Interactive effects of salinity, nitrogen and sulphur on the organic solutes in Spartina alterniflora leaf blades

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Abstract
Glycinebetaine, proline, asparagine, sucrose, glucose, and dimethylsulphoniopropionate (DMSP) were the major organic solutes in Spartina alterniflora leaf blades. To investigate the physiological role(s) of these solutes, the effects of salinity, nitrogen, and sulphur treatments on leaf blade solute levels were examined. Glycinebetaine was the major organic solute accumulated in leaf blades grown at 500 mol m⁻³ NaCl, although asparagine and proline also accumulated when the supply of nitrogen was sufficient. These solutes may play a role in osmotic adjustment. In contrast, DMSP levels either did not change or were reduced in response to the 500 mol m⁻³ NaCl treatment. Furthermore, elevated nitrogen supply decreased leaf blade DMSP levels, which was opposite to the response of glycinebetaine, proline, and asparagine. A 1000-fold increase in external sulphate concentration had no effect on the leaf blade levels of DMSP, glycinebetaine, proline, or asparagine. These findings suggest that the major physiological role of DMSP in S. alterniflora is not for osmotic adjustment, even under conditions of nitrogen deficit and excess sulphur. Instead, DMSP which was present at 45-130 μmol g⁻¹ dry weight, may play a role as a constitutive organic osmoticum.

Key words: Spartina alterniflora, dimethylsulphoniopropionate, glycinebetaine, nitrogen, salinity.

Introduction
Spartina alterniflora is a dominant plant in salt marshes on the east coast of North America (Wiegert et al., 1981), and has recently become well established in some west coast areas (Callaway and Josselyn, 1992). The salinity tolerance of S. alterniflora is presumably related to the regulation of its internal ion composition (Bradley and Morris, 1991), and the accumulation of organic solutes such as glycinebetaine, proline (Cavalieri and Huang, 1981; Cavalieri, 1983), and dimethylsulphoniopropionate (DMSP) (Dacey et al., 1987). However, while glycinebetaine and proline may function in the osmotic adjustment of many vascular halophytes (Rhodes and Hanson, 1993; Stewart and Lee, 1974), the physiological role of DMSP is less clear.

DMSP accumulation plays a role in the osmotic adjustment of some marine algae, as evidenced by an increase in intracellular DMSP levels in response to high external salinity (Dickson et al., 1980; Dickson and Kirst, 1986, 1987a, b). In addition, the growth of bacteria exposed to high NaCl concentrations was enhanced by adding 5 μM DMSP to the culture medium, presumably because its intracellular accumulation (up to 1.4 M) plays a role in osmotic adjustment (Paquet et al., 1994). The accumulation of DMSP in the non-vacuolate bacteria is evidence that a high concentration of DMSP is compatible with cytoplasmic processes (Paquet et al., 1994). Although a similar role for DMSP in osmotic adjustment has been suggested for some vascular plants (Dacey et al., 1987; Rhodes and Hanson, 1993; Storey et al., 1993), this hypothesis has been directly tested in only a few studies. High concentrations of NaCl had no effect on DMSP levels in the shoots of S. anglica (van Diggelen et al., 1986), and in the leaves of Melanthera biflora only modest increases (dry wt. basis) were observed (Storey et al., 1993).

An understanding of factors regulating DMSP levels
in *S. alterniflora* would improve our knowledge of osmotic adjustment in halophytes, and is critical for understanding the biogeochemical cycling of sulphur. DMSP is a precursor of dimethylsulphide (DMS) gas and *S. alterniflora*-dominated salt marshes are a major terrestrial source of DMS (Dacey *et al.*, 1987). Therefore, the major objective of the present study was to determine whether DMSP plays a role in the osmotic adjustment of *Spartina alterniflora* leaf blades under defined NaCl salinity, nitrogen, and sulphur regimes. The nitrogen (NO$_3^-$ and NH$_4^+$) and sulphur (SO$_4^{2-}$) treatments were included in this study because they are important environmental variables in coastal marshes, and their supply can affect the DMSP levels in some vascular halophytes (Dacey *et al.*, 1987; Storey *et al.*, 1993; Otte and Morris, 1994) and marine algae (Grone and Kirst, 1992).

