Effects of Salinity on Growth and Cation Accumulation of *Sporobolus virginicus* (Poaceae)

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Optimal growth of euhalophytes requires moderate concentrations of salt and, in dicotyledons, is associated with succulence and accumulation of Na\(^+\) in plant tissues. However, reports of salt-stimulated growth in monocotyledons are rare. Relative growth rate (RGR), biomass accumulation, and water content were studied in *Sporobolus virginicus* (Poaceae), a C\(_4\) chloridoid grass, grown hydroponically with different concentrations of NaCl. Cation concentrations were determined by atomic absorption spectrophotometry. Optimal growth occurred at 100–150 mmol/L NaCl and was not dependent on nitrogen levels or accompanied by accumulation of Na\(^+\) in leaves. Biomass accumulation and RGR in plants grown at 450 mmol/L NaCl were greater than in plants grown at 5 mmol/L. The Na : K ratios were lower in leaves than in roots, indicating discrimination in Na\(^+\) and K\(^+\) transport. Secretion of Na\(^+\) increased from 166.5 to 336.7 mmol \(\cdot\) g\(^{-1}\) dry biomass \(\cdot\) d\(^{-1}\) as the NaCl concentration of the nutrient solution increased from 125 mmol/L to 450 mmol/L. Water concentrations of leaves and shoots were significantly greater in plants grown at optimal levels of salinity than in plants grown at lower or higher salinities. These results demonstrate salt-stimulated growth in a monocotyledon.

**Key words:** cation accumulation; chloridoid grasses; halophyte; osmotic adjustment; Na : K ratio; Na\(^+\) secretion; Poaceae; salt-stimulated growth; *Sporobolus virginicus*.

Halophytes are plants that can complete their life cycles in habitats that have moderate to high concentrations of salts in their soils (Munns et al., 1983; Flowers et al., 1986). Greenway and Munns (1980) divide the growth response to salinity of plants into four groups. Group I\(_H\) (euhalophytes) have optimal growth (i.e., salt-stimulated growth) at moderate salinities (100–300 mmol/L NaCl) and continue to grow and survive at salinities up to 700 mmol/L. Group I\(_m\) (mihalophytes) have optimal growth at very low salt concentrations and continue to grow at reduced rates even at higher salinities. In group II halophytes and nonhalophytes, growth is greatly reduced even at moderate salinity, and salinities over 300 mmol/L are lethal. Group III are very salt-sensitive nonhalophytes that cannot survive when grown at salinities over 100 mmol/L. Halophytes occur in three subclasses of angiosperms (Kramer and Van Andel, 1995). In monocotyledons, halophytes are rare in the Alismatidae and clustered in a few related families in the Commelinidae; in the remaining angiosperms (dicotyledons, sensu lato), halophytes are found in fewer than 50% of families in the Carophyllidae (primarily within the Chenopodiaceae).

Euhalophytic members of the Chenopodiaceae include *Suaeda* (Yeo and Flowers, 1980), *Atriplex* (Greenway, 1968; Miyamoto et al., 1996), and *Salicornia* (Ayala and O’Leary, 1996; Pfister, 1999). In these genera, it is well established that maximum or optimal growth occurs at salinities ranging from 100 to 300 mmol/L and that growth is less under freshwater conditions (Chapman, 1960; Yeo and Flowers, 1980; Flowers et al., 1986). Optimal growth of dicotyledonous euhalophytes is associated with succulence of leaves and stems (Pfister, 1999), Na\(^+\) accumulation in (and, in some cases, secretion from) leaves (Munns et al., 1983; Miyamoto et al., 1996), and a high ratio of Na : K in plant tissues (at least 5.0 to 10.0) (Gorham et al., 1980; Rozema, 1991). Reports of salt-stimulated growth (by the definition of Greenway and Munns [1980]) are rare among grasses, and optimal growth of halophytic grasses is not associated with high Na\(^+\) accumulation (Gorham et al., 1980; Glenn, 1987).

Optimal growth under saline conditions has been observed to be nitrogen dependent in both halophytic dicotyledons (Rozema et al., 1983) and monocotyledons (Smart and Barko, 1980). The nitrogen dependence of halophytes is associated with production of quaternary ammonium compounds and free amino acids that are believed to contribute to osmotic adjustment and act as nitrogen sinks (Flowers et al., 1977).

Marcum and Murdoch (1992) evaluated *Sporobolus virginicus* (L.) Kunth for use as a turf grass or ground cover. At 150 mmol/L NaCl, the growth rate was 13% greater than at 1 mmol/L; however, at 450 mmol/L, the growth rate decreased to 66% of the growth at 1 mmol/L. Concentrations of shoot Na\(^+\), root Na\(^+\), and root K\(^+\) increased with increasing salinity. Shoot K\(^+\) declined although shoot K\(^+\) selectivity increased. Marcum and Murdoch (1992) and Naidoo and Naidoo (1998) have described salt secretion by salt glands on leaves of *S. virginicus*. Ramadan (2001) observed similar patterns of salt secretion in *S. spicatus*.

