

# Salinity Tolerance Mechanisms of Six C<sub>4</sub> Turfgrasses

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Additional index words. compatible solutes, ion secretion, ion uptake, salinity, salt tolerance

**Abstract.** Physiological responses to salinity and relative salt tolerance of six C<sub>4</sub> turfgrasses were investigated. Grasses were grown in solution culture containing 1, 100, 200, 300, and 400 mM NaCl. Salinity tolerance was assessed according to reduction in relative shoot growth and turf quality with increased salinity. Manilagrass cv. Matrella (FC13521) (*Zoysia matrella* (L.) Merr.), seashore paspalum (Hawaii selection) (*Paspalum vaginatum* Swartz), and St. Augustinegrass (Hawaii selection) (*Stenotaphrum secundatum* Walt.) were tolerant, shoot growth being reduced 50% at ≈400 mM salinity. Bermudagrass cv. Tifway (*Cynodon dactylon* × *C. transvaalensis* Burt-Davey) was intermediate in tolerance, shoot growth being reduced 50% at ≈270 mM salinity. Japanese lawngrass cv. Korean common (*Zoysia japonica* Steud) was salt-sensitive, while centipedegrass (common) (*Eremochloa ophiuroides* (Munro) Hack.) was very salt-sensitive, with total shoot mortality occurring at ≈230 and 170 mM salinity, respectively. Salinity tolerance was associated with exclusion of Na<sup>+</sup> and Cl<sup>-</sup> from shoots, a process aided by leaf salt glands in manilagrass and bermudagrass. Shoot Na<sup>+</sup> and Cl<sup>-</sup> levels were high at low (100 to 200 mM) salinity in centipedegrass and Japanese lawngrass resulting in leaf burn and shoot die-back. Levels of glycinebetaine and proline, proposed cytoplasmic compatible solutes, increased with increased salinity in the shoots of all grasses except centipedegrass, with tissue water levels reaching 107 and 96 mM at 400 mM salinity in bermudagrass and manilagrass, respectively. Glycinebetaine and proline may make a significant contribution to cytoplasmic osmotic adjustment under salinity in all grasses except centipedegrass.

Use of brackish groundwater or saline sewage effluent in many areas of the world for landscape irrigation has resulted in a need for salt-tolerant turfgrasses. Overuse of limited water resources in Florida and Hawaii has resulted in seawater contamination of freshwater wells, some of which are used for turfgrass irrigation (Adams, 1978; Murdoch, 1987).

Detrimental effects of salinity on plant growth result from direct effects of ion toxicity (Hasegawa et al., 1986) or indirect effects of saline ions on the soil water potential, which causes soil/plant osmotic imbalance. Avoidance of toxicity may involve ion exclusion at the root cortex (Jeschke, 1984), redistribution of excess ions to senescing leaves or other plant parts (Yeo and Flowers, 1984), and, in some halophytic plants, secretion or sequestration of ions into salt glands or bladders (Liphschitz and Waisel, 1982; Marcum and Murdoch, 1990a).

To avoid osmotic imbalance at high salinity, halophytic plants accumulate saline ions in shoots. Enzymes from halophyte leaves, however, are as sensitive to salinity as those from glycophytes and are not compatible with levels of NaCl found in expressed saps (Flowers et al., 1977). It has been proposed that salinity tolerance at the cellular level involves tight control of cytoplasmic ion levels, coupled with compartmentation of excess saline ions required for osmotic adjustment in vacuoles. Under these conditions, maintenance of osmotic equilibrium across the tonoplast would require accumulation in the cytoplasm of nontoxic "compatible solutes", above a basal cytoplasmic osmotic concentration (osmolality) of 300 to 400 mOsmol·kg<sup>-1</sup> (Gorham et al., 1985; Wyn Jones, 1981). Possible compatible solutes, found to accumulate in halophytic plants, include proline, glycinebetaine, and trigonelline (Wyn

Jones, 1981, 1984). Relatively little is known about mechanisms of salinity tolerance of turfgrasses. In C<sub>4</sub> turfgrasses, reported responses to salinity include differences in shoot growth with increased salinity in bermudagrass (Dudeck et al., 1983; Ramakrishnan and Nagpal, 1973; Youngner and Lunt, 1967) and seashore paspalum cultivars (Dudeck and Peacock, 1985). Osmotic adjustment under increased salinity occurred in seashore paspalum, St. Augustinegrass, bermudagrass, manilagrass, and Japanese lawngrass, concurrent with increased shoot Na<sup>+</sup> and Cl<sup>-</sup> concentrations, decreased shoot K<sup>+</sup> concentration, and decreased shoot succulence (Marcum and Murdoch, 1990b). Osmotic adjustment and maintenance of positive turgor under salt stress occurred in seashore paspalum (Peacock and Dudeck, 1985) and St. Augustinegrass (Dudeck et al., 1993). Salinity tolerance between two zoysiagrass species was related to shoot Na<sup>+</sup> and Cl<sup>-</sup> exclusion, due to differences in salt secretion from leaf salt glands (Marcum and Murdoch, 1990a). This study was conducted to compare the relative salt tolerances and physiological responses of six C<sub>4</sub> turfgrasses to salinity and to elucidate mechanisms of salinity tolerance.

