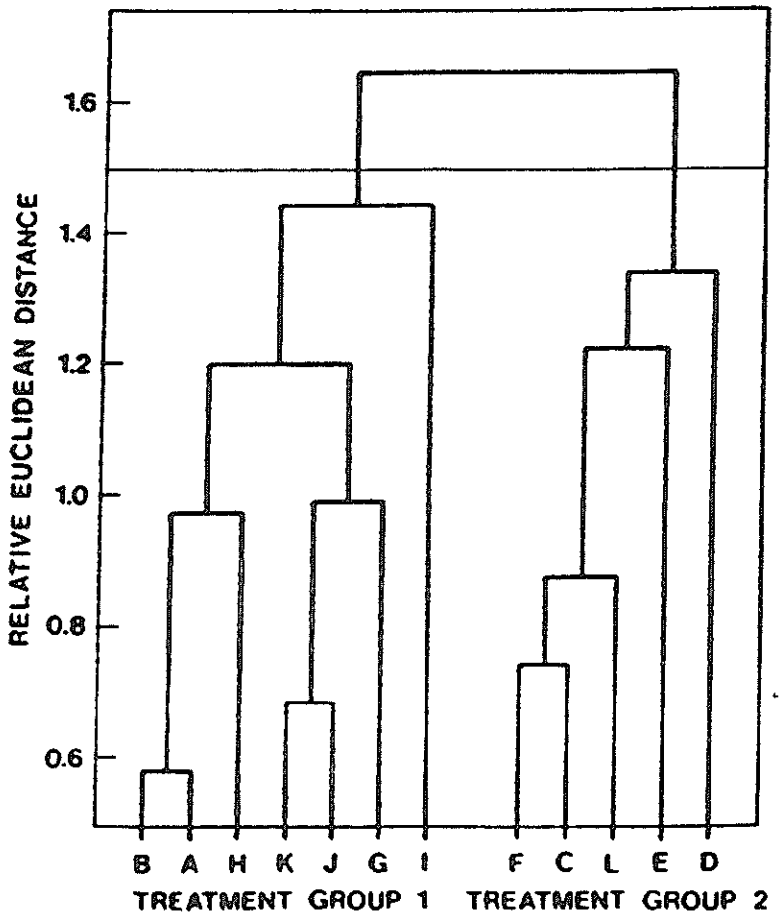

Figure 9. Phenogram resulting from the cluster analysis of the 12 growth treatments (A-L in Table 1). Two major clusters are identifiable: Cluster 1 (mainly low irradiance, high temperature and low salinity) and Cluster 2 (mainly high irradiance, low temperature and high salinity).

TABLE 16. Growth conditions used in controlled environment chambers. The letters A through L identify each of the 12 specific growth treatments: 2 growth temperatures (25 and 35 C) x 2 irradiances (140 and 270 W m^{-2}) x 3 salinity levels (0.1, 250 and 500 mM Na Cl).

Growth Temperature (C)	Irradiance (W m^{-2})	Salinity (mM Na Cl) 0.1	250	500
25	140	A	B	C
	270	D	E	F
35	140	G	H	I
	270	J	K	L

negative for cluster group 2 (circled letters), with a region of overlap near 0.0 (Figure 10).

In an attempt to interpret principal component I, the frequency of cases associated with the various growth treatments were tabulated at intervals of 0.1 along the first axis. The results for salinity and temperature are shown in Figure 11. Separation of the cases along this first axis is primarily a function of salinity treatment (Figure 11) although temperature also contributes to the separation (Figure 11). The latent root associated with principal component I accounts for 46.7 percent of the total sample variation in the original observations (41 estimates of the six model variables) (Table 17).

Correlations of the six photosynthetic model variables with the first three principal components are given in Table 17; such correlations can be extremely important in interpreting components (Maxwell 1977). P_{nmax} ($r = 0.92$), r_{smin} ($r = -0.81$), and r^m ($r = -0.72$) have the highest correlations with component I. The relationships between component I and each of these variables are plotted in Figure 10 (b-d).

The second principal component, which accounts for an additional 19.8 percent of the variation (Table 17), is related to growth treatment irradiance (Figure 12). Note that 77 percent of the high irradiance cases have scores greater than 0.0 and 68 percent of the low irradiance cases have scores less than 0.0 on principal component II (Figure 12). The variable most highly correlated with component II is ($r = -0.82$) (Figure 12, Table 17).

The only variable highly correlated with principal component III is K ($r = -0.74$) (Table 17). This final component accounts for an

TABLE 17. Correlations of the photosynthesis variables with the first 3 principal components (I, II, and III). Also given are the latent roots associated with each principal component and the percent of the total variance each represents. The first 3 latent roots account for a total of 80.5 percent of the sample variance. The proportion of the total variance (v^2) in each of the 6 variables is given; note that the sum of these variances is equivalent to the sum of the latent roots and represents 80.5 percent of the total variance in the variables.

	PRINCIPAL COMPONENT			
Variable	I	II	III	Variance (v^2)
	0.32	-0.82	0.31	.888
r^m	-0.72	-0.55	0.07	.844
R^d	0.61	-0.30	-0.10	.471
$p^{max,n}$	0.92	-0.04	-0.07	.845
K	-0.55	-0.30	-0.74	.950
r^{smin}	-0.81	0.10	0.41	.835
Variance (Latent Root)	2.80	1.19	.084	4.833
% of Total Variance	46.7	19.8	14.0	80.5
Accumulated Percent	46.7	66.5	80.5	

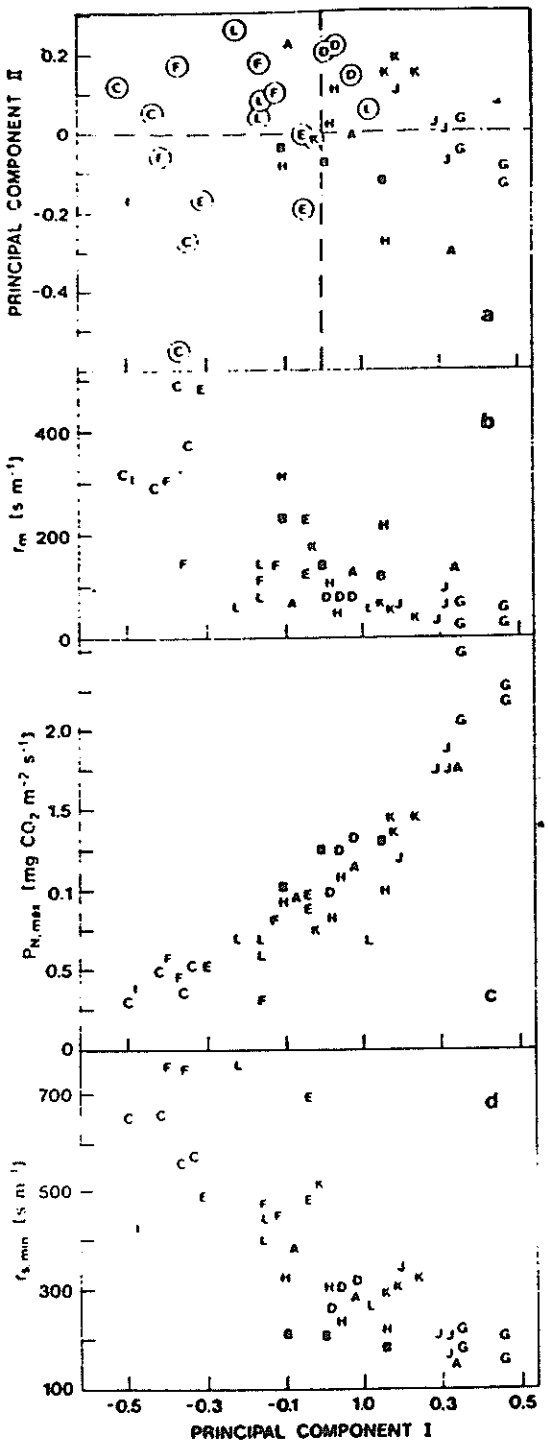

Figure 10. Results of the principal component analysis. (a). Projection of the 41 cases on axes representing the first 2 principal components. Each case is identified with the letter A-L, indicating its growth treatment (See Table 16). Uncircled letters are cases identified as cluster 1 (Fig. 9) and circled letters are those in cluster 2 (Fig. 9). (b)-(d). The relationships of the 3 photosynthesis variables having the highest correlation with principal component I.

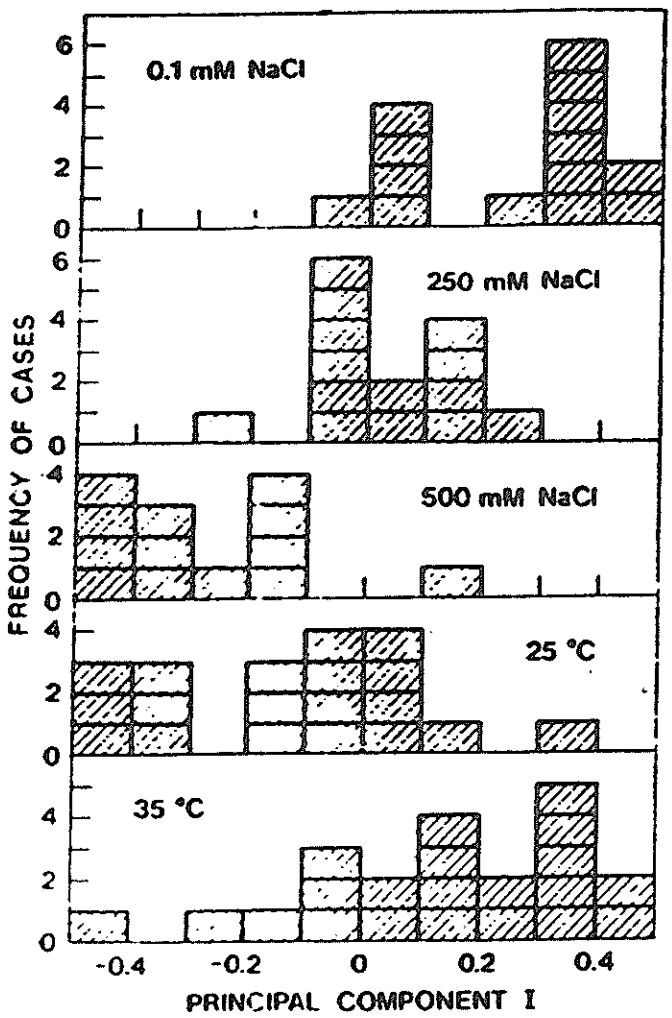

Figure 11. Frequency of cases associated with (a) the three salinity treatments and (b) the two temperature treatments tabulated along the first principal component.