*Sporobolus virginicus* (Poaceae) is a perennial, rhizomatous, C\(_4\) chloridoid grass with a broad distribution along subtropical shorelines (Hitchcock, 1971). Growth has been studied in eco-types of *S. virginicus* collected from populations in Georgia, Florida, and Hawaii, USA (Gallagher, 1979; Blits and Gallagher, 1991; Marcum and Murdoch, 1992) and Durban, South Africa (Breen et al., 1977; Naidoo and Mundree, 1993; Naidoo and Naidoo, 1998). The source of plant material, experimental design, and growth conditions differed in each study. No salt-stimulated growth of *S. virginicus* was observed in any study except the small increase found by Marcum and Murdoch (1992).
The present study was designed to verify and to amplify the results of Marcum and Murdoch (1992) and to answer the question, Is *S. virginicus* a euhalophyte? Four aspects of the response to salinity were investigated: (1) growth under a range of concentrations of NaCl, (2) the interaction of nitrate levels with levels of NaCl, (3) the relative accumulation of cations in plant tissues grown at different concentrations of NaCl, and (4) the water content and osmotic adjustment of *S. virginicus* grown at different concentrations of NaCl.

**MATERIALS AND METHODS**

**Plant material**—Rhizomes of *S. virginicus* (HA-4846) were supplied by the USDA Plant Materials Center in Hoolaheu, Hawaii, USA. The plant material was derived from the same accession; it is not known if the plants were clones. A voucher specimen is deposited with the herbarium of the University of Arizona (ARIZ).

**Growth conditions and protocols**—Rhizomes were planted in shallow trays filled with half sand/half Sunshine commercial planting mix (Sun Gro Horticulture, Bellevue, WA, USA) and watered weekly with half strength Hoagland’s solution #2 (Hoagland and Arnon, 1950) supplemented with 5 mmol/L NaCl (Brownell and Crossland, 1972). Plant material was periodically divided and planted in fresh sand/Sunshine commercial planting mix to increase stock.

Plants were grown in a greenhouse at the University of Arizona Campus Agricultural Center, Tucson, Arizona, USA. Temperatures were maintained between 20° and 31°C in the winter and 24° and 35°C in the summer. Relative humidity ranged from 25 to 50% (days) and 70 to 85% (nights). Mean photosynthetically active radiation (at noon, within the greenhouse) was 450 μmol·m⁻²·s⁻¹ in the winter and 1350 μmol·m⁻²·s⁻¹ in the summer. The roof and west side of the greenhouse were covered with a shade cloth that excluded 45% of natural light from June through September. The electrical conductivities (given in decisiemens per meter) of the nutrient solutions plus the following concentrations (in millimoles per liter) of NaCl were 1.9 (5), 6.8 (50), 11.7 (100), 16.1 (150), 30.3 (300), and 44.8 (450). Electrical conductivity was monitored daily until it was determined that changes in electrical conductivity from immediately after the change of solutions until the next weekly change were under 5%.

The hydroponic system of Marcum and Murdoch (1992) was used. Plastic tubs (10 L) were covered with duct tape to minimize algal growth in the nutrient solution. Tubs were topped with wooden boards each containing eight holes to support plastic cups filled with coarse sand. Bases of the cups were removed and replaced with pliable screening that was permeable to roots and the nutrient solution. Individual ramets were transferred from soil culture to the cups and allowed to acclimate to hydroponics. During acclimation and before the start of treatments, plants were maintained with half strength Hoagland’s solution #2 (Hoagland and Arnon, 1950) supplemented with 5 mmol/L NaCl (Brownell and Crossland, 1972). Plant material was prepared to tap water and were aerated constantly. As necessary, volumes were filled with half sand/Sunshine commercial planting mix to increase stock.

In experiment 1 during acclimation to hydroponics, shoots were cut back to the sand surface and allowed to regrow for 2 wk. Regrowth was cut back to the sand surface and dried to constant mass at 70°C. Based on the dry biomass harvested from each cup within tubs, cups were redistributed between tubs to equalize the total plant material in each tub and block. In experiments 2 and 3, the same procedure of shoot trimming and regrowth was followed but there was no redistribution of cups between tubs (S. Smith, University of Arizona, personal communication). The total dry biomass was pooled for each tub; analysis of variance was used to compare the values for tubs and blocks. There was no statistically significant difference in the starting total dry aboveground biomass between tubs or blocks in either experiment 2 or 3. It was assumed that these procedures, along with the use of a randomized complete block design, minimized the influence of random differences in potential for growth between individual ramets.

**Experimental design**—In experiment 1, the nutrient solution was changed to the formula used by Rozema et al. (1983) (supplemented with 5 mmol/L NaCl) 3 mo before the start of treatments. During acclimation, 3.5 mmol/L NaNO₃, equivalent to quarter strength Hoagland’s #2 (Hoagland and Arnon, 1950), was used for maintenance of plants. All other nutrients were supplied in amounts similar to half-strength Hoagland’s solution #2 except that N was only supplied as NO₃⁻. For experiments 2 and 3, the nutrient solution was half-strength Hoagland’s solution #2 supplemented with the designated concentration of NaCl.

In experiment 1, three levels of salinity were tested (5, 100, and 300 mmol/L NaCl) as well as three levels of nitrate (0.5, 3.5, and 14.0 mmol/L NaNO₃) (Rozema et al., 1983), giving nine treatment combinations. (Note: Since nitrate was supplied as the sodium salt, the treatment combination with the highest level of nitrate and lowest level of NaCl had 14.0 mmol/L Na⁺.) There were two tubs for each treatment combination and two control tubs harvested at the beginning of treatments. Treatments were started on 25 May 1999, and plants were harvested after 4 wk.