## Materials and Methods

**Growing conditions.** Six C<sub>4</sub> turfgrasses (Table 1) were grown in a glasshouse using a solution culture system. Grasses will be referred to by their common name (not by cultivar or accession name) in the text. Uniform sprigs of each grass were planted into 9 cm in diameter × 6-cm-deep plastic pots filled with coarse silica sand. Pots were suspended by white, 2-cm-thick plywood sheets over tubs containing 12 liters of a constantly aerified, modified Hoagland no. 2 solution (Hoagland and Arnon, 1950) in which 2 mg Fe/liter was supplied as Fe-EDDHA chelate (Sequestrene 138; Ciba-Geigy, Greensboro, N.C.). Pots had coarse screen bottoms allowing roots to grow into the nutrient solution. Grasses were clipped every 10 days at 2.5 cm (3.5 cm for St. Augustinegrass) and were fully established with a dense turf before treatments began.

Received for publication 21 Sept. 1993. Accepted for publication 29 Nov. 1993. Contribution of Hawaii Inst. for Trop. Agr. and Human Resource, Journal series no. 3872. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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Table 1. Turfgrasses evaluated with binomial classification and subfamily.

Common name	Classification	Subfamily
Bermudagrass cv. Tifway	<i>Cynodon dactylon</i> × <i>C. transvaalensis</i> Burt-Davey	Chloridoideae
Centipedegrass (common)	<i>Eremochloa ophiuroides</i> (Munro) Hack.	Panicoideae
Japanese lawngrass cv. Korean common	<i>Zoysia japonica</i> Steud	Chloridoideae
Manilagrass cv. Matrella (FC13521)	<i>Zoysia matrella</i> (L.) Merr.	Chloridoideae
Seashore paspalum (Hawaii selection)	<i>Paspalum vaginatum</i> Swartz	Panicoideae
St. Augustinegrass (Hawaii selection)	<i>Stenotaphrum secundatum</i> Walt.	Panicoideae

To avoid salinity shock, salinity levels were gradually increased by 50 mM NaCl (2.9 g NaCl/liter) every day until final treatment levels of 1, 100, 200, 300, and 400 mM NaCl were reached. A control solution contained 1 mM NaCl (0.054 g·liter<sup>-1</sup>), as C<sub>4</sub> plants require Na<sup>+</sup> as a micronutrient (Brownwell and Crossland, 1972). Solution volumes were maintained at 12 liters, and conductivities were monitored daily with a conductivity meter. Nutrient solutions were changed weekly, preventing any change in treatment conductivity levels. All grasses were represented in each salinity tub, and there were four replications.

**Relative salinity tolerance.** Shoots and roots (roots clipped at base of pot screens) were clipped and discarded 5 days after the highest salinity level was reached (400 mM), allowing plants to become equilibrated to treatment salinities before clippings were taken for analysis. Thereafter, shoots were clipped at 10-day intervals for a total of three harvests. Clipped shoots were immediately placed in glass bottles with air-tight lids for fresh weights, and subsequently dried at 70C for dry weight determination.

Turf quality was evaluated twice at 6 weeks following the initiation of the experiment. Pots were rated on a 1 to 9 scale for color and live shoot density (1 = completely brown turf, no live shoots; 9 = completely green turf, no dead shoots).

**Solute analysis.** Immediately before clipping, shoots were rinsed for 20 sec in deionized water and then allowed to dry. Clipped shoots were dried in a forced-air dryer at 70C, then ground in a mill (Wiley Mill; Arthur H. Thomas Co., Philadelphia) with a 20-mesh screen. For ion analysis, samples were placed in a 450C oven for 7 h to ash, and then dissolved in 1 M HNO<sub>3</sub>. Sodium and K<sup>+</sup> were determined by flame-emission spectrophotometry, Ca<sup>2+</sup> and Mg<sup>2+</sup> by atomic absorption spectrophotometry (model 3030B; Perkin-Elmer, Norwalk, Conn.), and Cl<sup>-</sup> by an Cl<sup>-</sup> ion activity electrode (Orion Research, Boston, Mass.). Ion secretion from leaf salt glands was determined as the difference in ion contents between unrinsed and rinsed shoots grown at 200 mM NaCl.

