A COMPARISON OF THE EFFECTS OF SALINITY ON PHOTOSYNTHETIC PHYSIOLOGY OF A SALT SENSITIVE GRASS, *Panicum obtusum*, AND A SALT TOLERANT GRASS, *Distichlis spicata*

by

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INTRODUCTION

In the arid regions of the southwestern United States agricultural productivity is limited by the supply of fresh water. There also has been a decline in productivity in some areas of the southwest due to increased soil salinity on irrigated fields. Thus, the utilization of crops tolerant of salinity offers a potential for increasing agricultural productivity in the southwest through the use of brackish water from vast underground resources or through increased growth on salt-damaged fields. The selection and breeding of salt tolerant crops will require an understanding of the physiological and anatomical mechanisms of salt tolerance.

There have been numerous studies of the effects of substrate salinity on physiology, anatomy and growth of plants (Bernstein and Hayward, 1958; Waisel, 1972; Poljakoff-Mayber and Gale, 1975; Jennings, 1976; Flowers, Troke and Yeo, 1977; DeJong, 1978; Longstreth and Nobel, 1979). However, most studies have dealt with mechanisms of salt tolerance in native halophytes or agronomic plants which exhibit some degree of salt tolerance. Another approach to understanding mechanisms of salt tolerance is to examine the sensitivity of various aspects of structure and function in plants which are not salt tolerant. This approach has been used to some extent, mostly in studies of crop plants such as Phaseolus vulgaris and Zea mays (Jennings, 1976; Bernstein et al., 1974; Jensen, 1975; Kennedy, 1977). In order to broaden the understanding of salt tolerance, particularly in plants native to the southwest United States we have designed a comparative experiment encompassing both of the above approaches. This experiment focuses on examining the effects of substrate salinity

on a grass which is tolerant of high salinity, Distichlis spicata, and a grass which is apparently intolerant of salt, Panicum obtusum. Both Distichlis spicata and Panicum obtusum grow abundantly in the southwestern United States. Both species have the C_4 dicarboxylic acid pathway of photosynthesis (Hansen et al., 1976; Cunningham et al., 1974) and both species occur in periodically flooded habitats (Ludwig, 1974; Cunningham, unpublished). Thus, the greatest differences between these species appears to be in their tolerance of salt. A comparative study of their responses to salinity should provide important details about which plant processes are susceptable to salinity and in what manner this occurs.

METHODS

Distichlis spicata was collected from near the Rio Grande, 5 km southwest of Las Cruces, New Mexico. Panicum obtusum was collected from a playa in the Jornada del Muerte, 25 km northeast of Las Cruces. The plants were collected as vegetative sods approximately 5 dm^2 in size. In the laboratory plants were washed free of soil and pieces of rhizome with shoots and associated roots were established in plastic pots with styrofoam lids. The pots contained 2 liters of deionized water, the appropriate amount of NaCl, and the following nutrient composition: 2 mM KNO_3, 0.8 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.3 mM $NH_4H_2PO_4$, 0.1 mM $Na_2H\ PO_4$, 0.04 mM Fe-EDTA, micronutrients, with a pH = 5.5. Solutions were replenished with deionized water regularly and completely replaced every 2 weeks. Preliminary experiments with D. spicata indicated that growth was equal in aerated and unaerated pots, thus pots were not aerated. Preliminary experiments with P. obtusum indicated that growing sufficient material in solution

culture for gas exchange and physiology experiments would be impossible. Plants grown in solution culture (either aerated or unaerated) produced very few roots and most of the above ground material produced was leafless stolons. Thus, experimental plants of P. obtusum were grown in 2 liter pots containing sand and watered every other day with deionized water or deionized water + NaCl, and every four days with the above nutrient solution.

There were 3 experimental treatments for each species. One treatment (or control) for both species consisted of no added NaCl. The NaCl concentration of the nutrient solution in deionized water was 0.1 mM. D. spicata plants were grown in two other treatment solutions: 250 mM NaCl (low salt) and 500 mM NaCl (high salt). P. obtusum rapidly died when watered with 500 mM NaCl. Thus, the 2 other treatments for P. obtusum plants were watering solutions of 125 mM NaCl (low salt) and 250 mM NaCl (high salt). All plants were grown in controlled environment chambers under 16 h day/8 h night photoperiods. Quantum flux densities at the leaf surface were 1200 ± 200 microeinsteins $m^{-2}s^{-1}$ (400-700 nm,PAR) provided by a combination of fluorescent, incandescent, and low pressure sodium vapor lamps. The thermoperiod (synchronous with the photoperiod) was 35/25 C.