For experiment 2, there were six blocks and six levels of treatments: 5, 50, 100, 150, 300, and 450 mmol/L NaCl. At each of three harvests (the beginning of treatments = control, 4 wk, and 8 wk) two cups were selected at random from each tub. A replicate consisted of the pooled dry biomass of the two cups from each tub for each harvest. At the 8-wk harvest, the above-ground plant material was rinsed with tap water and allowed to dry before harvesting. The roots and rhizomes were rinsed with an isotonic solution of LiCl (to remove surface cations) with 1.0 mmol/L Ca(NO₃)₂ added to maintain cell membrane integrity. The number of tillers per cup and the width of the two widest fully expanded leaves per cup were recorded at each harvest. Treatments were begun on 31 August 1999. *Sporobolus virginicus* bloomed in the fall; the first panicle appeared on 27 September 1999 during experiment 2. As panicles were exerted from the sheath, they were cut off just below the second subtending leaf because when the panicles mature the tiller bearing the panicle senesces (K. Marcum, University of Arizona, personal communication). Panicle dry mass was added to that of the tub from which they were removed.

In experiment 3, there were 12 blocks and three levels of treatment: 5, 125, and 450 mmol/L NaCl. Plants were scored for tiller number and leaf width (as in experiment 2) at the beginning and end of treatments. Treatments were started on 29 March 2000 and plants were harvested after 4 wk. The same procedure of rinsing plants prior to harvest (from experiment 2) was used in experiment 3.

**Cation content and secretion**—In experiments 2 and 3, concentrations of Na⁺, K⁺, Ca²⁺, and Mg²⁺ in leaves, rhizomes, and roots were determined by atomic absorption spectrophotometry (Perkin-Elmer 560, Perkin Elmer, Norwalk, Connecticut, USA). In experiment 2, dry plant samples were finely ground; in experiment 3, whole plant tissues were used. Tissue was digested in nitric acid and diluted to 25 mL with 0.25% La in 2.5% nitric acid prepared with deionized water. This solution was designated as full strength. If required to read within the range of the standards, samples were diluted 10× or 100×. The nitric acid concentration was brought to about 10% to match that of the blank and standards. La was added to the samples to improve the sensitivity of detection for Ca and Mg (Perkin-Elmer, 1976). The readings in parts per million (ppm) were converted to micromoles per gram dry biomass using the...
following formula: parts per million \cdot dilution volume \cdot sample mass\(^{-1}\) \cdot atom-
ic mass\(^{-1}\).

In experiment 3, secretion of Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) was determined by the difference in concentration of those cations in rinsed leaves and leaves that had been rinsed and allowed to secrete for 3 d. Plants were rinsed thoroughly with tap water and allowed to dry for 6 h. (It would have been preferable to rinse with distilled water but that was not available at the Campus Agricultural Center.) Four leaf blades per tub were removed, placed in tared Erlenmeyer flasks, sealed with Parafilm (American National Can, Greenwich, CT, USA), and transported to the laboratory in an ice chest. Fresh and dry biomass of leaves were obtained. The tissue was digested in the Erlenmeyer flasks and processed as in experiment 2. The procedure was repeated 72 h later. Salt crystals were visible on the leaves of plants grown at 125 and 450 mmol/L NaCl. Care was taken not to disturb the leaf surface during harvesting of leaves. The difference in cation concentration between the two samples of leaves was attributed to secretion. The cation concentrations of leaves were expressed both as micromoles per gram dry biomass and as micromoles per gram H\(_2\)O. Water content was determined as \((\text{fresh biomass} - \text{dry biomass})/\text{dry biomass}\) and expressed as gram H\(_2\)O per gram dry biomass.

Osmotic adjustment—About eight rinsed, dry leaves from plants in each tub were packed into 5-mL syringes and quickly frozen at \(-80^\circ\text{C}\). After thawing, the cell sap was expressed by the plunger and collected for measurement. The osmolality of the cell sap was determined by vapor pressure osmometry (Wescor Model 5500, Vapor Pressure Osmometer, Wescor, Logan, Utah, USA). Osmolality was multiplied by 2.48 to give osmotic pressure (Taiz and Zeiger, 1991, pp. 68–69). For monovalent cations, a balancing anion and dissociation factor of 92% were assumed; molality was multiplied by 1.84 to give osmotic pressure (Taiz and Zeiger, 1991, pp. 68±69). For monovalent cations, a balancing anion and dissociation factor of 92% were assumed; molality was multiplied by 1.84 to give osmotic pressure (Taiz and Zeiger, 1991, pp. 68±69). For monovalent cations, a balancing anion and dissociation factor of 92% were assumed; molality was multiplied by 1.84 to give osmotic pressure (Taiz and Zeiger, 1991, pp. 68±69).

Data and statistical analysis—Relative growth rate (RGR) was calculated using the classical method following Chiariello et al. (1989) and expressed as grams per gram per day; \((\ln(\text{final dry biomass}) - \ln(\text{initial dry biomass}))/\text{(final time} - \text{initial time)}\).

The root : shoot ratio was calculated as the ratio of the belowground to aboveground dry biomass. One- and two-way analyses of variance (ANOVA) were performed on the results using SigmaStat (Jandel Scientific, San Rafael, California, USA); in those cases in which data did not meet the requirements for normality and/or equal variances, a Kruskal-Wallis analysis of variance on ranks was performed (Zar, 1984). Differences between means were determined using a Student-Newman-Keuls (S-N-K) multiple comparison test.