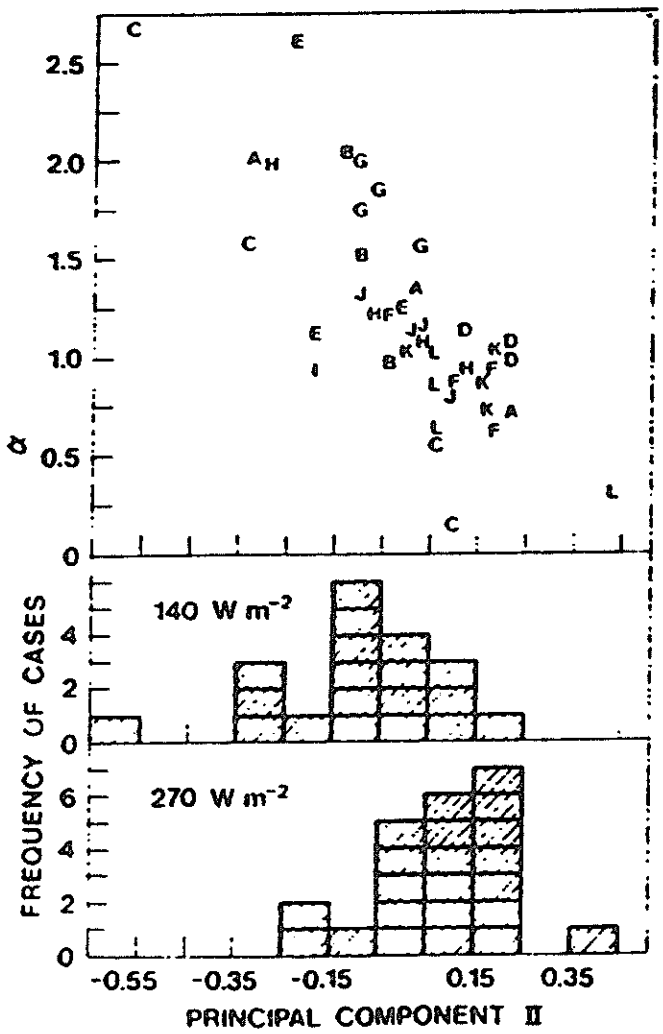

Figure 12. (a) The relationship between the model parameter for each case and principal component II. Cases are identified as in Fig. 10. (b) Frequency of cases associated with the two irradiance treatments tabulated along principal component II.

additional 14 percent of the total variation and separation of cases along the third principal component and is not clearly associated with any of the growth treatment conditions.

The results of the three-way factorial analysis of variance of the six photosynthetic model variables are summarized in Table 18. An interpretation of the analysis of variance results leads to conclusions which are consistant with those obtained from the cluster and principal component analyses. The initial slope of the photon fluence rate response curve ( ) was significantly affected by growth treatment irradiance (Table 18). There is a significant temperature and salinity interaction (Table 18); this is also apparent from the slight positive correlation (r = 0.32) (Table 17) of with component I. Hence, there is a tendency for to be maximum at low salinity and 35° C.

Mesophyll resistance (r^m), maximum rates of net photosynthesis (p^{nmax}), and minimum stomatal resistance (r^{smin}) were all significantly affected by both salinity and temperature (Table 18). Parameter K exhibits significant irradiance and salinity main effects (Table 18); this is reflected in the correlations of K with components I (a salinity gradient) and II (an irradiance gradient) (Table 17).

Dark respiration (R^d) is affected only by temperature (Table 18). The importance of R^d in accounting for the separation of the cases across the various treatments (components) is minor. The proportion of the total variance of R^d accounted for by the first three components is only 47.1 percent, compared to over 83 percent for the remaining variables (Table 17). These variances [column v^2, Table 17] are the sums of squares of the correlations of each variable across the three components

TABLE 18. F values from the analysis of variance of photosynthesis variables. Level of significance: * = ($P < 0.05$).

	VARIABLE					
Source[a]	a	r^m	R^d	Pn,max	K	r^{smin}
I	7.4*	4.1	0.0	3.2	19.6*	12.5*
T	0.1	13.6*	15.8*	23.5*	0.0	14.4*
S	0.7	9.1	0.8	58.6*	15.8*	25.4*
I x T	1.2	0.2	1.0	0.0	1.3	0.4
I x S	0.5	4.1*	0.8	1.4	4.4*	4.2*
T x S	3.4*	0.7	1.0	6.9*	3.0	2.7
I x T x S	0.4	3.8*	1.7	8.1*	3.2	4.5*

[a]I = irradiance, T = temperature, S = salinity

and would sum to one if all six principal components were extracted for analysis. R^d does show a positive correlation ($r = 0.61$) with component I, which is related to growth temperature (Figure 11).

DISCUSSION

The use of a simple model of net CO^2 exchange, in conjunction with a descriptive statistical analysis of the derived model parameters, provides some relevant insights on the role of growth conditions and their interactions in affecting photosynthesis and its partial processes. The maximum rate of net photosynthesis, P^{nmax}, had a high positive correlation with the first principal component. This axis exhibited a strong relationship in the positive direction with decreasing salinity and a moderate relationship with increasing temperature. Therefore, it can be concluded that P^{nmax} was decreased by increasing salinity and that increased temperature, to some extent, compensated for the detrimental effects of higher salinity. The analysis of variance confirmed these correlations. Growth irradiance appeared to have little influence on P^{nmax} as shown by its low correlation with the second principal component and lack of significant correlation with growth irradiance in the analysis of variance.

The incident photon-use efficiency at low irradiance, , was the only model parameter not influenced by salinity and temperature. Both the analysis of variance and principal component analysis showed a correlation of with growth irradiance. The negative correlation of with the second component and an increasing growth irradiance axis, indicated higher growth irradiance decreased the incident photon-use efficiency at low photon fluence rates.

The rate of dark (mitochondrial) respiration, R^d, showed a moderate positive correlation with the first principal component and the analysis of variance indicated this was primarily due to temperature. Decreased temperature resulted in a decrease in the dark respiration component of net photosynthesis (i.e., an increase in the rate of net photosynthesis). This effect must have been small, however, because it is opposite in direction from the effect of temperature on P^{nmax}.

The major effects of the various experimental treatments on the maximum rate of net photosynthesis, and the form of the photon fluence response relationship, were the results of the interaction of salinity and temperature on mesophyll resistance, r^m, the minimum stomatal resistance, r^{smin}, and K, the parameter describing the sensitivity of the stomata to photon fluence rate. The mesophyll resistance increased with increasing salinity and with decreasing temperature. It can be assumed that the CO^2 concentration (or partial pressure) at the site of carboxylation is essentially the same as that in the intercellular spaces of the leaf mesophyll (i.e., the actual diffusive resistance component of r^m is small) (Farquhar and Sharkey 1982). Therefore r^m is essentially equivalent to the reciprocal of a "carboxylation efficiency" and increased values of r^m indicate decreased carboxylation efficiencies. The major effect of higher salinity and lower temperature thus appears to be the reduction of carboxylation efficiency. Both K and r^{smin} increased with increasing salinity and with decreasing temperature as did r^m.

It cannot be determined from the present analysis whether the observed effects on the parameters describing stomatal resistance (r^{smin} and K) are a direct result of temperature and salinity effects on stomatal function or an indirect consequence of the effects on

carboxylation efficiency. This is a result of stomatal resistance responding to the experimental treatments in the same fashion as carboxylation efficiency (i.e., reduced carboxylation efficiency in the same treatments that yield greater stomatal resistance). It is likely that the stomatal responses are the result, and not the cause, of reduced rates of CO^2 assimilation.

CHAPTER 3

EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON DISTICHLIS SPICATA UNDER THREE SALINITY LEVELS

Distichlis spicata (L.) Greene (Gramineae) occurs widely in inland (Ungar 1974) and coastal (Duncan 1974) saline habitats of North America. This plant forms vesicular-arbuscular (VA) mycorrhizae in the field (Allen and Cunningham, personal observation), but most physiological studies on salt tolerance of D. spicata have been done in solution culture (e.g. Tiku 1976, Smart and Barko 1980 and Kemp and Cunningham 1981), where we observed no mycorrhizae, possibly due to low aeration (Allen and St. John 1982). The mechanisms suggested for its salt tolerance include high osmotic pressure in the cells (Ungar 1974) and excretion of salts through leaf glands (Hansen et al. 1976). We designed experiments to determine whether VA mycorrhizae play any role in salt tolerance.