For proline determination, clipped leaves were immediately placed in air-tight plastic vials and frozen in dry ice. Leaves were homogenized in 3% sulfosalicylic acid solution and assayed spectrophotometrically by an acid-ninhydrin method to detect proline (Bates et al., 1973).

Concentration of betaines (glycinebetaine and trigonelline) was determined by high-performance liquid chromatography (HPLC) (Gorham, 1984). The HPLC system consisted of a HPLC pump (model 410; Perkin-Elmer) with a 20µL injection loop and a variable wavelength ultraviolet (UV) detector (model LC-95; Perkin-Elmer). Separations were performed on a 250 × 5 mm I.D. stainless-steel column packed with Partisil 10-SCX and fitted with a direct-connect guard column packed with the same material.

**Statistical analysis.** All data were analyzed by regression using an approach similar to testing for heterogeneity of linear effects (Freund et al., 1986) with testing extended to quadratic effects (Marcum and Murdoch, 1990b). A model sequence approach was used for each response variable. The most general model included

terms for intercept, linear, and quadratic terms (Allen and Cady, 1982). Testing progressed until reduced models were found that described the data adequately. Overall goodness of fit of reduced models is described by both model SE and model R<sup>2</sup>. Single degree of freedom contrast coefficients were used to compare intercepts and regression coefficients among individual grasses. Grasses with responses not significantly different are presented as single regressions. Total mortality occurred in both centipedegrass and Japanese lawngrass at intermediate salinities. Regressions were not attempted when there was incomplete data, but means with accompanying SEs are presented. In all figures, labels for the grasses have been abbreviated to Ber. (bermudagrass), Cent. (centipedegrass), Japn. (Japanese lawngrass), Manl. (manilagrass), Pasp. (seashore paspalum), and Saug. (St. Augustinegrass).

## Results

Shoot growth rates of St. Augustinegrass and seashore paspalum were higher than other grasses at all salinity levels and shoot growth of St. Augustinegrass was stimulated at 100 to 200 mM NaCl (Fig. 1). Manilagrass and bermudagrass had intermediate growth rates, but bermudagrass growth rates decreased more rapidly as salinity increased. Centipedegrass and Japanese lawngrass shoot growth decreased to zero followed by total shoot mortality at ≈170 and 230 mM NaCl, respectively.

Relative shoot growth (as a percent of control) was reduced by 50% at predicted salinities of ≈400 mM NaCl in seashore paspalum and manilagrass, 395 mM NaCl in St. Augustinegrass, 272 mM NaCl in bermudagrass, 130 mM NaCl in Japanese lawngrass, and 77 mM NaCl in centipedegrass (Fig. 1). Relative shoot growth of St. Augustinegrass was stimulated 140% at intermediate salinity (≈150 mM NaCl) and then declined.

Turfgrass quality, as indicated by visual ratings, followed the same trends as did relative shoot growth (Fig. 2). At 400 mM NaCl seashore paspalum had a quality rating of 5.3, manilagrass 4.7, and St. Augustinegrass 4.0. These three grasses maintained relatively dense, green turf at high salinity. Bermudagrass quality decreased linearly to 2.0 at 400 mM NaCl. Japanese lawngrass and centipedegrass quality dropped to 1 (completely dead) at 230 and 170 mM NaCl, respectively.

Shoot Na<sup>+</sup> and Cl<sup>-</sup> concentrations (expressed as mM in tissue water) increased with increased salinity in all grasses (Fig. 3). Of the tolerant grasses, St. Augustinegrass had the highest shoot Na<sup>+</sup> concentration (400 mM), which was equal to the treatment solution concentration at that salinity. Japanese lawngrass accumulated shoot Na<sup>+</sup> and Cl<sup>-</sup> to higher levels under medium (200 mM NaCl) salinity than did other grasses. Centipedegrass accumulated high Na<sup>+</sup> and very high Cl<sup>-</sup> shoot levels at only 100 mM salinity where plant mortality occurred. Shoot K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> decreased with increased salinity in all grasses (Fig. 4). Seashore paspalum and manilagrass maintained higher shoot K<sup>+</sup> at 300 to 400 mM salinity than did other grasses, while bermudagrass maintained higher