RESULTS

Growth—Over the periods observed (4 and 8 wk), growth was significantly greater in plants grown at 100–150 mmol/L NaCl than in plants grown at lower or higher salinities. No effect of nitrate level or interaction between nitrate and NaCl was detected in any measured growth parameter (Table 1).

Because there was no interaction with nitrate, tubs from differing levels of nitrate and the same level of NaCl were pooled and analyzed by one-way ANOVA (Table 1). The RGR for total biomass was 0.05 g \(\cdot g^{-1} \cdot d^{-1}\) at 100 mmol/L NaCl and was significantly greater than RGR for plants grown at 5 mmol/L (0.04 g \(\cdot g^{-1} \cdot d^{-1}\)) and 300 mmol/L (0.04 g \(\cdot g^{-1} \cdot d^{-1}\)). At 300 mmol/L NaCl, belowground biomass increased more rapidly than aboveground biomass, resulting in a significantly higher ratio of below- to aboveground biomass than that found in plants grown at 5 or 100 mmol/L that were not significantly different from one another. Aboveground dry biomass was 15% greater and belowground dry biomass was 25% greater in plants grown at 100 mmol/L than at 5 mmol/L.

In experiment 2, two-way ANOVA for above- and belowground dry masses showed no significant differences in RGR due to blocks or salinity treatments at 0–4 wk (data not shown). However, at 4–8 wk (data not shown) and 0–8 wk, there were highly significant differences in RGR due to levels of NaCl. Aboveground dry biomass of plants grown at 100 and 150 mmol/L NaCl was significantly greater than that of plants grown at 5, 50, and 450 mmol/L NaCl (Table 2). Belowground biomass of plants grown at 50 and 300 mmol/L was significantly greater than that of plants grown at 5 and 450 mmol/L, but could not be distinguished statistically from one another because of the high variance of the data. Growth was highly variable between individuals, possibly due to a short period of acclimation in hydroponics. At 4 wk, 8% of individuals were no larger than the average at the start of treatments, and at 8 wk, 5% were no larger. No effects of blocking or interaction between blocks and and levels of NaCl were observed on growth patterns in experiment 2.

In experiment 2, the ratio of belowground to aboveground biomass was highest at the lowest level of salinity but not statistically different from those at 50, 300, and 450 mmol/L NaCl (Table 2). There was a significant decrease in the ratio from 5 to 100–150 mmol/L.

There was a fourfold increase in above- and belowground biomass between plants grown at 50 mmol/L and those grown at 100 and 150 mmol/L. Although not statistically significant, the difference in dry biomass between plants grown at 300 mmol/L and those grown at 50 mmol/L is striking (Table 2).

The increase in biomass at 100 and 150 mmol/L was associated with increases in leaf width, numbers of tillers, and panicles produced (Table 3). The increase in tiller number was particularly dramatic. At 8 wk, on average, tubs grown at 5 mmol/L NaCl had produced only two more tillers than they had at the start of treatments, while tubs grown at 100 and 150 mmol/L produced 46.7 and 48.7 more tillers than they
TABLE 3. Leaf width (in millimeters), increase in tiller number, and aboveground biomass (means ± SD) in *Sporobolus virginicus* grown at six concentrations of NaCl (in millimoles per liter) for 8 wk. The F and P values from two-way ANOVA are given. Within columns, values followed by different letters are significantly different at P ≤ 0.05 as separated by S-N-K, N = 6.

<table>
<thead>
<tr>
<th>NaCl concentration</th>
<th>RGR</th>
<th>Aboveground biomass</th>
<th>Belowground biomass</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>5</td>
<td>0.02 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>50</td>
<td>0.04 ± 0.02&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>1.89 ± 1.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60 ± 0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92 ± 0.37&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>100</td>
<td>0.07 ± 0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7.94 ± 6.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.39 ± 2.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.78 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>150</td>
<td>0.07 ± 0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>7.66 ± 6.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.26 ± 2.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>300</td>
<td>0.06 ± 0.01&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.48 ± 2.17&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>3.25 ± 0.71&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.97 ± 0.08&lt;sup&gt;AB&lt;/sup&gt;</td>
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<tr>
<td>450</td>
<td>0.04 ± 0.01&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>1.12 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98 ± 0.39&lt;sup&gt;AB&lt;/sup&gt;</td>
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Statistic

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<th>F</th>
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<tr>
<td>P</td>
<td>&lt;0.0001</td>
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 had at the start of treatments. The widest leaves were significantly wider in plants grown at 100, 150, and 300 mmol/L NaCl than those grown at 5, 50, or 450 mmol/L. A total of 10 panicles were produced in tubs grown at 5 mmol/L; a total of 43 panicles was produced in tubs grown at 100 mmol/L. Only two panicles were produced in tubs grown at 450 mmol/L.

Highly significant differences in growth parameters were observed in experiment 3 (Fig. 1). The RGR was highest at 125 mmol/L, and RGR at 450 mmol/L was significantly higher than at 5 mmol/L. All other parameters followed this pattern (Tables 3 and 4). Water content, fresh aboveground biomass, and dry biomass were all highest at 125 mmol/L, then at 450 mmol/L, and lowest at 5 mmol/L. Reflecting the higher water content, fresh aboveground biomass was fivefold higher at 125 mmol/L than at 5 mmol/L. Dry aboveground biomass was fourfold higher at 125 mmol/L than at 5 mmol/L. The fresh aboveground biomass was 1.7 times greater in plants grown at 450 mmol/L than at 5 mmol/L, while belowground biomass was 1.6 times larger (Table 4).