Physiological and anatomical parameters were measured on leaves and roots which had developed under the experimental conditions for 3 to 6 weeks with the exception of P. obtusum plants treated with 250 mM NaCl. These plants did not grow at that salinity, and therefore these leaves were developed on plants grown with no added NaCl and then maintained under the 250 mM NaCl treatment for 2 weeks.

Measurements of carbon dioxide and water vapor exchange were made using standard techniques (Sestak et al., 1971) with an infrared gas analyzer (Beckman model 215B) and a dew point hygrometer (EG&G model 880-c1) in an open system similar to that described by Williams and Kemp (1978). Resistances to CO_2 exchange were calculated according to the methods of Longstreth and Strain (1977). The air source was collected from outside the building and averaged 340 $\mu l\ l^{-1}$ CO_2. Temperature responses of net gas exchange (10 to 45 C) at 2000 microeinsteins $m^{-2}s^{-1}$ PAR were measured on 5 to 10 attached leaves that were sealed into an acrylic cuvette in which temperatures were regulated by circulating water through a surrounding water jacket. Leaf temperature was measured with a fine wire thermocouple inserted between the ligule of the leaf and the culm. Light was provided by a 300 W incandescent flood lamp filtered through 4 cm of acidified 0.06 M $Fe_4(NH)_2(SO_4)_2$. Leaf thicknesses were measured microscopically from cross sections cut at 5 to 10 mm from the ligule. Stomatal densities were measured microscopically from paradermal sections cut at 5 to 10 mm from the ligule. Leaf water potentials were measured just prior to illumination of the chamber ("predawn") and after midday ("afternoon"). Leaf water potentials were measured using a xylem tension pressure chamber (PMS Instruments) for D. spicata and using a thermocouple psychrometer (Wescor) for P. obtusum.

RESULTS AND DISCUSSION

Differences in salinity tolerance between these two species were conspicuously demonstrated in some preliminary experiments. D. spicata grows well in solution cultures up to 500 mM NaCl. P. obtusum rapidly dies when subjected to salinities over 250 mM NaCl

and it will not grow in salinity greater than 125 mM NaCl. Another difference between these species is the poor growth that P. obtusum exhibits in solution culture compared to D. spicata. Since both species occur in periodically flooded habitats, this difference was not expected and remains unexplained.

The temperature response curves of net photosynthesis indicate that maximal rates occur near 40 C for both species in the absence of added NaCl (Figs. 1 and 2) which is consistent with most other C_4 plants grown at warm temperatures (Downton, 1971; Black, 1973; Kennedy, 1977). However, rates of net photosynthesis were significantly different between species at all leaf temperatures when both were grown without added NaCl. Net photosynthesis rates of D. spicata were more than twice those of P. obtusum at all leaf temperatures. Maximal rates of net photosynthesis of D. spicata (41 mg CO_2 dm^{-2} hr^{-1}) fall at the lower end of the range reported for C_4 plants (Black, 1973). However, maximal rates of P. obtusum (18 mg CO_2 dm^{-2} hr^{-1}) are far below those reported for other C_4 grasses (Ludlow, 1970; Gifford, 1971; Doley and Yates, 1976), but very similar to rates reported previously by Cunningham et al. (1974). These very low rates of net photosynthesis in P. obtusum are due to both high stomatal and high internal resistances to CO_2 uptake (Figs. 3 and 4). Minimum stomatal and internal resistances in P. obtusum (at 40 C leaf temperature) were 556 s m^{-1} and 390 s m^{-1}, respectively, while minimum resistances in D. spicata (at 40 C leaf temperature) were 250 s m^{-1} for stomatal and 160 s m^{-1} for internal. Minimum resistances in other C_4 grasses range from 150 to 400 s m^{-1} for stomatal and 60 to 150 for internal (mesophyll) (Ludlow, 1970; Rawson et al., 1977; Kemp and Williams, 1980).