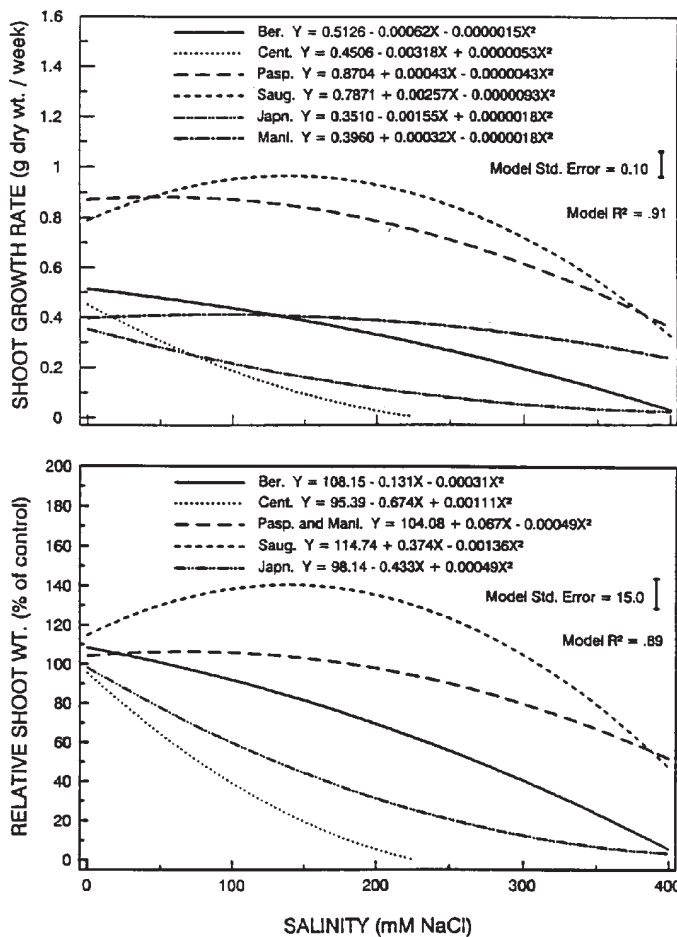


Fig. 1. Shoot growth rates and relative shoot weights (as a percent of control) of six  $C_4$  grasses as influenced by salinity between 0 and 400 mM NaCl.

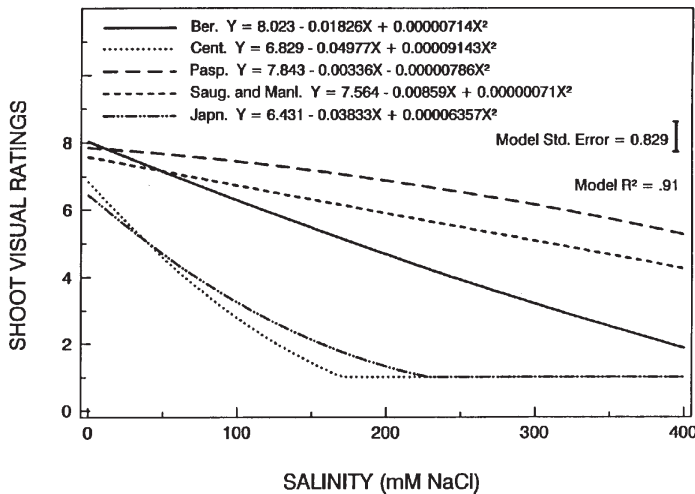


Fig. 2. Shoot visual quality ratings of six  $C_4$  grasses as influenced by salinity between 0 and 400 mM NaCl.

shoot  $Ca^{2+}$ . Shoot  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  concentrations were lowest at all salinities in St. Augustinegrass.

The presence of salt crystals on plants grown under saline conditions is indicative of active salt secretion by salt glands or bladders (Fahn, 1988). Salt crystals were observed on leaves of bermudagrass, manilagrass, and Japanese lawngress at all salinity levels. Salt secretion is shown in Figure 5 as the difference in ion concentrations between unrinsed and rinsed leaves grown at 200