Blocks were a significant factor (P = 0.003) in water content. However, there was no interaction between blocks and levels of NaCl, and blocks were not a significant factor for fresh biomass, dry biomass, or RGR. The blocks that were harvested early in the morning had higher water contents than the blocks that were harvested at midday. The two blocks with the highest water content were those that were located on the east wall of the greenhouse and were the last ones to be exposed to full light conditions.

A similar pattern (as in the previous two experiments) was observed in the ratio of belowground to aboveground dry biomass. The highest ratio was at 5 mmol/L, followed by 450 mmol/L and 125 mmol/L. No effect of blocking or interaction between blocks and levels of NaCl was observed in growth parameters in experiment 3.

The mean of the widest leaves was about 1.5 times greater in plants grown at 125 mmol/L that at 5 mmol/L (Table 3). For plants grown at 450 mmol/L, the mean of the widest leaves was 1.2 times greater than the mean of plants grown at 5 mmol/L. Tiller number increased more than fourfold in plants grown at 125 mmol/L vs. those grown at 5 mmol/L (Table 3).

**Cation content**—The results of cation analysis in experiment 2 were highly similar to the results from experiment 3 except that overall slightly higher concentrations of K<sup>+</sup> were observed in experiment 2. For space and simplicity, we will present only the results from experiment 3. The results from experiment 2 are available in Bell (2000).

The Na<sup>+</sup> content of roots grown at 5 mmol/L was very low and increased almost 10-fold in roots grown at 125 and 450 mmol/L (Table 5). The K<sup>+</sup> content of roots was not significantly different between roots grown at 5 and 450 mmol/L but was significantly higher in roots grown at 125 mmol/L. The Mg<sup>2+</sup> content was lowest in roots grown at 450 mmol/L and was significantly higher in roots grown at 125 and 450 mmol/L. The Ca<sup>2+</sup> content was significantly higher in roots grown at 5 mmol/L than in roots grown at 125 or 450 mmol/L. Total cation content was lowest in roots grown at 5 mmol/L, significantly higher in roots grown at 450 mmol/L, and highest in roots grown at 125 mmol/L. The Na : K ratio of roots was highest in roots grown at 450 mmol/L and significantly lower in roots grown at 5 and 125 mmol/L.

Na<sup>+</sup> content was higher in leaves from plants grown at 450 mmol/L than from those grown at 5 mmol/L (Table 5). K<sup>+</sup> contents slightly increased as NaCl concentrations increased. There were no significant differences in the Mg<sup>2+</sup> content, but...
a highly significant decrease in Ca\(^{2+}\) content. Total cation content was greatest in leaves grown at 450 mmol/L, and the Na : K was 1.17 (not significantly higher than the ratio of leaves grown at 5 and 125 mmol/L). The ratio of Na : K in the nutrient solution increased as follows: in 5 mmol/L, 1.6 : 1; in 125 mmol/L, 41.6 : 1; in 450 mmol/L, 150 : 1. The levels and patterns of cation accumulation observed in rhizomes were highly similar to those of leaves (data not shown).

**Cation secretion**—Salt crystals were visible on leaves of plants grown at 125 and 450 mmol/L NaCl. The most noticeable difference between the cation contents of freshly rinsed leaves and leaves that have secreted salts for 72 h is the large increase in Na\(^{+}\) content (Table 5). Leaves grown at 450 mmol/L secreted Na\(^{+}\) at the rate of 336.7 \(\mu\)mol · g dry biomass\(^{-1}\) · d\(^{-1}\); leaves grown at 125 mmol/L secreted Na\(^{+}\) at the rate of 166.5 \(\mu\)mol · g dry biomass\(^{-1}\) · d\(^{-1}\). Negligible amounts of Na\(^{+}\) were secreted from leaves grown at 5 mmol/L NaCl. Much smaller and highly variable amounts of K\(^{+}\), Mg\(^{2+}\), and Ca\(^{2+}\) were detected. The Na : K ratio of leaves harvested immediately after rinsing was not significantly different from 1 in plants grown at 5, 125, or 450 mmol/L NaCl. However, in leaves that had secreted for 72 h, the Na : K ratio increased significantly as the salinity of the nutrient solution increased.

Two-way ANOVA was performed on the cation contents of leaves harvested soon after rinsing and 72 h later with NaCl concentration and time as the fixed variables (Table 6). NaCl concentration in the nutrient medium and time and the interaction of NaCl and time were highly significant factors in the Na\(^{+}\) content of leaves. Both NaCl concentration and time were significant factors affecting K\(^{+}\) content, but there was no interaction between them. NaCl concentration had a highly significant effect on Ca\(^{2+}\) content and the interaction between NaCl and time was barely significant. There was no effect of NaCl, time, or the interaction of factors on Mg\(^{2+}\) content.