Research on VA mycorrhizae has generally focused on uptake of nutrients such as P (e.g., Hattingh et al. 1973, Pearson and Tinker 1975, Rhodes and Gerdemann 1975 and Allen et al. 1981a) and of water (Safir et al. 1972, Levy and Krikun 1980 and Allen et al. 1981b). The interaction of mycorrhizae with salts such as NaCl, which reduce plant growth in high concentrations, is less well understood, although mycorrhizae often occur in salt-tolerant plants (Khan 1974). Both Na and Cl reduce the germination of Gigaspora margarita spores (Hirrel 1981), and mycorrhizal spore numbers are inversely correlated with Na in Oregon desert soils

(Trappe 1981). Spore counts in saline Pakistani soils were fewer than 1 g^{-1} (Khan 1974). However, mycorrhizae may partially overcome the detrimental growth effects of NaCl in salt-tolerant plants such as onion and bell pepper (Hirrel and Gerdemann 1980). Atriplex canescens, a salt tolerant plant had improved growth with mycorrhizae, but was studied only in a soil of very low salinity (Aldon 1975). The object of our study was to measure the effects of mycorrhizae on dry mass, Na, K and P concentrations, Na and K excretion, and stomatal conductance of D. spicata grown under three NaCl levels (Figure 13).

MATERIALS AND METHODS

Whole plants from two populations of D. spicata were collected and maintained in hydroponic culture (Kemp and Cunningham 1981, Chapter 1). One was a coastal population near Oceanside, California, and the other was an inland population along the Rio Grande near Las Cruces, New Mexico . Mycorrhizal infection was assessed from five plants of each population by staining roots with cotton blue stain and scoring infection in 0.5 mm segments (Allen and Allen 1980). The coastal population had 16 percent infection in November 1980, while the inland population had 26 percent in October 1980. An additional assessment of the inland plants in March 1980 gave 77% infection, but no spring field samples were obtained from the coast. Plants grown in solution culture had no mycorrhizae after three months.

Spores were isolated from five 25 g soil samples at each site using a sucrose separation technique (Allen et al. 1979). Glomus fasciculatum (Thaxter sensu Gerdemann) Gerdemann and Trappe was the predominant spore type at both sites, with fewer than 1 spore g^{-1} dry soil.

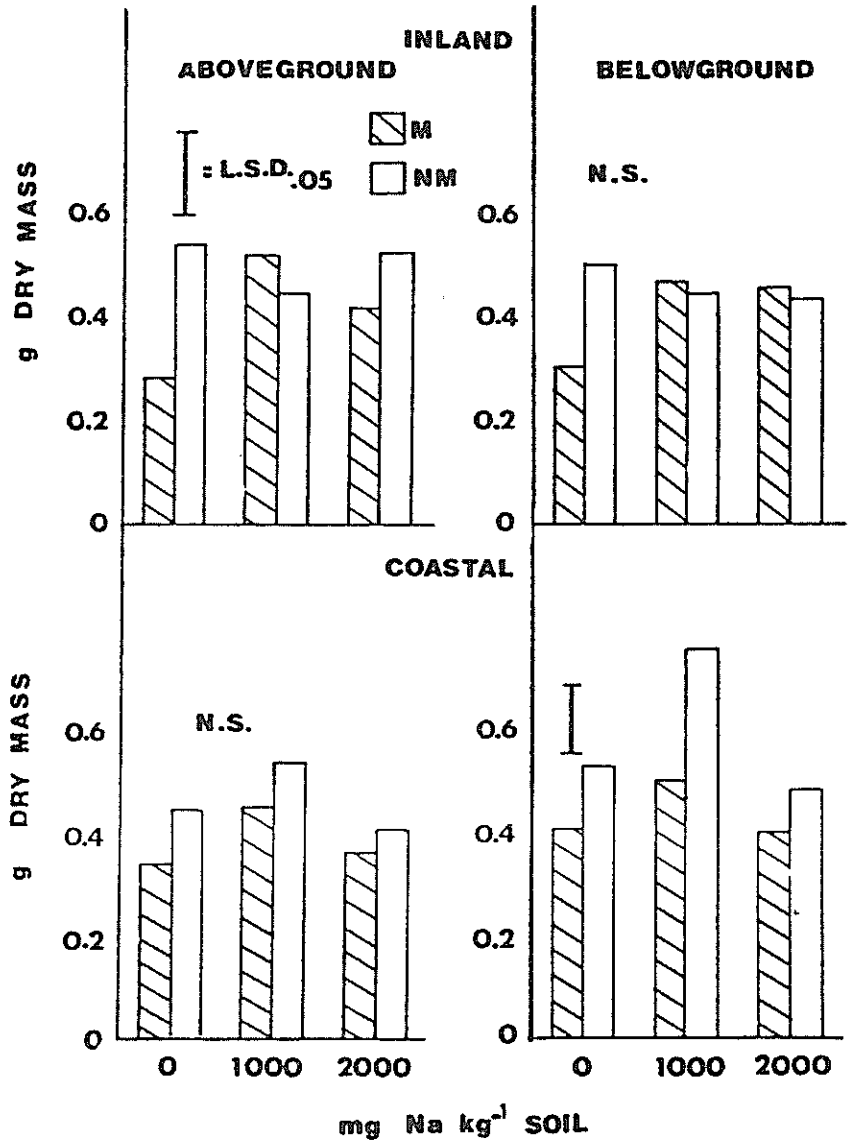

FIGURE 13. Above-ground (a), (c) and below-ground (b), (d) dry mass of plants from inland (a), (b) and coastal (c), (d) populations of D. spicata grown with three levels of Na added to soil and with ( ) and without ( ) VA mycorrhizae. N.S. = no significant difference. Bar. = L.S.D. 0.05.

NaCl was mixed with steam-sterilized coarse sand to give concentrations of 0, 1000 or 2000 mg Na^+kg^{-1} dry sand. The available Na^+ in the sand before NaCl amendment was 92 mg kg^{-1}, as determined by extraction with ammonium acetate and detection with a flame photometer. Analysis of soils indicated Na^+ concentrations of 192 and 398 mg kg^{-1} dry soil for the coastal and inland sites, respectively. Although D. spicata occurs at higher Na^+ concentrations than 2000 mg kg^{-1} dry soil, its growth is often stunted (Ungar 1974). Plants were difficult to establish at Na^+ concentrations higher than 2000 mg kg^{-1} dry soil.

Cuttings weighing from 0.5 to 1.0 g fresh mass were transferred to pots containing 570 g dry sand. For the mycorrhizal inoculum, 1 g fresh mass of chopped D. spicata roots from the inland population was added to half of the pots. There were 10 replicate pots of the mycorrhizal and non-mycorrhizal treatments at each of the three salinities, giving a total of 60 pots for each population. The plants were fertilized twice during the experiment with 50 ml per pot of a nutrient solution used for hydroponic culture containing 0.4 M PO_4^{-3} (Kemp and Cunningham 1981, Chapter 1). To avoid leaching Na^+ and nutrients, soil moisture was controlled by watering daily with 50 ml distilled water or 30 ml on occasional overcast days. Plants were grown for two months in a glasshouse with temperatures averaging 35°C in the day and 24°C at night.

The plants were monitored for stomatal conductance (C_S), leaf excretion of Na and K, tissue Na, K and P concentrations, and mycorrhizal infection. A diffusive resistance porometer (Li-Cor Model No. Li60) was used to measure C_S on the top unfolded leaves of five plants from each treatment for each population at midday before the plants received their daily watering. The rate of excretion by salt gland was measured by

first rinsing all salts from the surface of a leaf sample by vigorously shaking the attached leaves in a test tube filled with glass-distilled water. After 24 hours the leaf samples were again rinsed of salt in the same manner using 10 ml of glass-distilled water. These leaves were severed and their areas measured with a leaf area meter (Li-Cor Model No. LI-3000). Na and K concentrations in the rinse solutions were determined by flame photometry, and excretion was expressed as g ion excreted per unit leaf area per day. Above- and below-ground plant parts were harvested, including leaves used for excretion measurements, dried to constant mass at 65°C and weighed. Leaves and roots only, omitting culms and rhizomes, were ground in a Wiley mill. The leaves and roots were then digested in nitric acid and diluted. Na and K were measured with a flame photometer, and P colorimetrically. A second set of plants was grown under the same conditions to assess mycorrhizal infection (Allen and Allen 1980). Soil was taken from around the roots to measure Na concentrations at the end of the experiment.

Dry mass and element concentration data were subjected to a 2 x 3 factorial analysis of variance for each population separately. Statistical differences between treatments at the 0.95° confidence level are shown using the least significant difference ($L.S.D._{0.05}$).

RESULTS

Mycorrhizal infection reduced the dry mass of above-ground parts of D. spicata from the inland population under low salinity and of below-ground parts of plants from the coastal population at intermedite salinity. There were generally no changes in dry mass with increasing soil salinity.

Mychorrhizae did not change P concentrations of leaves, but the mycorrhizal roots had greater P concentrations at the low and high salinities for the inland population and the low salinity for the coastal population (Figure 14).

Leaves from both populations showed a four-fold greater concentration of K than Na, and there were few signficant differences resulting from the salinity or mycorrhizal treatments (Figure 15). The roots, however, exhibited increased Na accumulation at the higher soil salinity (Figure 16). Mycorrhizal infection significantly increased root Na and K concentrations in some cases. Specifically, the inland mycorrhizal roots had higher Na at the high salinity and the coastal mycorrhizal roots had higher K at the low and intermediate salinities. The tissue K/Na ratios were similar between the high and intermediate soil salinity treatment and highest for the low soil salinity treatment in all cases except for the coastal leaves (Figure 17). Mycorrhizal roots of both populations had a higher K/Na ratio at the low salinity treatment than did non-mycorrhizal roots.

Leaf excretion of Na increased with increasing soil salinity, but K excretion did not vary (Figure 18). The coastal plants which were mycorrhizal had a greater Na excretion rate than did non-mycorrhizal plants at the high salinity.

There were no significant differences in C_s among the mycorrhizal or salinity treatments for either population. The overall mean C_s, with standard error, for both populations and all salinity treatments was 0.23 $\pm$ 0.02 cm s^{-1} for non-mycorrhizal treatments. The C_s values were somewhat low because measurements were taken before the daily watering when plants would be at maximum moisture stress.