**Materials and methods**

*Nitrogen response curve at low and high NaCl concentrations*

*Spartina alterniflora* cuttings (0.25 m tall) were taken from near the San Bruno Slough in the South San Francisco Bay, CA, USA, on 16 September 1993 (Callaway and Josselyn, 1992). Cuttings were washed free of sediment before transplanting into 5.8 kg of silica sand in plastic pots. Each pot was flooded to the sand surface by submerging it in a bucket containing 12 l of 0.1 concentration modified Hoagland solution (pH 5.7) (Epstein, 1972) supplemented with 1 mol m$^{-3}$ NaCl and 5.0 mol m$^{-3}$ CaCl$_2$. The plants were maintained in this solution for 24 d (solutions were renewed every 7 d) to allow recovery from transplanting, during which time there was new shoot and root growth. The greenhouse temperature was 25.0 ± 0.4 °C/19.9 ± 0.2 °C day/night and the midday photon flux density was about 1100 μmol m$^{-2}$ s$^{-1}$.

All nutrient solutions used during the treatments contained in mol m$^{-3}$: K$^+$, 7.5; Ca$^{2+}$, 5.0; Mg$^{2+}$, 5.0; Na$^+$, 1.0; H$_2$PO$_4^-$, 0.2; SO$_4^{2-}$, 10.0; Fe-EDTA, 0.050; Cl$^-$, 8.3; and 0.25 concentration Hoagland micronutrients (Epstein, 1972). Two NaCl treatments were imposed by maintaining plants at 1 mol m$^{-3}$ or by increasing the NaCl concentration to 500 mol m$^{-3}$ in 100 mol m$^{-3}$ d$^{-1}$ increments. Nitrogen treatments (7.1 molar ratio of NO$_3^-$:NH$_4^+$) were 0, 0.1, 0.5, 1.0, 5.0, and 10.0 mol m$^{-3}$ total concentration of NO$_3^-$ plus NH$_4^+$ supplied as a combination of NH$_4$NO$_3$, KNO$_3$, and NaNO$_3$. The K$^+$ concentration in all solutions was kept constant at 7.5 mol m$^{-3}$ by decreasing the amount of KCl used as more KNO$_3$ was supplied. There were 3 replicates of each treatment and solutions were renewed every 7 d. Plants were harvested after 56 d of exposure to the treatments.

*Interactive effects of sulphate, nitrogen and NaCl-salinity*

Seeds of *Spartina alterniflora* (Environmental Concern Inc., St Michaels, MD, USA) were germinated on a screen over 0.1 concentration Hoagland solution (pH 5.7) (Epstein, 1972), supplemented with 1 mol m$^{-3}$ NaCl and 5.0 mol m$^{-3}$ CaCl$_2$. After 7 d, seedlings were planted into 1.3 kg of silica sand in plastic pots flooded to their surface with the same solution (2.1 l per pot, renewed every 7 d). Thirty-five days after imbibition, sulphate and nitrogen treatments were imposed in the same base solution as described above for the nitrogen dose–response experiment. However, SO$_4^{2-}$ concentrations for the low and high treatments were 0.1 mol m$^{-3}$ (4.9 mol m$^{-3}$ of Mg$^{2+}$ was supplied as Cl$^-$ salt) and 100 mol m$^{-3}$ (200 mol m$^{-3}$ of NaCl was replaced by 100 mol m$^{-3}$ Na$_2$SO$_4$), respectively. The low and high SO$_4^{2-}$ treated plants were salinized with 100 mol m$^{-3}$ NaCl d$^{-1}$ or 60 mol m$^{-3}$ NaCl plus 20 mol m$^{-3}$ Na$_2$SO$_4$ d$^{-1}$, respectively, to a final Na$^+$ concentration of 500 mol m$^{-3}$. Plants in each SO$_4^{2-}$ treatment were supplied with either 0.5 or 10.0 mol m$^{-3}$ final concentration of NO$_3^-$ plus NH$_4^+$ (7:1 molar ratio of NO$_3^-$:NH$_4^+$). There were 3 replicates of each treatment and solutions were renewed every 5 d. Plants were harvested after 28 d of exposure to the treatments.

*Harvests*

Shoots were rinsed for 30 s in deionized water, after which the leaves were excised, blotted to remove surface water, weighed, frozen in liquid N$_2$, and lyophilized. The dry weights of the lyophylized tissues were then recorded.

*Field sampling*

*S. alterniflora* leaf blades were collected from near the San Bruno Slough, South San Francisco Bay, California (Callaway and Josselyn, 1992), for subsequent analysis of solutes. Leaf blades were taken from older plants (0.8–0.95 m tall) near the centre, and younger plants (0.35–0.50 m tall) at the edge of the same clonal stand at afternoon low tide on 15 November 1993 ('autumn') and 22 May 1994 ('spring'). Stems were excised 0.05 m above the sediment surface, shoots were washed three times (10 s each wash) in deionized water, leaf blades were excised, blotted to remove surface water, frozen in liquid N$_2$, transported to the laboratory on dry ice, and lyophilized. Five replicates of each plant type were taken for each season.