Treating plants with NaCl caused significant declines in net photosynthesis in both species (Figs. 1 and 2), although rates of net photosynthesis in D. spicata grown in 500 mM NaCl were still greater or equal to rates of net photosynthesis of P. obtusum grown without added NaCl. Decreased net photosynthesis in plants grown at low salt was caused by stomatal closure in D. spicata (Fig. 5) and both stomatal closure and increased internal resistance to CO_2 uptake in P. obtusum (Figs. 3 and 4). Growing plants at high salinity further reduced net photosynthesis in both species by causing increases in both stomatal and internal resistances to CO_2 assimilation (Figs. 3 to 6). It should be noted that high salinity was only 250 mM in P. obtusum, compared to 500 mM in D. spicata and yet net photosynthesis was reduced almost to zero in P. obtusum. Net photosynthesis is generally reduced in plants not tolerant of salinity (Gale, 1975; Kennedy, 1977), but usually not so severely. Kennedy (1977) found that rates of net photosynthesis in corn were reduced to 43% of controls when grown in solution culture of approximately 250 mM NaCl. Thus, photosynthesis in P. obtusum is apparently much more sensitive to salt stress than even corn. Photosynthesis in some salt tolerant plants is unaffected by salt concentrations equivalent to 500 mM NaCl (Longstreth and Strain, 1977; Mallott et al., 1975). However, photosynthesis in other salt tolerant species declines at salinities equivalent to 350-500 mM NaCl, (Abdulrahman and Williams, 1981; DeJong, 1978). Thus, net photosynthesis in D. spicata, while far more resistant to salt stress than P. obtusum, is not as resistant as some salt tolerant species.

This comparative study identified several factors which may underlie the differential effects of salinity on the primary processes of CO_2 assimilation in these species. Both stomatal and internal aspects of CO_2 assimilation in P. obtusum are adversely affected by salinity to a strong degree. The great increases in stomatal resistance in P. obtusum with added NaCl were not due to changes in stomatal density (Table 1) as there was no significant difference in stomatal density with increased salinity. Likewise, salinity did not influence stomatal density in D. spicata. There was also no significant difference in stomatal density between species, indicating that this factor did not account for the much higher stomatal resistance of P. obtusum compared to D. spicata in 0.1 mM NaCl.

Internal resistances of P. obtusum were higher than D. spicata under all treatments and were greatly increased by salinity. Thus, D. spicata has a more efficient internal CO_2 assimilation mechanism and this mechanism is more resistant to or more protected from salinity than in P. obtusum. This difference does not appear to be related to internal mesophyll anatomy, which has been shown to be affected by salinity in many species and which can affect internal CO_2 resistances (Jennings, 1976; Longstreth and Noble, 1979). In P. obtusum and D. spicata microscopic examination showed no change in mesophyll cell size or arrangement and little change in leaf thickness (succulence) (Table 1). Thus, in neither species does leaf succulence appear to affect CO_2 assimilation. However, one anatomical feature distinctly different between these species is the external salt glands found on D. spicata leaves (Hansen et al., 1976). No structure exists on P. obtusum for excreting salt from leaves. Thus, part of the ability of D. spicata to resist internal effects of salt may be

related to its ability to excrete NaCl, since NaCl can have detrimental effects on enzyme systems even in salt tolerant plants (Flowers et al., 1977). Experiments are underway now to examine concentrations of NaCl in the leaves of these two species. It is also necessary to determine the intracellular distribution of NaCl. Mechanisms for sequestering salts in the vacuole could protect enzyme systems in the cytoplasm and other organelles from high salt (Flowers et al., 1977). It is possible that P. obtusum is not able to exclude salt form cells by either of the above mechanisms.

An investigation of the internal ion localization is necessary to understand the water relations in these two species. An apparent difference between glycophytes and halophytes is that the former tend to restrict the movement of salts into the leaves, whereas the latter tend to accumulate salts in the leaves (Flowers et al., 1977). If plants are to grow and extract water from saline substrates they must have sufficiently low leaf water potentials. Water potentials in both species examined here declined with increasing salinity (Table 1). However, there were differences between the species. Distichlis spicata exhibited a significant diurnal change in leaf water potential (as measured with a pressure bomb), whereas P. obtusum showed no diurnal change in leaf water potential (as measured with a thermocouple psychrometer). This difference may be due to the different measuring techniques or may reflect the fact that P. obtusum has extremely high stomatal resistance and avoids afternoon water depletion. On the other hand the "predawn" water potentials of P. obtusum are much lower than in D. spicata at corresponding salinity treatments. This may reflect a state of dehydration or stress in

P. obtusum or a very low osmotic potential accomplished through ion accumulation or organic solute accumulation. Further investigation in this area will be needed to evaluate these questions.

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TABLE 1. Leaf water potentials, leaf thickness, and stomatal density of plants grown at different salinities. Values are means ± one standard error of the mean (n=4 for all but stomatal density where n=12).