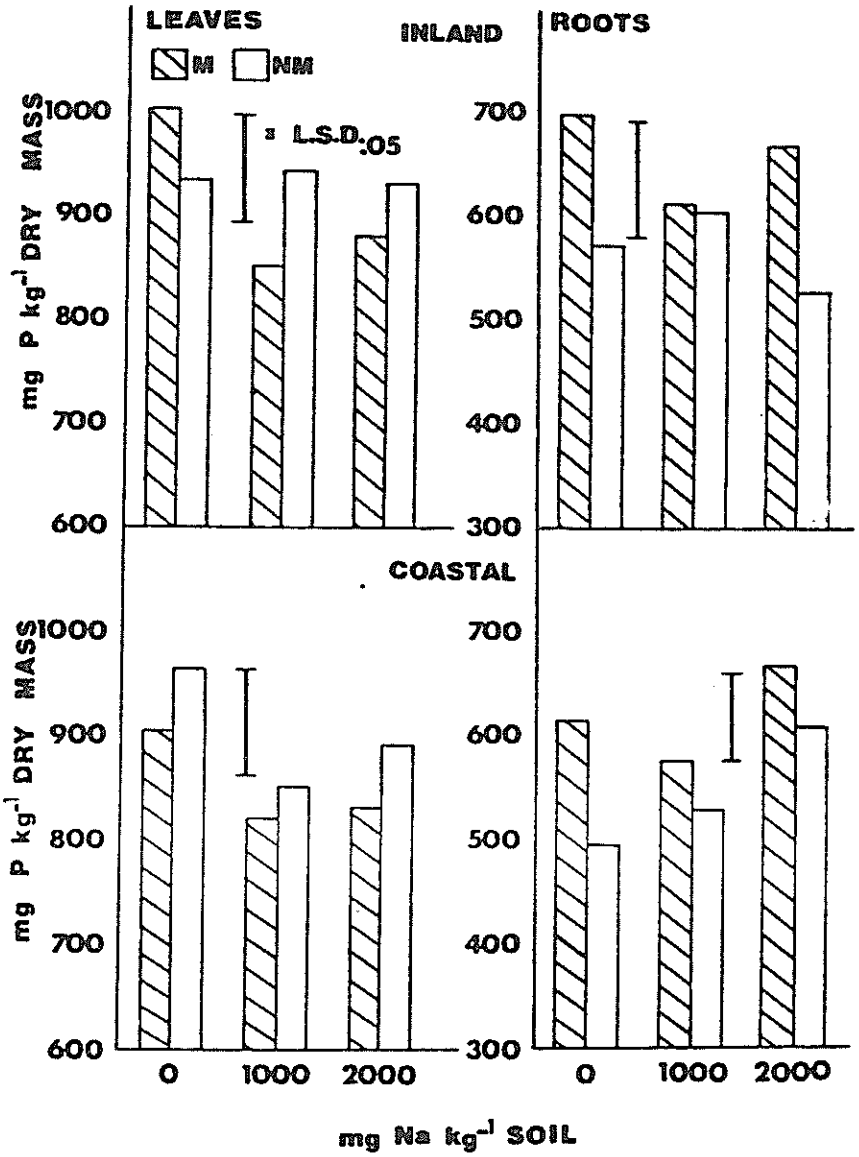

FIGURE 14. Leaf (a), (c) and root (b), (d) P concentrations in inland (a), (b) and coastal (c), (d) populations of D. spicata grown with three levels of Na added to soil, and with ( ) and without ( ) mycorrhizae. Bar. = L.S.D. 0.05

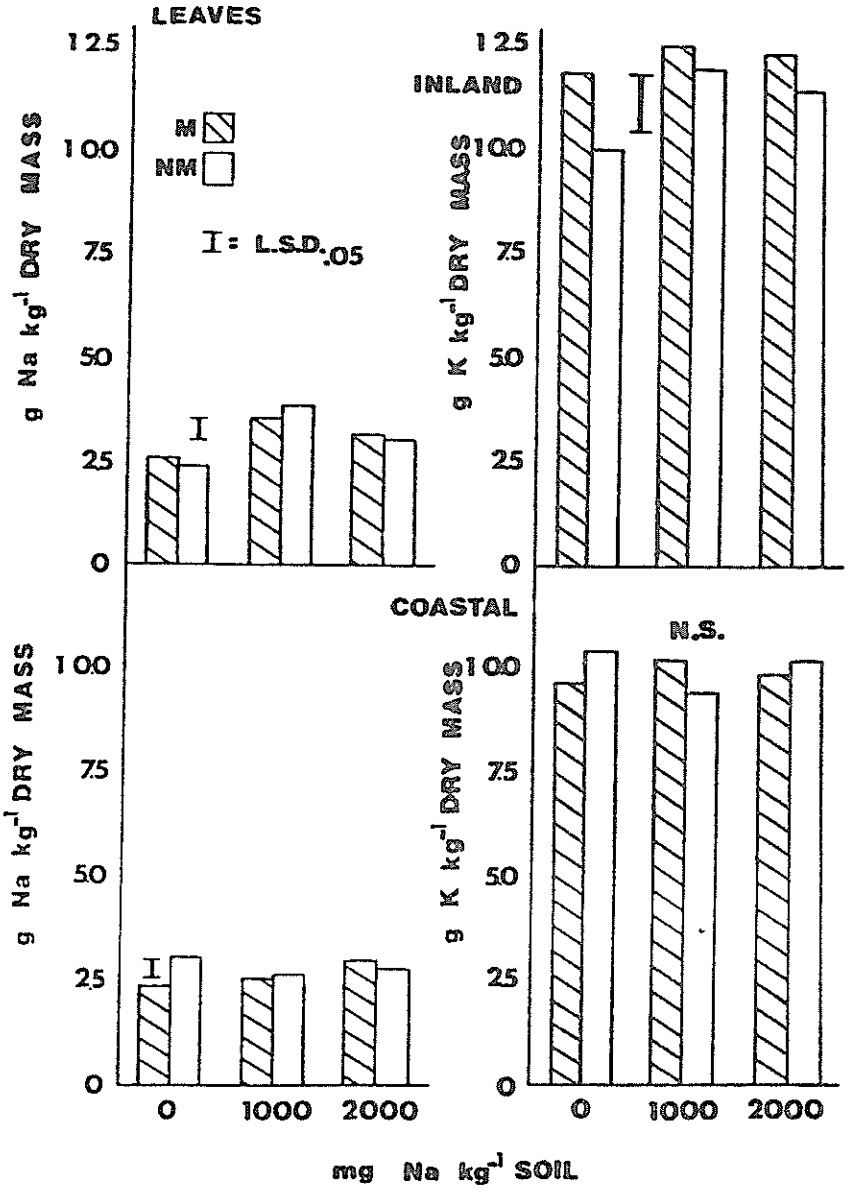

FIGURE 15. Leaf Na and K concentrations in inland (a), (b) and coastal (c), (d) populations of D. spicata grown with three levels of Na added to soil, with ( ) and without ( ) mycorrhizae. N.S.= no significatant differences. Bar. = L.S.D. 0.05.

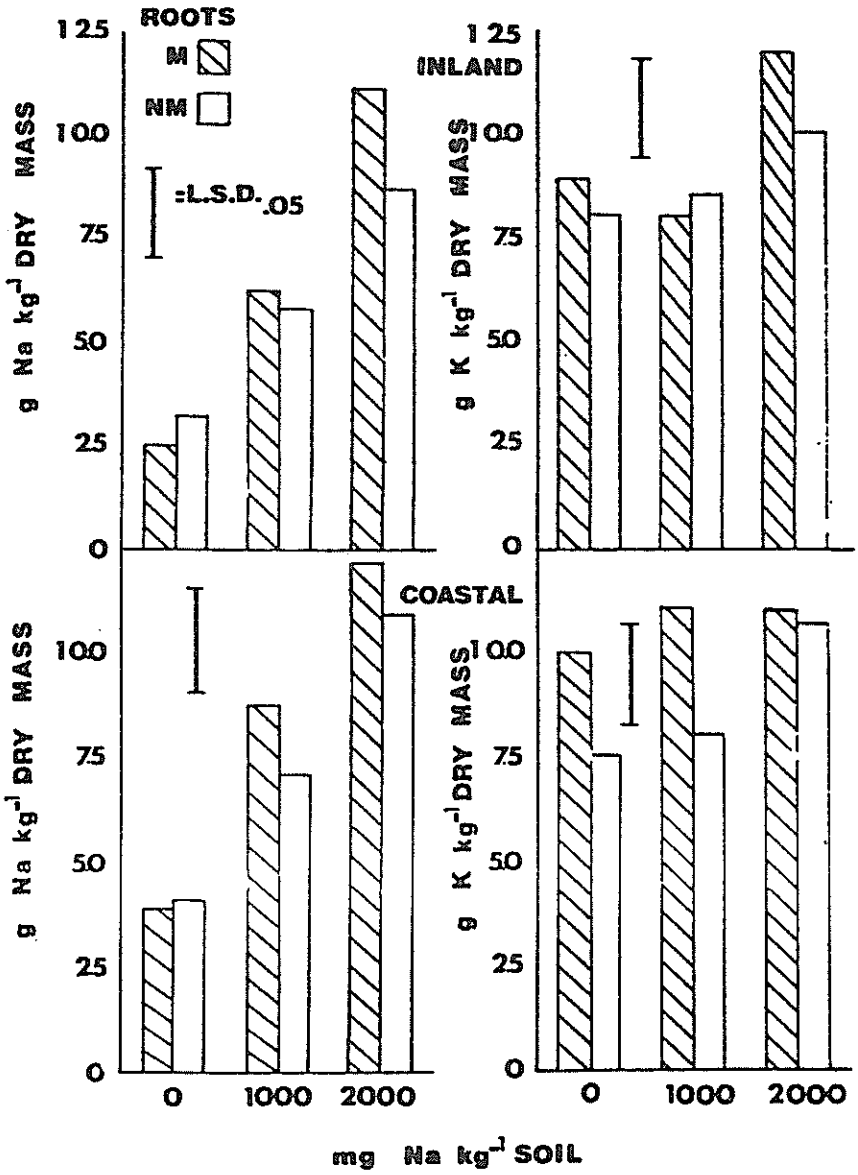

FIGURE 16. Root Na and K concentrations (g kg^{-1}) in inland (a), (b) and coastal (c), (d) populations of D. spicata grown with three levels of Na added to soil, and with ( ) and without ( ) mycorrhizae. Bar. = L.S.D. 0.05.