Sediment samples (0–0.05 m depth) were also taken from near the *S. alterniflora* stand using 'plug samplers' made from 0.051 polyethylene syringes. Five replicate samples of sediments were sealed in syringes with a polyethylene stopper at each end to prevent gas exchange. Sediments were stored at 4 °C for 12 h, prior to collecting the 'pore water' by centrifuging at 15,000 rpm for 60 min. The pore water (supernatant) was passed through a 0.22 μm filter and then acidified to pH 2–2.5 with concentrated HCl. Concentrations of various ions in the pore water were determined as follows: Na$^+$ and K$^+$ by atomic emission flame photometry; SO$_4^{2-}$ by the anion exchange resin/inductively coupled plasma spectroscopy method of Littlefield *et al.* (1990); NO$_3^-$ and NH$_4^+$ by the diffusion/conductivity method of Carlson *et al.* (1990).

*Analysis of leaf blade inorganic ions*

Lyophilized leaf tissues were pulverized to 1–3 μm particles with an agate ball in a teflon chamber using a Braun Mikro-Dismembrator II (Melsungen, Germany). Na$^+$ and K$^+$ were extracted from 100 mg of leaf tissue by shaking in 0.015 l of 500 mol m$^{-3}$ HCl for 2 d (Hunt, 1982). Atomic emission flame photometry was used to measure K$^+$ and Na$^+$ (Model IL157, Instrumentation Laboratory Inc., Wilmington, DE, USA).

SO$_4^{2-}$ was extracted from 100 mg of leaf tissue by shaking in 0.015 l of 2% acetic acid for 60 min (modified from Littlefield *et al.*, 1990). The extract was passed through a 0.22 μm Millipore filter prior to analysis for SO$_4^{2-}$ using an ion chromatograph equipped with an Omnipac PAX-500 analytical column with guard, and a Pulsed Electrochemical Detector set to the conductivity mode ( Dionex Corp., CA, USA). The eluant flow rate was at 0.001 l min$^{-1}$ (1 mol m$^{-3}$ NaOH: 100 mol m$^{-3}$ NaOH: 5% methanol in the volume ratio 55:40:5).
SO\(_4^{2-}\) was quantified by comparing the peak areas from sample extracts with those of standards.

**Perchloric acid extraction of organic solutes**

The concentrations of several organic solutes, including glycinebetaine, amino acids, glucose, and sucrose, in perchloric acid extracts of leaf tissue powders were quantified using the combined gas/liquid chromatography and \(^1\)H NMR spectroscopy procedure described by Fan et al. (1993).

Briefly described, 100 mg of leaf tissue was extracted twice with 0.003 l of 3% (w/v) perchloric acid, at 4 °C. Extracts were titrated to pH 3.25±0.05 with K\(_2\)CO\(_3\) to precipitate the perchlorate as KClO\(_4\) and centrifuged at 15 000 rpm, after which 200 \(\mu\)l aliquots of the supernatants were put into GC vials and lyophilized. The remainder of each extract was prepared for \(^1\)H NMR analysis by titration to pH 7.25±0.05 with KOH, passage through 3–4 g of Chelex 100 resin (200–400 mesh, Bio-Rad, Richmond, CA, USA) to remove paramagnetic ions, and then lyophilization.

**Gas/liquid chromatography**

Gas/liquid chromatography was used to measure the concentrations of amino acids in the extracts after derivatization with 400 \(\mu\)l of \(N\)-methyl-(\(\tau\)-tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA):acetonitrile (1:1, v/v), and sonication at 60 °C for 3 h. A Varian 3300 gas chromatograph (Varian Instruments, Palo Alto, CA, USA) with flame ionization detector (FID) and DB-1 open tubular column of 0.18 mm i.d. x 40 m length (J&W Scientific, Folsom, CA, USA) was used. The injector, column, and FID temperature programmes were as described by Fan et al. (1993). FID signals were acquired and processed using Peaksimple III software (SRI Instruments, Torrance, CA, USA). Amino acid concentrations were quantified by comparing peak areas from the sample extracts with those of standards.