	Leaf water potential (MPa)		Leaf thickness (mm)	Stomatal Density (mm^{-2})
	"predawn"	"afternoon"		
Distichlis spicata				
0.01 mM	-0.4 ± .03	1.6 ± .10	.21 ± .01	
250 mM	-1.4 ± .04	2.7 ± .10	.22 ± .01	150 ± 9
500 mM	-2.4 ± .12	3.7 ± .11	.22 ± .01	
Panicum obtusum				
0.01 mM	-1.4 ± .33	1.4 ± .07	.18 ± .02	
125 mM	-2.2 ± .41	2.2 ± .44	.17 ± .01	161 ± 13
250 mM	-3.0 ± .51	2.9 ± .55	.16 ± .01	

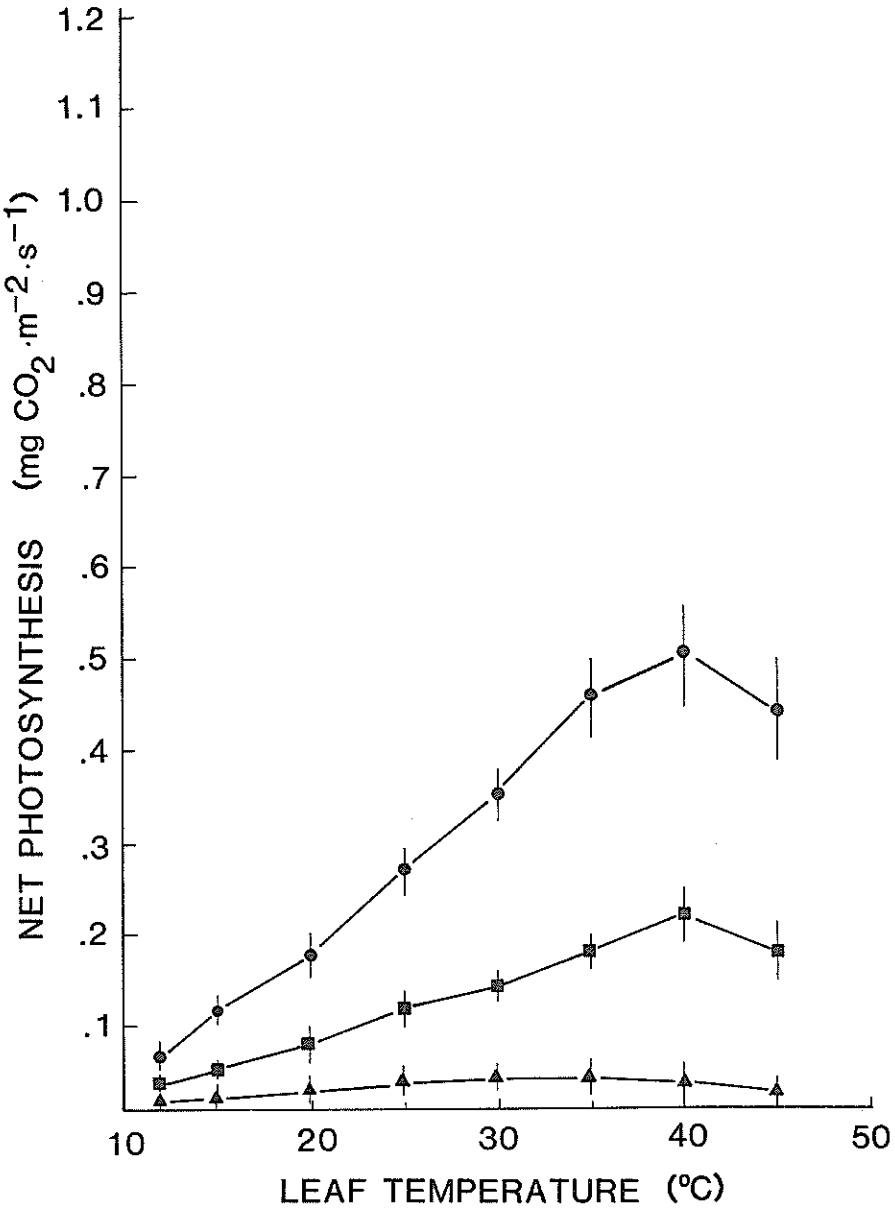

Figure 1. Response of net photosynthesis to leaf temperature in Panicum obtusum grown at 0.1 mM NaCl (●), 125 mM NaCl (■), and 250 mM NaCl (▲). The vertical bars in this and subsequent figures indicate ±1 standard error of the mean (n = 4).