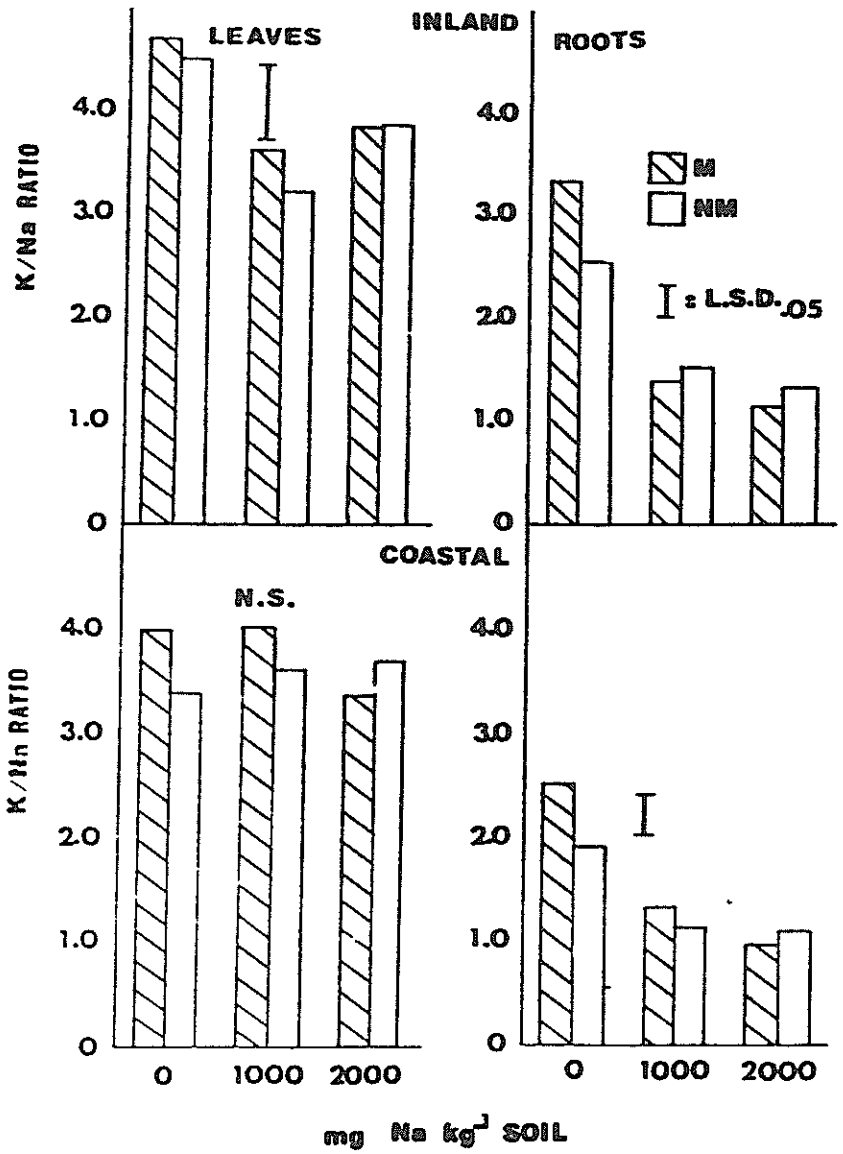

FIGURE 17. Leaf (a), (c) and root (b), (d) K/Na ratios in inland (a), (b) and coastal (c), (d) populations of D. spicata grown with three levels of Na added to soil, and with ( ) and without ( ) mycorrhizae. N.S.= no significant differences. Bar.= L.S.D. 0.005.

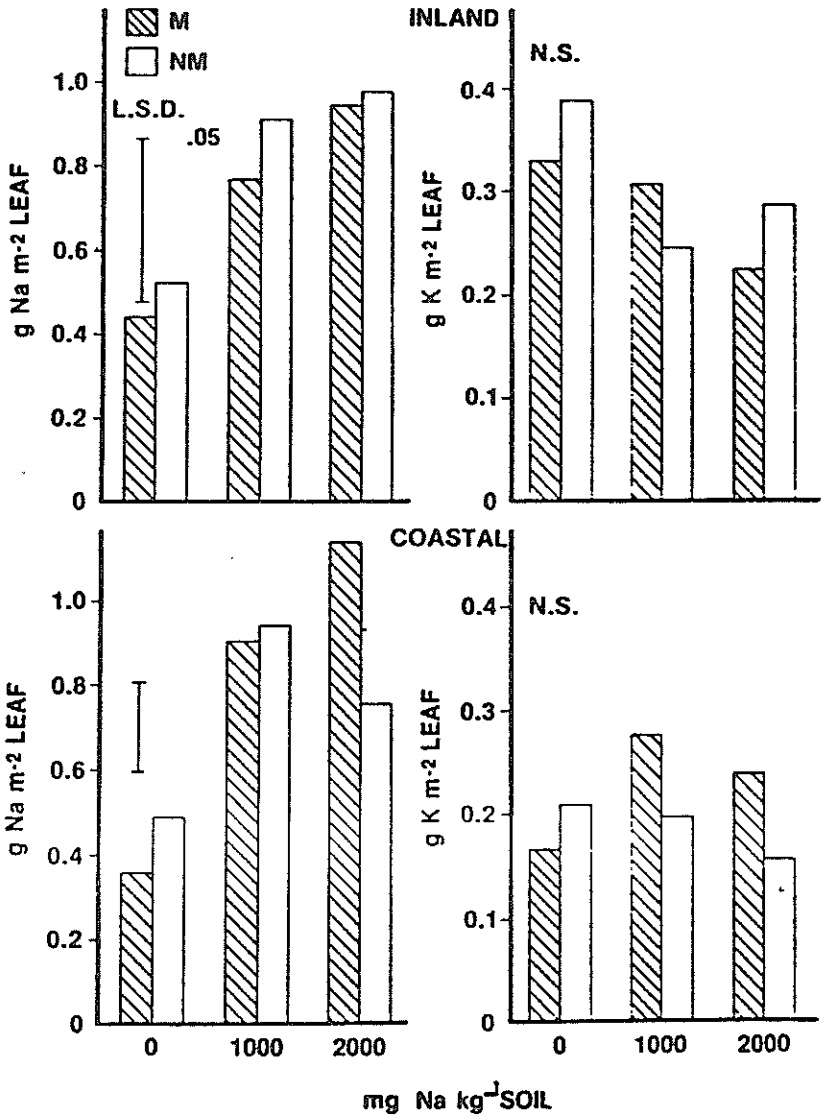

FIGURE 18. Daily excretion of Na and K by leaves of inland (a), (b) and coastal (c), (d) populations of D. spicata grown with three levels of Na added to soil, and with ( ) and without ( ) mycorrhizae. N.S. = no significant difference. Bar. = L.S.D. 0.005.

Percentage mycorrhizal infection was lower for the coastal than the inland population, but there were no significant differences among salinities within a population. Mean percentage infection for all three salinities was 28 ± 4 for the inland plants and 9 ± 3 for the coastal plants.

Soil Na concentrations decreased during the course of the experiment at the two higher salinities. The treatment with no added Na had 94 mg exchangeable Na kg^{-1} soil at the end of the experiment, similar to the 92 mg kg^{-1} measured initially. The 1000 mg Na kg^{-1} treatment dropped to 295 $\pm$ 16 mg kg^{-1}, and the 2000 mg Na kg^{-1} treatment dropped to 479 $\pm$ 25 mg kg^{-1}.

DISCUSSION

Dry mass of D. spicata did not increase with VA mycorrhizal infection, in contrast to a study where mycorrhizae partially overcame the detrimental growth effects of NaCl on salt-tolerant species (Hirrel and Gerdemann 1980). In two cases mycorrhizal infection reduced dry mass. While mycorrhizae sometimes increased root Na concentrations, these may have been offset by increases in P and K concentrations. Na toxicity is reduced by the K-Na pump, which replaces cytoplasmic Na with K. Increased K, and P for ATP formation, might be needed to balance the increased Na for proper functioning of the K-Na pump (Rains 1972).

Mycorrhizae did not increase stomatal conductance as has been demonstrated for other species (Levy and Krikun 1980 and Allen et al. 1981b). Mycorrhizal infection was low in both populations, and may not have provided the increased surface area of hyphae which some workers have suggested may reduce root resistance and increase water uptake

(Hardie and Leyton 1981 and Allen 1982). Improved P nutrition by mycorrhizae did not increase conductance of D. spicata as was hypothesized for onion (Nelson and Safir 1982).

The generally constant leaf Na concentrations with increasing soil salinity may be due to increased Na excretion from leaves, as was suggested by Hansen et al. (1976). The leaves selectively excreted more Na but excreted relatively constant amounts of K with an increasingly saline medium. The same phenomenon was exhibited by Tamarix aphylla leaves, which selectively excreted the most abundant ion in different nutrient solutions (Berry 1970).

By contrast, the roots had increased Na concentrations with increasing salinity, but these higher concentrations were not reflected in reduced below- or above-ground dry mass. D. spicata grown in the solution culture at about 0.5 M Na (and higher) had decreased mass (Tiku 1976, Smart and Barko 1980, and Kemp and Cunningham 1981). The lack of a dry mass change in our study may be due to the lowered soil salinity at the end of the study, although the final salinities were still higher than soil salinities from the plant collection sites (see Introduction). Plant uptake and excretion contributed somewhat to reduced soil salinity, but a visible salt crust which formed on the soil surface, edges of the pots, and around drainage holes due to evaporation may have also contributed to the Na concentration reduction in the rhizosphere. The roots were concentrated in the pot center where salinity was lowest.