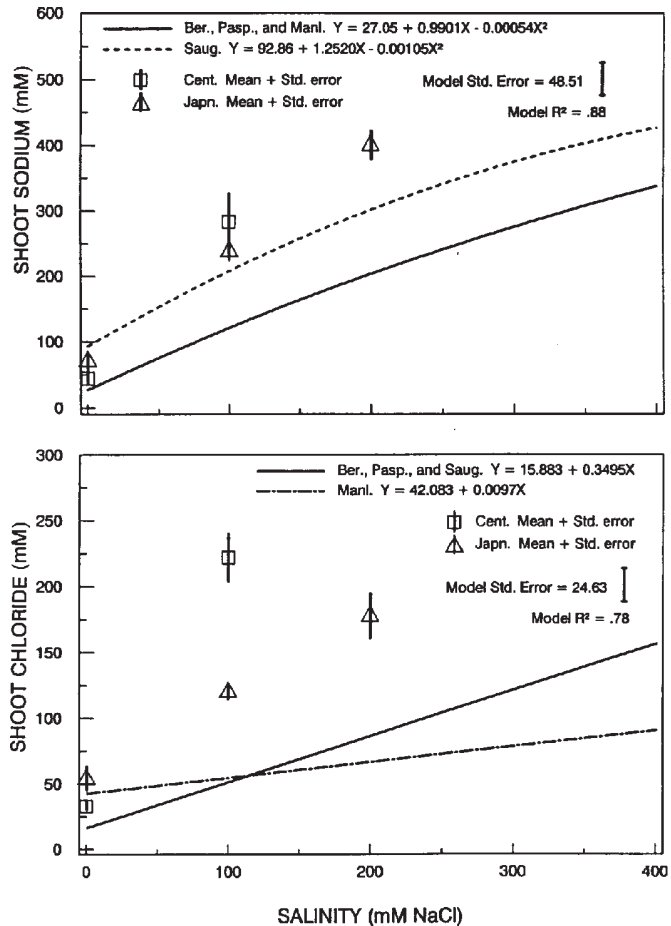


Fig. 3. Shoot sodium and chloride concentrations of six  $C_4$  grasses as influenced by salinity between 0 and 400 mM NaCl. Means are presented for centipedegrass and Japanese lawngress.

mm NaCl. Sodium and  $Cl^-$  secretion occurred in the above three grasses. A slight  $K^+$  secretion is indicated for bermudagrass and manilagrass, but the trend was not significant for Japanese lawngress. Manilagrass, bermudagrass, and Japanese lawngress shoots secreted 0.72, 0.66, and 0.36  $mmol \cdot g^{-1}$  leaf dry weight  $Na^+$ , and 0.42, 0.43, and 0.24  $mmol \cdot g^{-1}$  leaf dry weight  $Cl^-$ , respectively, over a 10-day period.

Proline accumulated to a level of 103 mm in bermudagrass at 400 mM NaCl (Fig. 6). Proline has been previously reported to accumulate in common bermudagrass under both salinity and drought stress (Manetas et al., 1986; Stewart and Lee, 1974). Proline increased only slightly in St. Augustinegrass, seashore paspalum, manilagrass, and Japanese lawngress with increased salinity.

Glycinebetaine accumulated to highest levels in bermudagrass and manilagrass (Fig. 6). These levels were higher than those reported in wheat, sorghum, and other glycophytic and mesophytic grasses (Hitz and Hanson, 1980; Grieve and Maas, 1984), but were similar to the levels in the halophytic grass *Spartina townsendii* (Wyn Jones and Storey, 1981). No significant accumulation of glycinebetaine occurred in centipedegrass. Trigonelline (nicotinic acid betaine) accumulated with increased salinity only in St. Augustinegrass (Fig. 6).