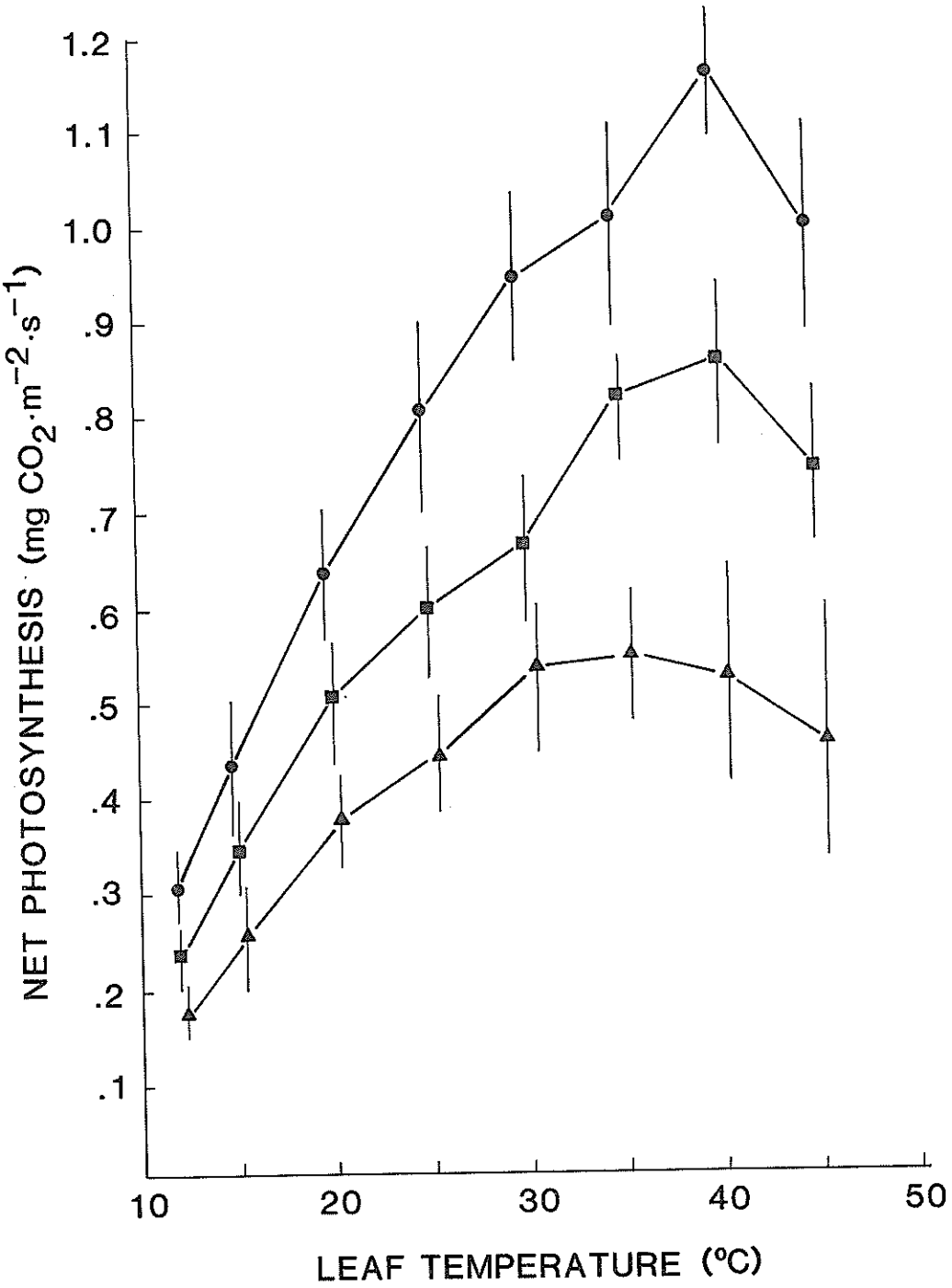

Figure 2. Response of net photosynthesis to leaf temperature in Distichlis spicata grown at 0.1 mM NaCl (●), 250 mM NaCl (■), and 500 mM NaCl (▲).