The importance of VA mycorrhizae varies from plants which are obligately mycorrhizal to non-mycorrhizal (Janos 1980). The large group in between, the facultatively mycorrhizal plants, may differ greatly in their response to mycorrhizae and require individual experimentation to

determine the physiological benefits of mycorrhizae. D. spicata lies in this latter group and probably gains no net benefits under the conditions tested. However, in other studies mycorrhizae caused increased growth in onion and bell pepper under three initial Na levels which were one-third lower than ours (Hirrel and Gerdemann 1980), and the halophyte A. canescens exhibited increased growth with mycorrhizae in a non-saline soil (William et al. 1974 and Aldon 1975). Because our experiments were conducted under one set of environmental conditions in the greenhouse, further experimentation may be necessary. The physiological response to mycorrhizae changes with such variables as moisture (Allen et al. 1981b) and nitrogen (Wallace 1981 and Wallace et al. 1982). Long-term field experimentation under naturally variable conditions would determine whether mycorrhizae have any role in the growth and survival of D. spicata.

CHAPTER 4

DIFFERENTIAL RESPONSES OF *DISTICHLIS SPICATA* POPULATIONS TO CHLORIDE AND SULFATE SALINITY

Previous work with halophytes has usually dealt with physiological tolerance for sodium chloride, regardless of the predominate ions of the substrate in the plant's natural habitat (Milford et al. 1977,Preibe and Jaser 1978, Ahmed et al. 1980 and Kemp and Cunningham 1981). Frequently only the cations have been of concern (Storey and Wyn Jones 1976 and Pollak and Waisel 1979). Hansen et al. (1976) investigated the effects of various ions found in the natural habitat on the growth of *D. spicata*. The growth responses of several halophytic species to sodium chloride have been shown to differ from the growth response to sodium sulfate (Dorph-Peterson and Steebjerg 1950, Ovadia 1969, and Gale and Poljakoff-Mayber 1970).

Selective pressure on a plant population experiencing growth-limiting conditions such as high salinity can result in genetically based adaptation by the population. Ecotypes resulting from such variations in selective pressure have been shown for species of halophytes with both coastal and inland populations (Waisel 1972). The differences between coastal marsh and inland salinity regimes resulting from tidal flushing could provide sufficient selective pressure for ecotype formation. High sulfate concentrations characteristic of many inland *D. spicata* habitats could also provide a selective pressure for ecotype formation. Therefore, it is hypothesized that populations of *D. spicata* have formed ecotypes in response to the types of ions which predominate in their

environments. If this is the case, individuals from a habitat where sulfate is the predominate anion should respond differently, in terms of ion balance, from a population native to a habitat where chloride is the predominate anion if sulfate rather than chloride is the major anion in the growth substrate.

This hypothesis was tested by comparing an inland population from a habitat where sulfate is the predominate anion and a coastal populaton from a habitat where chloride is the predominate anion with respect to their tissue ion content and ion exudation.

MATERIALS AND METHODS

Vegetative material was collected from two populations of Distichlis spicata. Coastal population material was collected from a salt marsh on the north shore of Bodega Bay, California. Inland population material was collected from the banks of the Rio Grande river southwest of the Mesilla Dam, near Las Cruces, New Mexico. Soil ion concentrations at the collection sites are given in the introduction. The soils of the inland site relative to the coastal site contained twice the sodium, an equal amount of chloride, and seven times the sulfate.

Plants were maintained in a greenhouse with environmental conditions approximating the natural environment of the inland population until the beginning of this experiment. Fertilizer and water were applied as needed.

Individual ramets of the two populations were subjected to five different salinity treatments. The ramets were grown hydroponically using the nutrient solution described by Kemp and Cunningham (1981,

Chapter 1), with no additional salt, low sodium chloride, low sodium sulfate, high sodium chloride, and high sodium sulfate. The sodium chloride treatments were 125mM (low NaCl) and 250 mM (high NaCl) for both sodium and chloride ions. These concentrations are equivalent to one-quarter and one-half the sodium and chloride ion concentrations of sea water. The sodium sulfate treatments were designed to provide 100 meq of sulfate ions (50 mM) and 200 meq of sulfate ions (100 mM). These values bracket the sulfate level of the soil solution at the inland site. This required 50 mM Na_2SO_4 and 100 mM Na_2SO_4 in the low and high sodium sulfate treatments, respectively. These gave sodium ion concentrations of only 100 mM and 200 mM, respectively. The sodium ion concentrations were brought to the same level as in the sodim chloride treatments by adding 25 and 50 mmoles/l of sodium ions as sodium acetate. Ten replicate pots containing four to five ramets were prepared for each treatment level.

The plastic two liter pots were thoroughly washed and rinsed in double-distilled water. Plants were removed from the greenhouse pots, divided at the rhizomes, rinsed in distilled water, in double-distilled water, and then placed in the hyroponic solutions. Thus, any salt on the plant surface was removed prior to experimentation. A circular styrofoam lid with holes for plants was placed over the solution, thus, evaporation was reduced although not eliminated. Plants were grown in a controlled environment chamber with a daytime temperature of 30°C and a nightime temperature of 20°C, a 14/10 day/night photoperiod, and a relative humidity of 40 percent. Light was maintained at a quantum flux density of 1200 mol $m^{-2}s^{-1}$ (400-700nm, PPFD) and was provided by a combination of fluorescent, incandescent and low pressure sodium lamps.

Plants were grown for 21 days for the control and sodium chloride treatments and for 42 days for sodium sulfate treatments. Initial shoot dieback occurred within a week of transfer into the sodium sulfate solutions. The period of time necessary for regrowth to a size comparable to the control and the sodium chloride treatments resulted in the longer growth period for the plants in the sodium sulfate solutions. Bacterial contamination occurred in the sodium sulfate/sodium acetate solutions. This contamination could have resulted in some loss of sulfate from the solutions but sulfate contents of the plant tissue indicated this was not a major problem.

A measure of leaf exudation was obtained by removing a culm with three to five blades and rinsing it in a known volume of double-distilled water for ten minutes. This extended time period was necessary to dissolve the salts on the leaf surface. Care was taken to avoid rinsing the cut end of the culm. The leaves and culm were dried at 70° for 24 hours before weighing. After removal of the culm for exudation measurement, the plant was removed from the culture solutions, separated into leaves, culms, roots, and rhizomes, rinsed in double-distilled water, and dried at 70°C for 24 hours before weighing.

Each plant organ and exudation solution was analyzed for sodium, chloride, and sulfate ion content. Ground plant organs were ashed for three hours at 470-500°C with magnesium nitrate and calcium oxide added to reduce the volatilization of sulfate and chloride ions (Chapman and Pratt 1961 and Allen et al. 1974). Ashed material was extracted with 0.02 M nitric acid.

Sodium was analyzed with an Instrumentation Laboratory Atomic Absorption Spectrophotometer model 451. Chloride was measured with a Markson chloride electrode IL-1005 or with an Altex chloride electrode, coupled with a Corning pH meter model 125. Sulfate sulfur was evaluated using a turbidimetric method from Allen et al. (1974) which is a modification of the method described by Butters and Chenery (1959). A Beckman model 26 spectrophotometer was used to measure turbidity.

To evaluate the regulation of the internal ionic balance by the plant, a relative exudation rate (RER) was calculated. Relative Exudation Rate was defined as the ratio of the increase in ion exudation over the exudation of the control plants to the increase in ion content over the ion content of the control. The method of calculation of RER was modified from Pollak and Waisel (1978).

If there was no increase in exudation over the control the RER would be zero. Conversely, if there was no increase in the accumulation of ions in the plant, RER would be infinitely large. A value of one indicated equal exudation and accumulation of excess ions. A relative exudation rate greater than one indicates exudation was the major ion regulation mechanism and a value less than one suggests accumulation.

Tissue ion content data were subjected to an analysis of variance using a factorial completely randomized split-plot design. This design was utilized because of the inability to eliminate the interrelationship of the individual plant organ. The entire plant of which there were 10 replicates per treatment was considered the whole unit or plot. The leaves, the culms, the roots, and the rhizomes were considered subunits or split plots. A completely randomized factorial design was used to analyze the sodium ion exudation. Chloride and sulfate ion exudation

analysis was a completely randomized design of the three salinity treatments, control, low, and high sodium chloride for the chloride ion and control, low and high sodium sulfate for the sulfate ion. All statistical procedures were performed using the Statistical Analysis System (Blair 1979).

RESULTS

Ion Content of Plant Tissue

The three experimental factors that possibly could have affected the ion content of the plant tissue were the population from which the plant tissue was taken, the salinity treatment the plant was grown in, and the organ the tissue was taken from. The influence of each of these main factors will be considered in turn.

Population. The significant F values for the population factor indicated that chloride (Table 19) and sulfate (Table 20) but not sodium (Table 21) ion contents of the whole plant varied significantly between the populations. The tissue chloride ion content of the inland population was not significantly different from the coastal population except for the higher chloride content of the roots of the inland population at the low salinity treatment and the higher chloride content of the leaves, culms, and roots of the inland population at the high salinity treatment (Table 22). In the treatments with sulfate as the major anion, the sulfate ion content for the low salinity treatment was higher in the coastal population although it was only significantly higher in the rhizomes and at the high salinity treatment it was highest in the inland population for the culms and roots (Table 23).

TABLE 19. Analysis of variance table for tissue chloride ion contents.