## Discussion

A common response of plants to salinity is shoot dehydration

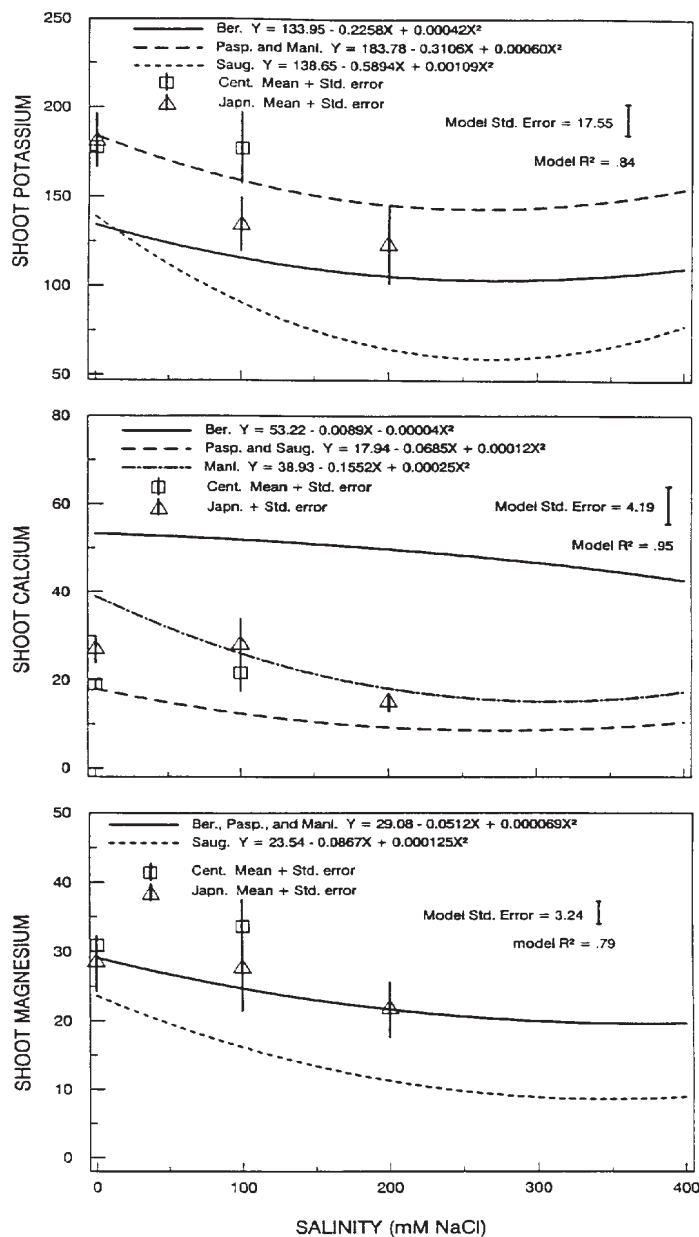


Fig. 4. Shoot K, Ca, and Mg concentrations of six *C*<sub>4</sub> grasses as influenced by salinity between 0 and 400 mM NaCl. Means are presented for centipedegrass and Japanese lawngrass.

and loss of cell turgor, resulting in reduced growth rate (Neumann et al., 1988). High shoot growth rates of St. Augustinegrass and seashore paspalum (6 g/pot per week fresh weight at 100 mM NaCl in St. Augustinegrass compared to 1.8 g/pot per week for Tifway bermudagrass) may have resulted from maintenance of shoot tissue succulence. Shoot fresh/dry weights in St. Augustinegrass and seashore paspalum were twice those of bermudagrass, manilagrass, and Japanese lawngrass when grown in salinities from 0.7 to 30 dS·m<sup>-1</sup> (Marcum and Murdoch, 1990b). St. Augustinegrass and seashore paspalum are in the subfamily Panicoideae, whereas bermudagrass, manilagrass, and Japanese lawngrass are in the subfamily Chloridoideae (Gould and Shaw, 1983). This may indicate basic differences in physiological responses to salinity of the two subfamilies.

Rather than comparing absolute growth rates under salt stress, salinity tolerance is better expressed as relative shoot growth

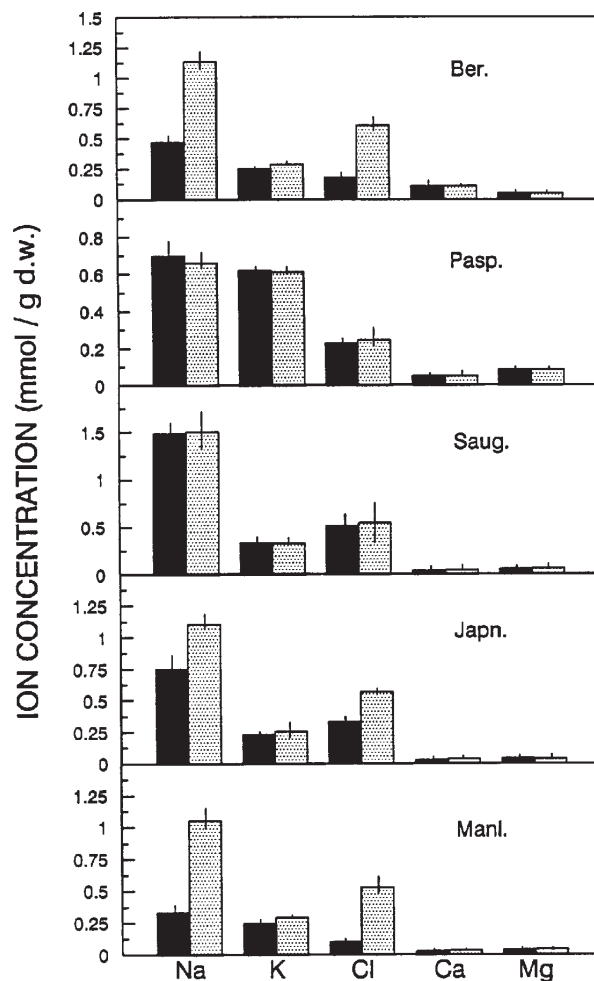


Fig. 5. Ion concentrations of leaves of six *C*<sub>4</sub> grasses grown in 200 mM NaCl. Light bars represent unrinsed leaves, dark bars represent rinsed leaves. Differences in ion secretion for 1 week are depicted. Results are means  $\pm$  SE of three plants.

reduction (as a percent of control) with increased salinity, which is an indication of relative plant vigor under stress (Maas and Hoffman, 1977). Using 50% relative yield reduction as a criteria, seashore paspalum, manilagrass, and St. Augustinegrass were most salt tolerant, followed by bermudagrass, Japanese lawngrass, and centipedegrass.