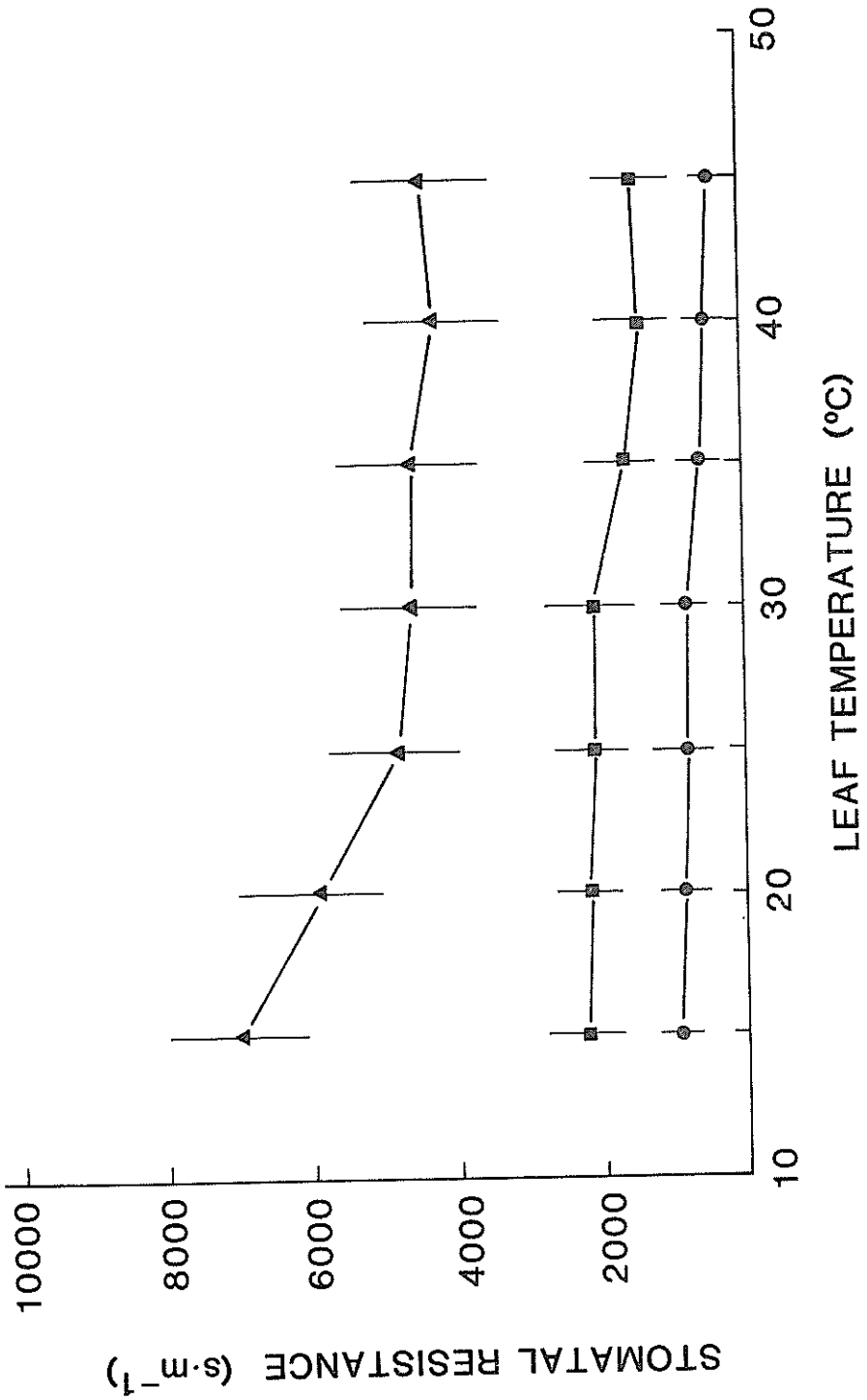

Figure 3. Response of stomatal resistance to leaf temperature in Panicum obtusum grown at 0.1 mM NaCl (●), 125 mM NaCl (■), and 250 mM NaCl (▲).