**Water content and osmotic adjustment**—The water content of leaves of *S. virginicus* was highest in plants grown at 125 mmol/L NaCl, followed by the leaves grown at 5 mmol/L and the lowest water content was in leaves grown at 450 mmol/L (Table 7). Leaf water content was lower than that of whole shoots (Table 6). In whole shoots, the highest water content was found in plants grown at 125 mmol/L and the next highest in plants grown at 450 mmol/L. When expressed as molar quantities (in micromoles per gram H\(_2\)O), there were no significant differences in the Na\(^{+}\) and K\(^{+}\) concentrations in leaves grown at 5 and 125 mmol/L, while leaves grown at 450 mmol/L had Na\(^{+}\) and K\(^{+}\) concentrations that were one-third higher (Table 7). The Ca\(^{2+}\) concentration was lowest in plants grown at 125 mmol/L and highest in plants grown at 5 mmol/L. There was not a significant difference between the Mg\(^{2+}\) concentration of leaves grown at 5 and 450 mmol/L but leaves grown at 125 mmol/L had lower Mg\(^{2+}\) concentrations. Total cations were highest in plants grown at 450 mmol/L.

There were no significant differences in the measured osmolality of cell sap from leaves grown at 5 or 125 mmol/L, but leaves grown at 450 mmol/L had osmotic potentials that were 80% higher (Table 8). Contributions of Na\(^{+}\) and K\(^{+}\) to osmolality were 62.7% for plants grown at 5 mmol/L NaCl, 67.5% for plants grown at 125 mmol/L, and 54.2% for plants grown at 450 mmol/L.
**Table 4.** Relative growth rate (RGR; in grams per gram per day), water content of aboveground biomass (in grams H₂O per gram dry biomass), fresh and dry biomass accumulation (in grams), and ratio of below- to aboveground biomass (below : above; means ± SD) in *Sporobolus virginicus* grown at three concentrations (in millimoles per liter) of NaCl for 4 wk. The P values were obtained from two-way ANOVA. Within rows, values followed by different letters are significantly different at P ≤ 0.05 as separated by S-N-K. N = 12.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5</th>
<th>125</th>
<th>450</th>
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<tbody>
<tr>
<td>RGR total</td>
<td>&lt;0.0001</td>
<td>0.04 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Water content</td>
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<td>2.35 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Fresh aboveground biomass</td>
<td>&lt;0.0001</td>
<td>2.28 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.78 ± 1.43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry aboveground biomass</td>
<td>&lt;0.0001</td>
<td>0.88 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.53 ± 0.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry belowground biomass</td>
<td>&lt;0.0001</td>
<td>2.34 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99 ± 0.96&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Below : above</td>
<td>&lt;0.0001</td>
<td>2.79 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.41 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The present study offers the first demonstration of growth stimulation of a grass (Poaceae) by NaCl that qualifies as euhalophytic and that fits the I<sub>1</sub> halophyte classification of Greenway and Muirns (1980). Growth stimulation was similar to that observed with euhalophytic dicotyledons (Flowers et al., 1986); plants of *S. virginicus* grown at 100–150 mmol/L NaCl were visibly larger than those grown at 5 mmol/L. In experiment 3, RGR was 100% greater in plants grown at 125 mmol/L NaCl than in those grown at 5 mmol/L, accumulation of aboveground dry biomass was four times greater, and tiller production was about 23 times greater. These results support the assertion that 100–150 mmol/L NaCl is optimal for growth of *S. virginicus*. Not only can *S. virginicus* survive at NaCl levels of 450 mmol/L, biomass accumulation and tiller production were greater at 450 mmol/L than in plants grown at 5 mmol/L.

Other researchers have not observed growth stimulation by NaCl in *S. virginicus* (Gallagher, 1979; Blits and Gallagher, 1991; Naidoo and Mundree, 1993; Naidoo and Naidoo, 1998), except for the small increase found by Marcum and Murdoch (1992). Marcum and Murdoch (1992) did not measure the entire aboveground increase in biomass from tiller production, but only the growth above 10 cm. Blits and Gallagher (1991) used dune and marsh ecotypes of *S. virginicus* in their study and found significant differences in the response to salinity between the ecotypes. Rhizome biomass increased strikingly in the dune ecotype grown with full seawater.

Reports of growth stimulation by NaCl in halophytic monocotyledons are rare and have usually involved small changes relative to the increases observed for halophytic dicotyledons (Rozema, 1991). For Distichlis palmeri, Glenn (1987) observed a 15% increase in RGR in plants grown at 180 mmol/L NaCl vs. those grown with no NaCl. In that study, 15 species of grasses survived at 540 mmol/L NaCl although in all cases RGR was reduced to 50% or less of RGR with no NaCl. Distichlis spicata (Kemp and Cunningham, 1981), Cynodon dactylon (Ramakrishnan and Nagpal, 1973; Dudeck et al., 1983), Paspalum vaginatum (Dudeck and Peacock, 1993; Marcum and Murdoch, 1994), Spartina alterniflora (Bradley and Morris, 1991), and Stenothaphrum secundatum (Marcum and Murdoch, 1994) are grass species that have been reported to sur-

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**Table 5.** Cation content (in micromoles per gram dry biomass; means ± SD) of roots and leaves (0 and 72 h after rinsing) of *Sporobolus virginicus* grown at three concentrations of NaCl (in millimoles per liter) for 4 wk. The P values from one-way ANOVA are given. Within columns, means followed by different letters are significantly different at P ≤ 0.05 as separated by S-N-K. For interaction of NaCl and time on cation contents of leaves, see Table 6. N = 12.