Clipping yield is not a critical factor in turfgrass management in that reduced growth rate may actually result in lower maintenance costs. Turfgrass quality, which is primarily influenced by live shoot density, is more important. Quality rankings followed the same trend as relative shoot reductions, which were best in seashore paspalum and St. Augustinegrass across all salinities, but quality ratings were slightly better in seashore paspalum due to higher live shoot densities. Bermudagrass was intermediate in quality with low live shoot density at 400 mM NaCl. Leaf burn and shoot die-back occurred in centipedegrass and Japanese lawngrass at 100 mM NaCl. There were few live shoots remaining at the end of the experiment for Japanese lawngrass at 200 mM NaCl and for centipedegrass at 100 mM, respectively. However, seashore paspalum, St. Augustinegrass, and manilagrass continued to produce new shoots through the last harvest at 400 mM NaCl.

Salinity tolerance in grasses has been associated with exclusion of Na<sup>+</sup> and Cl<sup>-</sup> from shoots (Gorham et al., 1986; Marcum and Murdoch, 1990a). Salinity tolerance in the two grass subfamilies was associated with shoot Na<sup>+</sup> and Cl<sup>-</sup> exclusion. In the

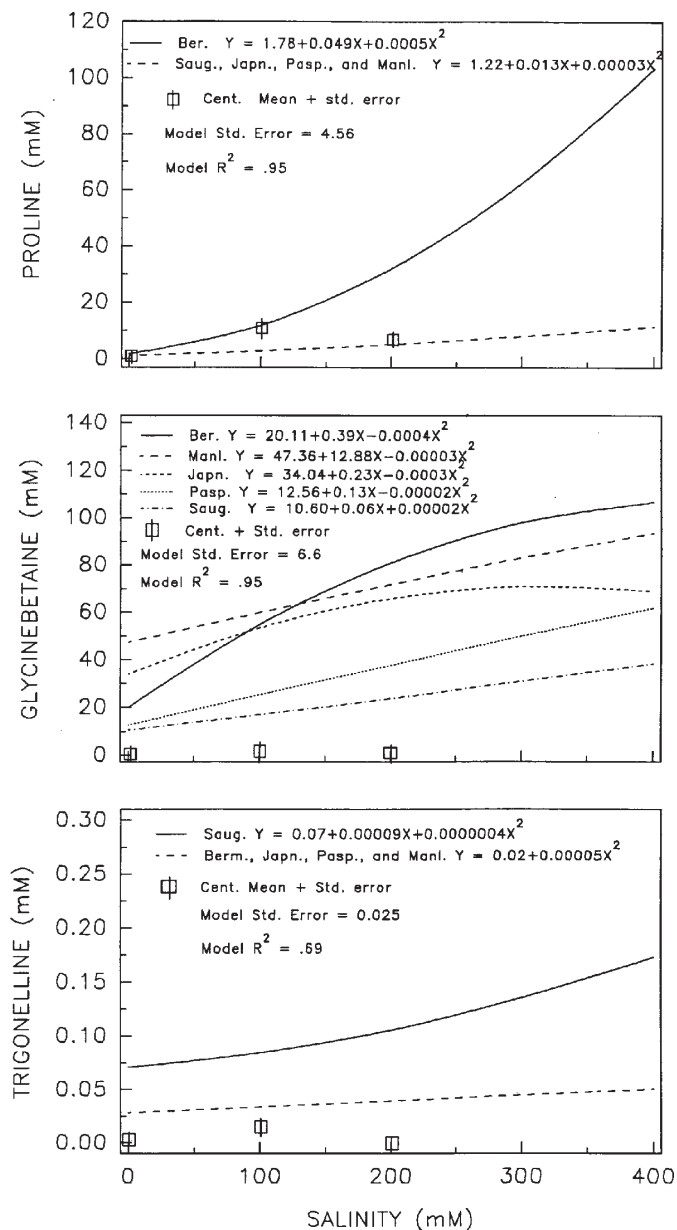


Fig. 6. Shoot proline, glycinebetaine, and trigonelline concentrations of six  $C_4$  grasses as influenced by salinity between 0 and 400 mM NaCl. Means are presented for centipedegrass and Japanese lawngrass.