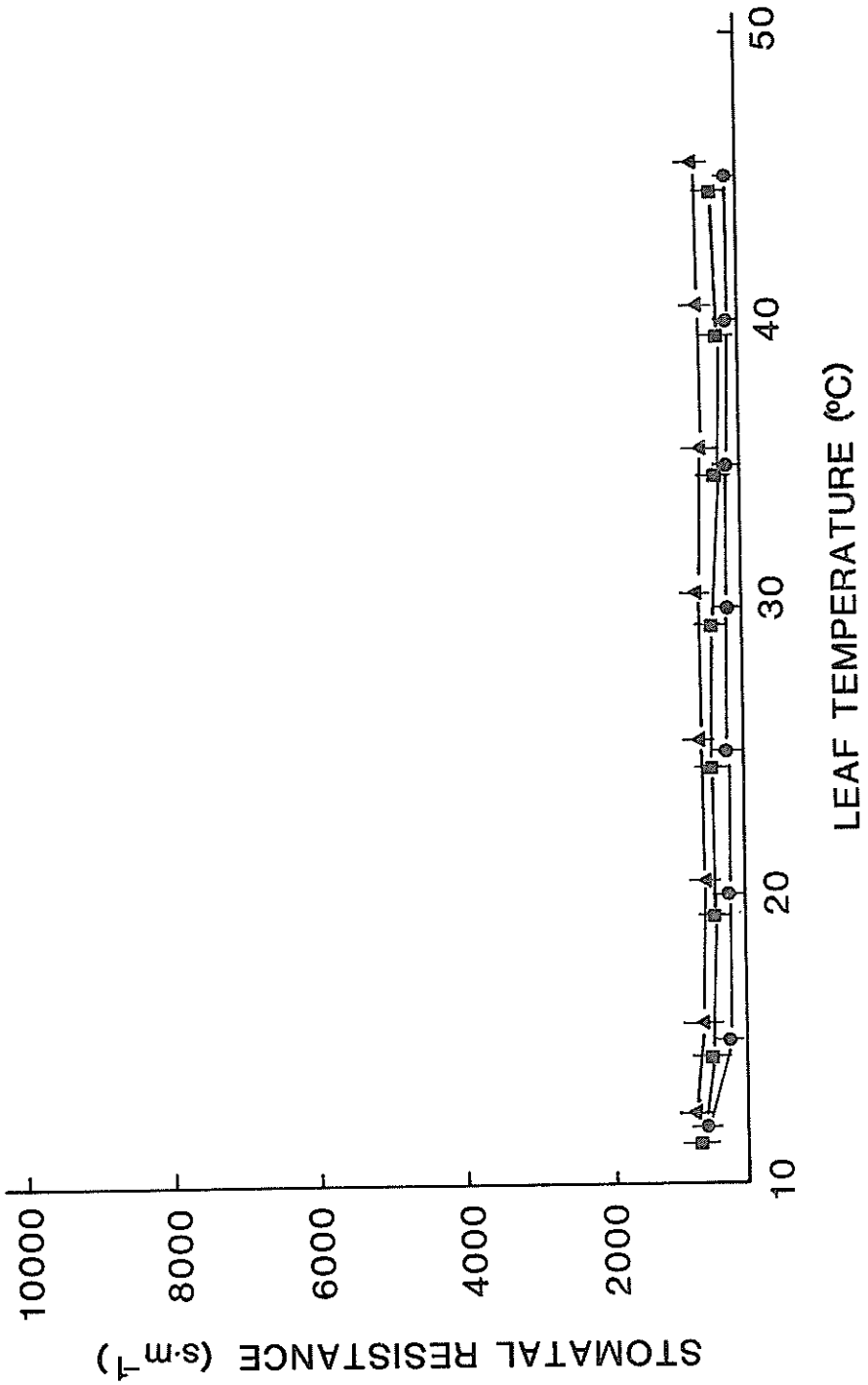

Figure 4. Response of stomatal resistance to leaf temperature in Distichlis spicata grown at 0.1 mM NaCl (●), 250 mM NaCl (■), and 500 mM NaCl (▲).