<table>
<thead>
<tr>
<th>NaCl concentration</th>
<th>Na&lt;sup&gt;a&lt;/sup&gt;</th>
<th>K&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mg&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ca&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Na&lt;sup&gt;b&lt;/sup&gt; : K&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.08 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.87 ± 0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.39 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.21 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.39 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>125</td>
<td>1.41 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99 ± 0.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.58 ± 1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>450</td>
<td>2.87 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.41 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.58 ± 1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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*a* One-way analysis of variance.

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*b* One-way analysis of variance.
vive at high salinities. Greipps and Davy (1996) observed an increase in tiller number as NaCl concentrations increased but a decrease in aboveground biomass in a study of *Leymus arenarius*. Stelzer and Läuchli (1977) report a spike in growth stimulation of *Puccinellia peisonis*; optimal growth occurs at 100 mmol/L NaCl but plants cannot survive at salinities greater than 300 mmol/L NaCl. *Puccinellia peisonis* is a C₃ grass lacking salt glands. Similar results have been found for *P. maritima* (Munns et al., 1983). Interestingly, in these experiments, as in Kemp and Cunningham (1981) where no growth stimulation of *Distichlis spicata* by NaCl was observed, the optimal growth of these grasses occurred in nonaerated hydronomic culture. Hester et al. (1996) observed wide intraspecific variation in salt tolerance among clones of *Spartina patens*. Macke and Ungar (1971) observed optimal growth of *Puccinella nuttalliana* seedlings in 100 mmol/L NaSO₄; growth was greatly reduced by 350 mmol/L and no plants survived at 550 mmol/L. *Halopyrum mucronatum* showed a similar pattern of growth (Khan et al., 1999); after 90 d, seedlings showed a maximum RGR at 90 mmol/L NaCl. However, no plants survived at 360 mmol/L. There were genotypic, ecotypic, and developmental stage differences in the actual plant material used in these studies as well as differences in experimental protocols that may, in part, account for the differences in growth responses observed.

There are reports of growth stimulation by NaCl in two other monocotyledons. Bourn (1935) reported that *Ruppia maritima* (Potamogetonaceae), an aquatic species found in salt marshes of temperate zones, required about 10% seawater for optimal growth and “appeared quite healthy” at 150% seawater. Growth in seawater positively correlated with increased production and retention of leaves of *Triglochin maritima* (Juncaginaceae) (Rudmik, 1983). The cation content and Na : K ratio of fully expanded young leaves of *T. maritima* is similar to that of the halophytic grasses; older leaves with high Na⁺ contents are shed.

Allocation of biomass to plant organs forms characteristic patterns in different species. These patterns are subject to perturbations by environmental conditions (Brouwer, 1983). Higher allocations to roots and belowground structures are symptomatic of nonoptimal nutritional conditions in the root environment and of preparation for dormancy (i.e., storage of carbohydrates). The pattern of allocation observed in these three experiments showed that the lowest ratio of belowground : aboveground biomass was found in plants grown at 100–150 mmol/L NaCl.

In contrast to the results of Rozema et al. (1983), no significant effect of nitrate levels or interaction between nitrate and NaCl was observed in this study. *Sporobolus virginicus* may have a lower requirement for nitrate than the three dicotyledons studied by Rozema et al. (1983). Smart and Barko (1980) found that growth of *Distichlis spicata* and *Spartina alterniflora* were nitrogen limited in freshwater, brackish, and marine sediment culture. Limitations in nitrogen stimulated root growth in *S. alterniflora* but not in *D. spicata*. Both species showed the greatest biomass accumulation on the fresh-water sediments. Field studies by Gallagher (1979) did not find that pulses of NH₄NO₃ influenced the growth of *S. virginicus* in saline soil. However, both Marcum and Murdoch (1992) and Naaido and Naaido (1998) observed a high production of compatible solutes (which would require N) in *S. virginicus*. It is possible that the large volume of nutrient solution in the present study (10 L) and weekly changing of solutions was sufficient to meet the nitrogen needs of *S. virginicus*.

When expressed in terms of dry biomass, the cation contents of halophytic dicotyledons were higher than those observed for *S. virginicus*. In *Salicornia bigelovii* grown at optimal salinity of 200 mmol/L NaCl, Pfister (1999) found over 3000 μmol Na⁺/g dry biomass. These results are consistent with other studies on halophytic dicotyledons (Gorham et al., 1980; Glenn and O’Leary, 1984). However, when expressed in terms of water content, the differences between halophytic dicotyledons and monocotyledons are not as great. The more significant difference appears to be in the relative proportions of Na⁺ and K⁺. Among dicotyledons, the cation contribution to osmolality comes primarily from Na⁺ (Glenn and O’Leary, 1984), while in the present study, Na⁺ and K⁺ made roughly equivalent contributions to osmolality.

Pfister (1999) observed a Na : K ratio of 10 in shoots of *Salicornia bigelovii* grown at optimal salinity; in contrast the present study found a Na : K ratio of about 1 in leaves of *S. virginicus* grown at optimal salinity. The Na : K ratio in roots grown at optimal salinity was 1.6 to 2.5. Sodium increased dramatically in leaves that secreted for 3 d. These observations taken together suggest that Na⁺ is actively secreted from leaves and retained in roots. Further, it appears that there is

### Table 6. Effects of NaCl and time on the secretion of cations by leaves of *Sporobolus virginicus* grown at three concentrations of NaCl for 4 wk. The *P* values from two-way ANOVA are given. *N* = 12.