SOURCE	DF	SUM OF SQUARES	F VALUE
CORRECTED TOTAL[a]	397	81571277839	
MODEL	129	56642953570	4.7****
WHOLE UNIT			
POPULATION	1	2844671907	26.6****
SALT	4	23263316760	54.3****
Chloride vs. sulfate		———	
Control vs. Trts.	1	1660000000	310.0****
Low salt vs. High	1	419000000	78.0****
Remainder	2	247300000	
POP X SALT	4	7173873047	16.8****
ERROR A	90	9632700000	
SUBUNIT			
ORGAN	3	4078665501	14.6****
POPULATION X ORGAN	3	912778797	3.3*
SALT X ORGAN	12	5908268362	5.3****
POP. X SALT X ORGAN	12	2828652131	2.5**
ERROR	268	24928324269	

a Two missing observations
* Significant at $P < 0.05$
** Significant at $P < 0.01$
**** Significant at $P \leq 0.0001$

TABLE 20. Analysis of variance table for tissue sulfate ion content.

SOURCE	DF	SUM OF SQUARES	F VALUE
CORRECTED TOTAL[a]	397	4120567	
MODEL	129	3517091	12.11****
WHOLE UNIT			
POPULATION	1	66189	15.8****
SALT	4	1310943	78.2****
Chloride vs. Sulfate		———	
Control vs. Trts.	1	880200	418.0****
Low salt vs. High	1	134000	64.0****
Remainder	2	296743	
POP X SALT	4	109318	6.5****
ERROR A	90	377338	
SUBUNIT			
ORGAN	3	560111	82.9****
POPULATION X ORGAN	3	45957	6.8***
SALT X ORGAN	12	1023627	37.9****
POP. X SALT X ORGAN	12	23604	0.9NS
ERROR	268	603476	

a Two missing observations
*** Significant at $P < 0.001$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

TABLE 21. Analysis of variance table for tissue sodium ion contents.

SOURCE	DF	SUM OF SQUARES	F VALUE
CORRECTED TOTAL[a]	397	30520657147	
MODEL	129	23642563205	7.14****
WHOLE UNIT			
POPULATION	1	16555818	0.04NS
SALT	4	5635248092	37.2****
Chloride vs. Sulfate	1	954500000	25.2****
Control vs. Trts.	1	648690720	171.4****
Low salt vs. High	1	170000000	52.0****
Remainder	1	23370000	
POP X SALT	4	196182895	1.3NS
Chloride vs. Sulfate	1	192000000	50.4****
Remainder	3	4182000	
ERROR A	90	3406200000	
SUBUNIT			
ORGAN	3	7988869642	103.8****
POPULATION X ORGAN	3	280903798	3.6**
SALT X ORGAN	12	3610844674	11.7****
POP. X SALT X ORGAN	12	2522640263	8.2****
ERROR	268	6878093942	

a Two missing observations
** Significant at $P < 0.01$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

TABLE 22. Mean chloride content of organ and whole plant tissue (mg/gm dry mass) of the inland and coastal populations of *D. spicata*.

Salinity	Leaves	Culms	Roots	Rhizomes	Whole Plant
Control					
Inland	136.01	108.31	101.03	53.85	95.85
Coastal	107.93	52.70	51.25	26.96	38.17
Low Na_2SO_4					
Inland	98.76	95.36	212.15	76.59**	120.77
Coastal	120.27	107.90	249.93	157.48**	161.81
High Na_2SO_4					
Inland	149.18	150.21**	469.05**	147.65	219.21
Coastal	161.78	98.39**	388.06**	143.06	187.63

** Significant *l.s.d.* test at $P < 0.01$, Comparison of inland and coastal populations for each organ, $t = 2.326$.

TABLE 23. Mean sulfate content of organ and whole plant tissue (mg/g dry mass) of the inland and coastal populations of *D. spicata*.

Salinity	Leaves	Culms	Roots	Rhizomes	Whole Plant
Control					
Inland	2.149	6.236	5.412	5.558	5.032
Coastal	4.100	4.305	4.525	3.847	4.157
Low NaCl					
Inland	5.105	11.839	26.674**	8.488	11.526
Coastal	4.091	9.950	15.122**	9.007	9.122
High NaCl					
Inland	33.018**	46.316**	46.461**	10.828	30.349
Coastal	5.196**	10.582**	23.139**	9.370	10.857

** Significant *l.s.d.* test at $P < 0.01$, Comparison of inland and coastal populations for each organ, $t = 2.326$.

Salinity. The salinity treatments significantly affected the ion contents of the whole plant (Table 24). Valid contrasts could be made between the sodium chloride and sodium sulfate treatments with respect to their effects on tissue sodium content but not with respect to their effects on tissue chloride or sulfate content. Because the amount of chloride in the sodium sulfate solutions and the amount of sulfate in the control solution was the same as in the sodium chloride treatment solutions, any differences in the tissue contents of these ions was a result of the ion ratio of the treatment solution and not a result of regulation by the plant. The chloride vs sulfate contrast (Table 24) indicated that the effect of the sodium chloride and sodium sulfate treatments on the sodium content of the whole plant was highly significant, ($P < 0.0001$). Inspection of the sodium content of the whole plant and of the root tissue (Table 25) suggests a complex relationship between type of salinity treatment and sodium ion content. Sodium content tended to be higher in the plants grown in sodium sulfate and lower in the plants grown in sodium chloride (Table 25).

The level of salinity as well as the types of salinity significantly affected whole plant ion contents.

Treatments were significantly different from controls (Table 24, Control vs Treatment line) in the contents of all three ions. Low and high salt treatments were also significantly different for all ions (Table 24, Low vs High line).

The interaction between the population and salinity factors were not significant for the sodium content of the whole plant but was significant for both the chloride and sulfate ion contents (Table 24). Inspection of

TABLE 24. Summary of F values for tissue sodium, chloride and sulfate ion contents.

WHOLE UNIT	Sodium	Chloride	Sulfate
Population	NS	26.6****	15.8****
Salt	37.2****	54.3****	78.2****
Chloride vs. Sulfate	25.2****	----	----
Control vs. Trts.	17.1****	310.0****	418.0****
Low salt vs. High	52.0****	78.0****	64.0****
Population x Salt	NS	16.8****	6.5****
Chloride vs. Sulfate	50.4****	----	----
SUBUNIT			
Organ	103.8****	14.6****	82.9****
Population x Organ	3.6**	3.3*	6.8***
Salt x Organ	11.7****	5.3****	37.9****
Population x Salt x Organ	8.2****	2.5**	NS

* Significant at $P < 0.05$
** Significant at $P < 0.01$
*** Significant at $P < 0.001$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

TABLE 25. Mean sodium content of organ and whole plant tissue (mg/g dry mass) of inland and coastal populations of *D. spicata*.

Salinity	Leaves	Culms	Roots	Rhizomes	Whole Plant
Control					
Inland	1.544	1.770	4.148	2.997	2.369
Coastal	1.918	2.003	2.794	1.868	2.353
Low NaCl					
Inland	4.312	8.529	24.348**	6.775	9.433
Coastal	5.933	7.282	13.963**	6.500	4.177
High NaCl					
Inland	3.658	16.064**	8.642*	11.993	11.300
Coastal	5.110	7.428**	20.274**	7.681	10.421
Low Na_2SO_4					
Inland	2.079	3.998	14.698	6.497	3.215
Coastal	3.367	5.315	13.680	11.640	13.619
High Na_2SO_4					
Inland	3.023	5.585**	34.323**	11.228	12.845
Coastal	6.274	11.129**	22.249**	17.222	27.560

** Significant l.s.d. test at $P < 0.01$, Comparison of inland and coastal populations for each organ, $t = 2.326$.

mean sulfate content (Table 23) suggested the coastal population contained more sulfate for the low salinity treatment whereas the inland population contained more sulfate at the higher salinity. Although the salt by population interaction was not significant for the whole plant (Table 24), the large differences in the mean sodium ion content of the individual organs between the sodium chloride and sodium sulfate treatments (Table 25) suggested possible differences. Therefore, the population by salinity interaction was divided into the contrast of sodium chloride vs sodium sulfate treatments (Table 24, Chloride vs Sulfate). This contrast was found to be highly significant. At the highest salinity treatment level the sodium content was greatest in the roots of the inland population treated with sodium sulfate and was the lowest in the roots of the inland sodium chloride treated plants. In addition, the sodium content of the roots of the inland population was higher in the low sodium chloride treatment than in the high sodium chloride treatment.

Organ. Sodium, chloride, and sulfate ion contents differed significantly between the four plant organs (Table 24). In general, ion content was highest in the roots and lowest in the leaves (Tables 22, 23, and 25). The extremely low sodium content of the inland population's root tissue in the high sodium chloride (Table 25) and the significant population by organ interaction (Table 24) suggested a shift in the ion accumulation from one organ to another with changes in sodium chloride concentration. At the higher sodium chloride salinity level, the sodium ions were concentrated in the culms rather than in the roots as in other treatments (Table 25). Analysis of the population by organ interaction of the

chloride, sodium, and sulfate ion contents (Tables 22, 23, and 25) by an l.s.d. test suggested that the roots and culms, both areas of apoplastic transport, were the organs that differ between the populations. The significant salt by organ interaction (Table 24) was largely the result of increased ion content of the culms as the salinity level increased (Table 22, 23, and 25). The population by salt by organ interaction for the chloride ion was significant (Table 24) due to the high leaf chloride content of the inland population in the high sodium chloride treatment (Table 22). The significant three-way interaction for the sodium ion content (Table 24), was more complex. Inland plants had higher culm sodium contents in the high sodium chloride treatment but lower culm sodium contents in the high sodium sulfate treatments. Root sodium contents showed the opposite pattern (Table 25).

Ion Content of Leaf Exudate

The major factor influencing the ion content of the solution exuded from the leaf was the salinity of the treatment solution (Table 26). The populations exhibited no differences in chloride exudation (Table 27) and there was no population by salinity interaction. However, the amount of sulfate ion exuded was significantly different between the two populations (Table 28). The coastal population exuded a lesser amount of sulfate ion. In addition, the population by salinity interaction for the low salinity treatment was not significant (Table 28). This lack of significance may have been a result of the high variability in the sulfate measurements for the coastal population grown in low sulfate.