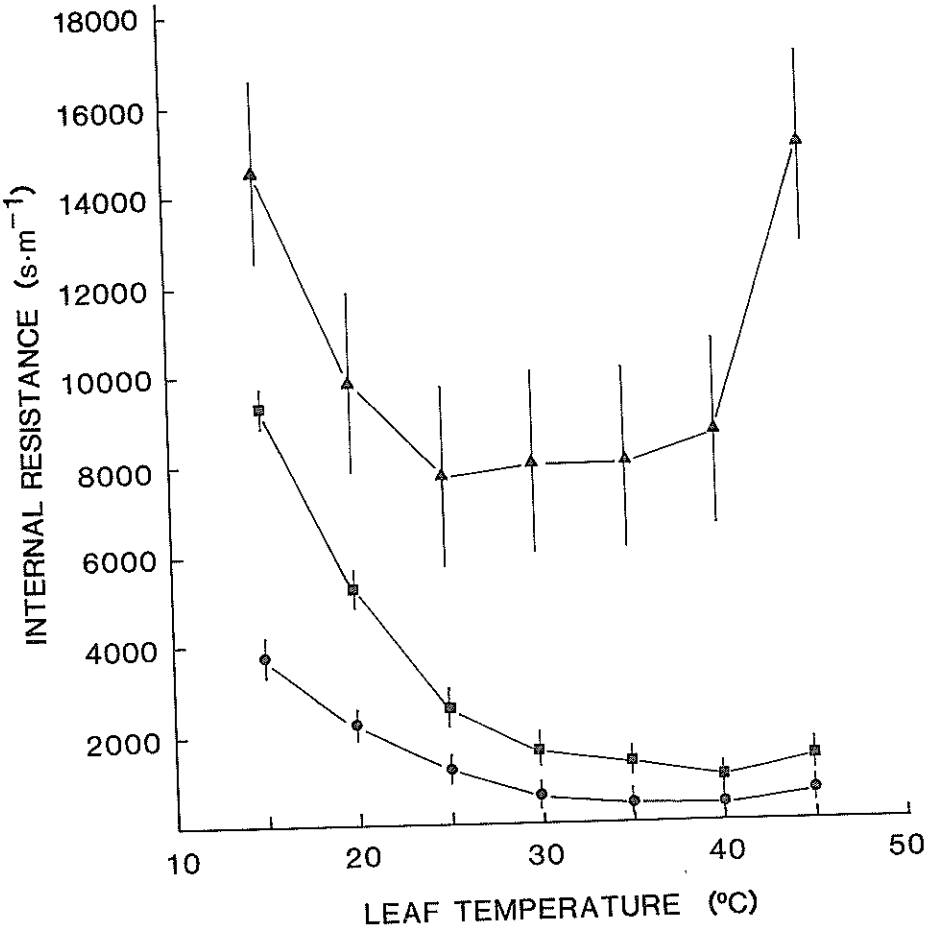

Figure 5. Response of internal (residual) resistance to leaf temperature in Panicum obtusum grown at 0.1 mM NaCl (●), 125 mM NaCl (■), and 250 mM NaCl (▲).

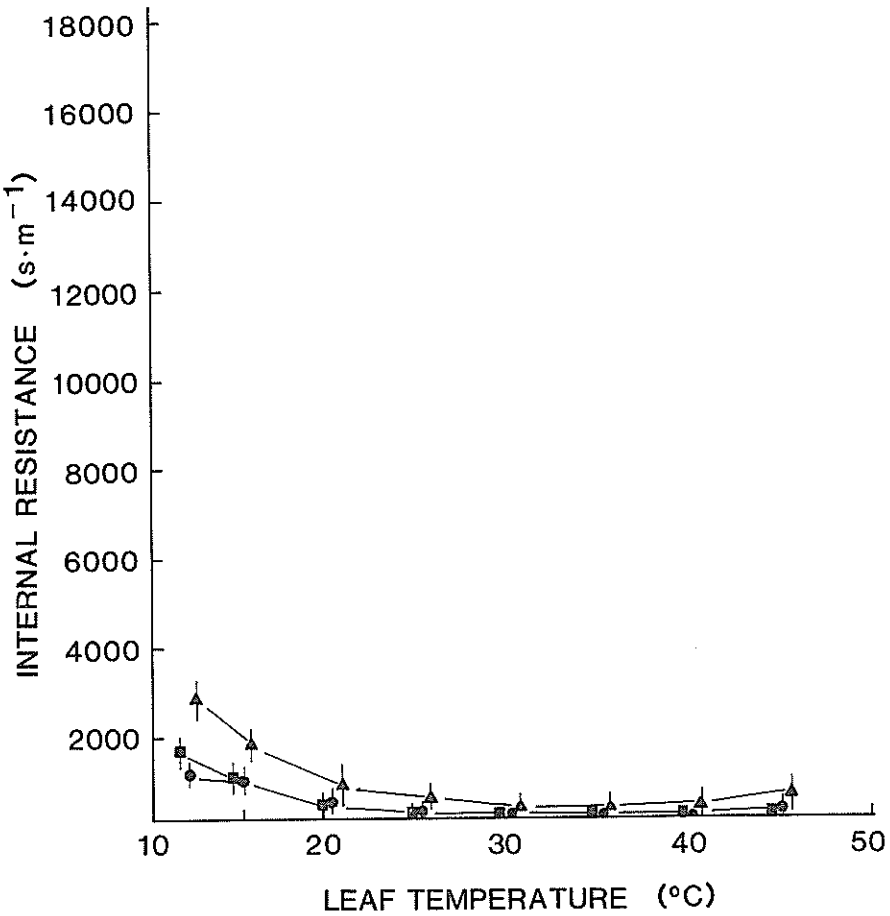

Figure 6. Response of internal (residual) resistance to leaf temperature in Distichlis spicata grown at 0.1 mM NaCl (●), 250 mM NaCl (■), and 500 mM NaCl (▲).