**\(^1\)H NMR spectroscopy**

\(^1\)H NMR spectroscopy was used to determine the concentrations of glycinebetaine, glucose, and sucrose extracts redissolved in 700 \(\mu\)l of \(\text{D}_2\)O. One-dimensional \(^1\)H NMR spectra were acquired at 7 T on a General Electric QE-300 spectrometer, at 25 °C. Acquisition parameters included a 90° pulse with gated solvent suppression, a 1.0 s interpulse delay, a 1.36 s acquisition time, a 3003 Hz spectral width, 8192 sampling points, and 128 passes. The data were zero-filled to 16 384 points and apodized using a line broadening of 1 Hz prior to Fourier transformation. The spectral assignment of the various components and calculations of glycinebetaine, glucose, and sucrose concentrations were as described by Fan et al. (1993).

**Analysis of leaf blade DMSP**

DMSP in leaf tissues was measured using a modification of the procedure by White (1982). Three mg of lyophilized leaf powder was incubated with 100 \(\mu\)l of 1 N NaOH for 2 h in a gas tight 0.002 l vial at room temperature, to decompose the DMSP to dimethylsulphide (DMS). Vials were warmed to 60 °C for 2 min immediately before DMS in the vial headspace was analysed using a gas chromatograph (SRI Instruments, Torrance, CA, USA) with direct injection into a 0.53 mm i.d. x 30 m GSQ (a divinylbenzene homopolymer) open tubular column (J&W Scientific, Folsom, CA, USA) at 200 °C, with \(\text{H}_2\) as the carrier gas, and a FID. Leaf DMSP levels were quantified by comparing the DMS released from leaf powders with that from authentic DMSP standards (Research Plus, NJ, USA). The assay recovered 101±2% of DMSB added to leaf powder, and did not cause detectable release of DMS from S-methylmethionine in rice leaf powders.

**Statistical analysis**

Treatment comparisons of growth and tissue solute data were examined statistically by analysis of variance for one- or two-factor designs. Means were compared using least significant differences.

**Results**

**Effects of salinity, nitrogen and sulphate on leaf growth**

Exposure to 500 mol m\(^{-3}\) NaCl reduced the leaf growth of field transplanted \(S.\ alterniflora\) (Fig. 1; \(P<0.05\)). Additional nitrogen stimulated leaf growth of plants exposed to low or high salinity. The experiment with plants raised from seed confirmed that high nitrogen increased leaf growth, and plants exposed to 0.1 or 100 mol m\(^{-3}\) sulphate had a similar response (data not shown).

**Effects of salinity, nitrogen and sulphate on leaf blade ion relations**

The Na\(^+\) levels in leaf blades of \(S.\ alterniflora\) grown at 500 mol m\(^{-3}\) NaCl were 10-fold higher (Fig. 2A), but the K\(^+\) levels were only about 50% (Fig. 2B) of the values in leaf blades grown at 1 mol m\(^{-3}\) NaCl. Increasing the nitrogen supply reduced the Na\(^+\) levels in leaf blades of plants grown at 500 mol m\(^{-3}\) NaCl by as much as 200 \(\mu\)mol g\(^{-1}\) dry wt., but did not affect Na\(^+\) levels in plants grown at 1 mol m\(^{-3}\) NaCl (Fig. 2A). Increasing the nitrogen supply also resulted in slightly higher levels of K\(^+\) in leaf blades of plants grown at both concentrations of NaCl (Fig. 2B).

Plants raised from seed had similar leaf blade Na\(^+\) and

**Fig. 1.** Growth response of \(S.\ alterniflora\) leaf blades to nitrogen supply, for plants exposed to 1 or 500 mol m\(^{-3}\) NaCl. Cuttings were transplanted into a flooded non-saline sand culture for 24 d, after which the nitrogen and NaCl treatments were imposed for 56 d. Leaf blade dry wt. (g plant\(^{-1}\)) at the start of the treatments was 0.78±0.02. Fresh to dry wt. ratios were 3.43:1 and 2.97:1 for leaf blades grown at 1 and 500 mol m\(^{-3}\) NaCl, respectively. Values given are means of 3 replicates with standard errors. Symbols: (–O–) 1 mol m\(^{-3}\) NaCl; (–●–) 500 mol m\(^{-3}\) NaCl.
Effects of salinity, nitrogen and sulphate on leaf blade organic solutes

Glycinebetaine accumulated in response to the 500 mol m⁻³ NaCl treatment for plants under all nitrogen regimes, but proline and asparagine levels were only elevated when the nitrogen supply was greater than 0.1 mol m⁻³ (Fig. 3A, B, C; P < 0.05). The 500 mol m⁻³ NaCl treatment also increased the levels of glucose from 2–5 μmol g⁻¹ dry wt. up to 13–15 μmol g⁻¹ dry wt., and sucrose from 10–40 μmol g⁻¹ dry wt. up to 60–110 μmol g⁻¹ dry wt. in the leaf blades, for plants under all nitrogen regimes (data not shown). In contrast, the levels of dimethylsulphonopropionate (DMSP) in leaf blades of plants grown at 500 mol m⁻³ NaCl were the same as, or even lower than those in plants grown at 1 mol m⁻³ NaCl, depending on the nitrogen supply (Fig. 3D). More specifically, the high NaCl treatment reduced DMSP levels in plants with 1.0 mol m⁻³ or less, of nitrogen (Fig. 3D; P < 0.05).