<table>
<thead>
<tr>
<th>Cation</th>
<th>NaCl</th>
<th>Time</th>
<th>NaCl × time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>K</td>
<td>0.004</td>
<td>0.01</td>
<td>0.38</td>
</tr>
<tr>
<td>Mg</td>
<td>0.92</td>
<td>0.83</td>
<td>0.89</td>
</tr>
<tr>
<td>Ca</td>
<td>&lt;0.0001</td>
<td>0.55</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### Table 7. Water content (in grams per gram dry biomass) and cation content (in micromoles per gram; means ± SD) of leaves of *Sporobolus virginicus* grown at three concentrations of NaCl (in millimoles per liter) for 4 wk. Within columns, values followed by different letters are significantly different at *P* ≤ 0.05 as separated by S-N-K. *N* = 12.

<table>
<thead>
<tr>
<th>NaCl concentration</th>
<th>Water content*</th>
<th>Cations</th>
<th>Total cations*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
<td>Mg⁺</td>
</tr>
<tr>
<td>5</td>
<td>1.46 ± 0.15</td>
<td>174.0 ± 33.1</td>
<td>193.5 ± 43.0</td>
</tr>
<tr>
<td>25</td>
<td>1.72 ± 0.13</td>
<td>183.2 ± 34.6</td>
<td>190.8 ± 27.5</td>
</tr>
<tr>
<td>450</td>
<td>1.26 ± 0.11</td>
<td>310.5 ± 43.8</td>
<td>285.1 ± 131.9</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

*One-way analysis of variance.

*Kruskal-Wallis one-way analysis of variance on ranks.
discrimination in the transport of K\(^+\) from roots to leaves and rhizomes.

Results of the present study regarding cation content in tissues of \textit{S. virginicus} are in general agreement with those made by Marcum and Murdoch (1992) and Naidoo and Naidoo (1998) and with studies of other halophytic grasses (Stelzer and Läuchli, 1977; Gorham et al., 1980; Miyamoto et al., 1996; Ramadan, 2001). Secretion of Na\(^+\) from leaves of \textit{S. virginicus} increased from 166.5 to 336.7 \textmu mol \cdot g dry biomass\(^{-1} \cdot d^{-1}\) as the NaCl concentration in the nutrient solution increased from 125 to 450 mmol/L. In contrast, secretion of K\(^+\) was about 10 times lower than Na\(^+\) and decreased in a nonsignificant manner. The patterns for secretion observed by Naidoo and Naidoo (1998) were similar to the present study; however, the results of Marcum and Murdoch (1992) differ from both. Marcum and Murdoch (1992) observed slightly higher levels of secretion for both Na\(^+\) and K\(^+\).

As a percentage of fresh biomass, water content of tillers of \textit{S. virginicus} ranged from 61.7\% at 5 mmol/L to 69.8\% at 125 mmol/L and 63.3\% at 450 mmol/L. These figures are at the low end of the normal range for grasses (65–85\%) (Tiku and Snaydon, 1971; Howard and Mendelsson, 1999) and considerably lower than those of succulent halophytic dicotyledons (Glenn and O’Leary, 1984). Pfister (1999) observed water contents of 84\% for \textit{S. bigelovii} grown at 5 mmol/L NaCl and 90\% when grown at 200 mmol/L. The water content of whole tillers of \textit{S. virginicus} was slightly higher than that of leaves alone.

When expressed in terms of \textmu mol/g H\(_2\)O, there was no significant difference between the Na\(^+\) or K\(^+\) concentrations in leaves of \textit{S. virginicus} grown at 5 or 125 mmol/L NaCl. It is possible that the increase in water content serves as a mechanism to dilute Na\(^+\). The Na\(^+\) concentration in leaves grown at 450 mmol/L was 1.7 times higher and the K\(^+\) concentration was 1.5 times higher. The absolute amounts of Na\(^+\) and K\(^+\) were higher in plants grown at 450 mmol/L. When expressed as the Na\(^+\) and K\(^+\) contributions to osmolality, in plants grown at 5 mmol/L, the contribution was 62.7\%; at 125 mmol/L, 67.5\%; and at 450 mmol/L, 54.2\%. Marcum and Murdoch (1992) estimated the contributions of Na\(^+\) and K\(^+\) to mobility to be between 40 and 45\%.

The structure of the \textit{C}_\text{i} leaves and the relatively rigid cell wall (Esau, 1977) of grasses limits the ability of halophytic grasses to change cell volumes in response to salinity. When grown at optimal salinity, \textit{S. virginicus} increases its RGR and tillering. Within the time frame of these experiments, the water content of leaves increases by 18\% and that of shoots by 46\% when grown at optimal salinity. The increase in water content can be attributed to an increase in actively growing young tissues. \textit{Sporobolus virginicus} appears to regulate cation uptake and retention; K\(^+\) is selectively taken up and retained in leaves, while Na\(^+\) is retained in roots and secreted from leaves. Both Na\(^+\) and K\(^+\) contribute to osmotic adjustment. Secretion of Na\(^+\) allows \textit{S. virginicus} to maintain transpiration while avoiding toxic buildup of Na\(^+\). \textit{Sporobolus virginicus} is a euhalophytic grass that does not accumulate Na\(^+\) in response to salinity.

**LITERATURE CITED**


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