Chloridoideae subfamily, manilagrass and bermudagrass limited shoot  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations due to the presence of leaf salt glands, which secreted 0.50 and 0.46 mmol  $\text{Na}^+$ /g leaf dry weight per week, respectively, when grown at 200 mM NaCl. Though salt glands were also present in Japanese lawngrass, they were only half as efficient as those in manilagrass and bermudagrass, and secreted about half the  $\text{Na}^+$  and  $\text{Cl}^-$  per leaf weight. Consequently, Japanese lawngrass did not limit shoot  $\text{Na}^+$  and  $\text{Cl}^-$  levels as effectively, which resulted in high shoot ion levels at intermediate salinities. Centipedegrass of the subfamily Panicoideae was the most salt-sensitive grass, and accumulated  $\text{Na}^+$  and  $\text{Cl}^-$  to very high levels in shoots under low salinity. Japanese lawngrass and centipedegrass showed moderate to severe foliar NaCl toxicity at 100 mM NaCl.

When tissue concentration of NaCl exceeds  $\approx 200$  mM, ion compartmentation in the vacuole becomes necessary to avoid enzyme deactivation and subsequent cell death (Wyn Jones, 1981). Under these conditions, maintenance of osmotic equilibrium across the tonoplast requires accumulation in the cytoplasm of nontoxic organic solutes, or "compatible solutes." The most likely candidates in the Poaceae are glycinebetaine and proline (Gorham et al., 1985).

Glycinebetaine and proline occur predominately in the cytoplasm (Gorham and Wyn Jones, 1983; Leigh et al., 1981). If it is assumed that glycinebetaine and proline are located entirely in the cytoplasm and that the cytoplasmic volume in mature mesophyll cells in the grasses studied is  $\approx 10\%$  of total cell volume (Leigh et al., 1981), then estimates of the relative contributions of glycinebetaine and proline to the osmotic adjustment of the cytoplasm can be made (Table 2). On this basis, the concentrations of glycinebetaine and proline in bermudagrass, manilagrass, sea-shore paspalum, and Japanese lawngrass would be sufficient for complete cytoplasmic osmotic adaptation above a normal basal osmolality level of 300 to 400  $\text{mOsmol}\cdot\text{kg}^{-1}$  (Gorham et al., 1985). This accommodated all the increase in cytoplasmic osmotic pressure necessary to balance that of the whole cell. Though the levels in St. Augustinegrass would contribute substantially to osmotic adjustment, they would still be 60 to 160 mM short of fully adjusting cytoplasmic osmotic potentials. However, the high shoot succulence of St. Augustinegrass (Marcum and Murdoch, 1990b) may result in a dilution effect on a whole-cell basis. Glycinebetaine and proline levels in centipedegrass were too low to affect any significant osmotic adjustment of the cytoplasm. Lack of compatible solutes may indicate that centipedegrass was unable to compartmentalize ions in shoot vacuoles. This, coupled with high  $\text{Na}^+$  and  $\text{Cl}^-$  shoot concentrations due to an inability to restrict these

Table 2. Estimated contributions to cytoplasmic osmotic adjustment (osmolality) of compatible solutes glycinebetaine and proline in comparison to whole-cell osmotic adjustment. Data are from grasses grown at 200 mM NaCl.

Grass	Shoot tissue content (mM)		Contributions to cytoplasmic osmotic adjustment <sup>z</sup> ( $\text{mOsmol}\cdot\text{kg}^{-1}$ )	Whole-plant cell osmotic adjustment <sup>y</sup> ( $\text{mOsmol}\cdot\text{kg}^{-1}$ )
	Glycinebetaine	Proline		
Bermudagrass	89	34	1230	913
Manilagrass	77	3	800	833
Paspalum	39	3	420	669
St. Augustine	25	6	310	770
Japanese lawn	71	5	760	1076
Centipedegrass	2	9	110	660

<sup>z</sup>Osmolality calculated assuming that glycinebetaine and proline are located within the cytoplasm comprising 10% of the cell volume. Both compounds are given an osmotic coefficient of 1.

<sup>y</sup>Osmolality of expressed shoot sap ( $\text{mOsmol}\cdot\text{kg}^{-1}$ ) measured with a vapor pressure osmometer (model 5100C; Wescor, Logan, Utah).

ions from the shoots, may be responsible for salt sensitivity of centipedegrass.

In conclusion, salinity tolerance of the C<sub>4</sub> turfgrasses in this study was related to Na<sup>+</sup> and Cl<sup>-</sup> restriction from shoots. This process was aided by leaf salt glands in grasses of the subfamily Chloridoideae (manilagrass, bermudagrass, and Japanese lawngrass). Shoot Na<sup>+</sup> and Cl<sup>-</sup> ion concentrations under saline conditions were sufficiently high in all grasses to necessitate vacuolar ion compartmentation and cytoplasmic compatible solute accumulation, which it is hypothesized, avoids ion toxicity. Proposed compatible solutes proline and glycinebetaine accumulated sufficiently for cytoplasmic osmotic adjustment in all grasses except centipedegrass.

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