The amount of sodium exuded from the leaf tissue was not different for the two populations (Table 29) but was influenced by the major anion

TABLE 26. Summary of F values for leaf sodium, chloride, and sulfate ion exudation of *D. spicata*.

	Sodium	Chloride	Sulfate
Population	NS	NS	5.3^{*}
Salt	18.4^{****}	6.9^{****}	16.9^{****}
Population x Salt	2.5^{*}	NS	NS

* Significant at $P < 0.05$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

TABLE 27. Analysis of variance table of chloride exudation.

SOURCE	DF	SUM OF SQUARES	F VALUES
CORRECTED TOTAL	59	197993721193	
MODEL	5	5451563147	3.72^{***}
POPULATION	1	202921101	0.8^{NS}
SALT	2	45260591716	6.9^{****}
POPULATION X SALT	2	9052118330	1.4^{NS}
ERROR	54	14347809046	

*** Significant at $P < 0.001$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

TABLE 28. Analysis of variance table of sulfate exudation.

SOURCE	DF	SUM OF SQUARES	F VALUES
CORRECTED TOTAL	59	135095	
MODEL	5	63906	9.7^{****}
POPULATION	1	7623	5.8^{*}
SALT	2	44357	16.6^{****}
POPULATION X SALT	2	5962	2.3^{NS}
ERROR	54	71189	

* Significant at $P < 0.05$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

TABLE 29. Analysis of variance table of sodium exudation.

SOURCE	DF	SUM OF SQUARES	F VALUES
CORRECTED TOTAL[a]	97	72045300088	
MODEL	9	35133761881	9.31****
POPULATION	1	218687614	0.52NS
SALT	4	30719997451	18.4****
POPULATION X SALT	4	4195176816	2.5*
ERROR	88	36911538207	

a Two missing observations
* Significant at $P < 0.05$
**** Significant at $P < 0.0001$
NS Not Significant at $P \leq 0.05$

of the treatment solution. Plants grown in the sodium sulfate solutions exuded much lower amounts of sodium ion than those grown in sodium chloride solutions. The signficant population by salt interaction (Table 26) was due to the low sodium exudation of the inland population grown in sodium sulfate.

The efficiency of ion exudation was evaluated using the RER. The sodium RER values, which were less than one, indicated that more sodium was accumulated in the plant tissue than was exuded onto the leaf surface (Table 30). Both populations were quite similar in sodium RER although the inland population exuded relatively more sodium than the coastal population when chloride was the major anion of the solution. There were large differences in the sodium RER between the sodium chloride and sodium sulfate treatments. Both sodium and sulfate ions were accumulated in the tissues rather than exuded when plants were grown in the sodium sulfate solution (Tables 30 and 31). With the exception of the inland high sodium chloride treatment, chloride ions were exuded rather than stored in the plant tissues (Table 32). This suggests that chloride ions were transported through the plant and actively exuded onto the leaf while the sodium ion moved passively as an ionic charge balancer.

DISCUSSION

The inland and coastal populations did not exhibit any significant differences in the regulation of the internal ionic balance when grown in a substrate high in sulfate. Although population was a significant factor in the tissue sulfate content and sulfate exudation, the RER were similar in the two populations. The RER similarity indicates that while

TABLE 30. Effect of salinity type and level of treatment solution on sodium RER of inland and coastal populations of *D. spicata*.

SOLUTION MOLARITY	INLAND	COASTAL
Low NaCl	0.95	0.60
High NaCl	0.81	0.48
Low Na_2SO_4	0.14	0.16
High Na_2SO_4	0.01	0.07

TABLE 31. Effect of sulfate concentration of treatment solution on sulfate RER of inland and coastal populations of *D. spicata*.

SOLUTION	INLAND	COASTAL
Low Na_2SO_4	0.14	0.07
High Na_2SO_4	0.06	0.04

TABLE 32. Effect of chloride concentration of treatment solution on chloride RER of inland and coastal populations of *D. spicata*.

SOLUTION	INLAND	COASTAL
Low NaCl	1.46	1.54
High NaCl	0.56	1.36

the inland population accumulated and exuded more sulfate ions than the coastal population, it was not more efficient in sulfate exudation.

The two populations do appear to differ in their regulation of sodium chloride. The inland population at the high sodium chloride salinity was less efficient in the exudation of chloride ions than the inland population at the lower salinity or the coastal population at either salinity level. In addition, the coastal population accumulated more sodium, i.e., it had lower RER, than the inland population. The accumulation of sodium ions in halophyte tissue is generally considered to be balanced by organic acids, amino acids, or carbohydrates (Flowers et al. 1977). At high salinity levels the inland population produced less organic acid than the coastal population. These factors possibly could be explained by the ability of the coastal population to produce organic acids in response to salinity, whereas the inland population retained chloride ions as opposed to manufacturing metabolically costly organic anions to maintain charge balance.

In addition to the different responses of the inland and coastal populations to substrate sodium chloride and sodium sulfate, further inspection of the results suggested that the regulation of sodium varies with the anion of the substrate. This variation is suggested by the chloride salinity vs sulfate salinity analysis of tissue sodium content and the population salinity, and population by sulfate salinity interaction, as well as the complex nature of the sodium ion exudation. However, this interaction between the sodium and the substrate anion was most clearly seen in the relative exudation rates. The high RER for chloride and sodium ions in the sodium chloride treatments, relative to

the RER for sulfate and sodium in the sodium sulfate treatments, suggests that chloride may be actively exuded by the salt gland and that sodium is passively exuded as an ionic balancer. The easily metabolized sulfate ion, in contrast, tended to accumulate, along with sodium, which probably serves as a charge balancing cation.

In general, ions accumulated in the roots and culms of both populations with the photosynthetically active leaves having the lowest content. Major differences in ion content between the populations were seen in the roots and culms. The inland population did, however, consistently maintain a lower ion content in the leaves than did the coastal population.

The different responses of the inland population from the coastal population with respect to sulfate and chloride accumulation, sulfate exudation, chloride exudation efficiency, and differential ion accumulation in the plant organs suggests genetic adaptations to diverse environments. These findings are supported by photosynthetic response differences of the same populations (Chapter 1). Thus, coastal and inland ecotypes of Distichlis spicata exist.

Ungar (1970) found the species composition of halophytes in soils high in chloride was 90 percent similar to those found in high sulfate soils. Thus, he proposed that the total ionic content of the soil, not the major anion, was important in determining the species composition. The above results indicate that while either ecotype of D. spicata can survive in sodium chloride or sodium sulfate, growth is severely stunted in sodium sulfate. The coastal population was the most stunted and had the highest tissue sodium content (Table 25). This suggested that the

anion, in this case sulfate, may indeed have a major effect on growth and survival of this species.

CONCLUSION

In the preceding four chapters we have presented an evaluation of our progress along four lines of investigation designed to provide some of the ecophysiological information necessary for the effective domestication of Distichlis spicata as a salt tolerant forage crop.

In Chapter 1 clear ecotypic differences among populations from habitats of contrasting salinities and temperatures were shown. The northern coastal population from Bodega Bay, California was found to have the greatest relative growth rate at all salinity and temperature combinations evaluated. This higher relative growth rate was partially a result of its greater overall photosynthetic capacity. Its higher photosynthetic capacity did not, however, completely account for its ability to maintain high growth rates under the more stressful conditions of low temperature and high substrate salinity. The ability of the northern coastal ecotype to increase its relative allocation of new biomass to leaf structure appeared to be an important characteristic in allowing it to maintain higher relative growth rates. In addition the northern coastal population did not produce more energetically costly, higher protein, leaf tissue with increased salinity as did the other populations. This characteristic would also contribute to its ability to maintain higher relative growth rates under more saline conditions than the other populations.

In Chapter 2 a modeling approach was used to evaluate the effects of growth irradiance, salinity and temperature on net photosynthesis, and its partial processes in the higher photosynthetic capacity of the

northern coastal population. Salinity decreased the maximum photosynthetic capacity but increased temperature tended to compensate to some extent for the depressing effect of salinity. The major effects of both salinity and temperature were on the carboxylation efficiency. Growth at lower irradiance increased the incident photon-use efficiency at low photon fluence rates but had no effect on the maximum photosynthetic capacity at saturating photon fluence rates. It would appear from these results that the northern coastal population of D. spicata has considerable acclimation potential in its photosynthetic mechanisms. We are currently evaluating data for the other populations to assess the generality of these conclusions for the species as a whole.

In Chapter 3 the role of mychorrhizae in influencing salinity tolerance of coastal and inland populations of D. spicata was evaluated. This investigation indicated that although mychorrhizae occur in both inland and coastal habitats they have no appreciable influence on biomass accumulation, ion balance or plant water relationships. This study confirmed the validity of using hydroponic culture, where mychorrhize do not occur, in investigations of ecophysiological differences among populations.

In Chapter 4 responses of an inland and coastal population to chloride and sulfate were compared. Ion balances and exudation characteristics were sensative to the major anion species in the culture solution. More importantly the populations differed in their responses to the anions. It will, therefore, be important to consider the ion compositions of the soils and irrigation waters of sites where D. spicata is introduced and possibly match them with those of the natural habitat from which the material for introduction is taken.

Distichlis spicata is a highly productive native halophyte with excellent forage characteristics. It should be possible to establish highly productive pastures of this species on salinized lands and/or to irrigate these pastures with poor quality water. The success of such establishments, will, however be greatly affected by the extent to which the environment of the establishment site corresponds to the natural habitat of the source plant material. The conditions that should be matched as nearly as possible are: (1) temperature, (2) frost free period, (3) total salinity, and (4) ion composition. The data on which this report is based is being used to develop a simulation model of plant production, which should serve as a guide to matching potential establishment conditions with the physiological characteristics of potential establishment source ecotypes.

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