Glycinebetaine, proline, and asparagine levels in leaf blades grown at 500 mol m⁻³ NaCl were generally increased by a higher nitrogen supply (Fig. 3A, B, C). The glycinebetaine and proline responses, however, levelled off between 1 and 5 mol m⁻³ nitrogen, while asparagine levels continued to rise as nitrogen was increased to 10 mol m⁻³. For leaf blades grown at 1 mol m⁻³ NaCl, the levels of glycinebetaine (Fig. 3A) and asparagine (Fig. 3C) also showed a positive response to nitrogen (peaking at 5 mol m⁻³ nitrogen), while those of proline did not (Fig. 3B). In contrast to the nitrogen-containing solutes, leaf blade DMSP levels were reduced

Table 1. Levels of selected solutes in Spartina alterniflora leaf blades grown at 500 mol m⁻³ NaCl with low or high sulphate and nitrogen

<table>
<thead>
<tr>
<th>Solutes (μmol g⁻¹ dry wt.)</th>
<th>Sulphate treatments (mol m⁻³)</th>
<th>0.1</th>
<th>0.1</th>
<th>100</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nitrogen treatments (mol m⁻³)</td>
<td>0.5</td>
<td>10</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>Na⁺</td>
<td></td>
<td>1033±50</td>
<td>996±95</td>
<td>1095±41</td>
<td>1105±35</td>
</tr>
<tr>
<td>K⁺</td>
<td></td>
<td>555±43</td>
<td>599±32</td>
<td>505±25</td>
<td>552±6</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td></td>
<td>29±3 ± a</td>
<td>35±3 ± a</td>
<td>116±13</td>
<td>138±10</td>
</tr>
<tr>
<td>Glycinebetaine</td>
<td></td>
<td>195±26c</td>
<td>331±16d</td>
<td>206±27c</td>
<td>381±39d</td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td>21±2 ± e</td>
<td>78±2 ± f</td>
<td>15±3 ± e</td>
<td>74±4 ± f</td>
</tr>
<tr>
<td>Asparagine</td>
<td></td>
<td>12±3 ± h</td>
<td>60±1 ± j</td>
<td>8±3 ± h</td>
<td>94±13±i</td>
</tr>
<tr>
<td>DMSP</td>
<td></td>
<td>85±5 ± i</td>
<td>54±2 ± k</td>
<td>83±2 ± i</td>
<td>50±6 ± k</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>110±20</td>
<td>103±13</td>
<td>109±6</td>
<td>81±7</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>17±2</td>
<td>21±5</td>
<td>19±1</td>
<td>22±2</td>
</tr>
</tbody>
</table>

Fig. 3. Response of S. alterniflora leaf blade (A) glycinebetaine, (B) proline, (C) asparagine, and (D) DMSP levels to nitrogen supply, for plants exposed to 1 or 500 mol m⁻³ NaCl. Plants were grown as described in Fig. 1. Leaf blade organic solute levels (μmol g⁻¹ dry wt.) at the start of the treatments were: glycinebetaine, 264.7±31.2; proline, 2.4±0.2; asparagine, 27.5±5.9; and DMSP, 109.9±13.3. Values given are means of 3 replicates with standard errors. Symbols: (-O-) 1 mol m⁻³ NaCl; (-●-) 500 mol m⁻³ NaCl.
by increasing the supply of nitrogen to plants grown at either 1 or 500 mol m\(^{-3}\) NaCl (Fig. 3D).

For plants raised from seed and grown at 500 mol m\(^{-3}\) Na\(^+\), a 1000-fold increase in external sulphate concentration had no effect on the levels of DMSP or the other organic solutes (Table 1). However, an increase in nitrogen supply from 0.5 to 10 mol m\(^{-3}\) reduced the levels of DMSP by about 50%, but increased those of glycinebetaine by 70–80%, of proline by 2.7–3.9 times, and of asparagine by 4.9–12.4 times (Table 1; \(P<0.05\)). These effects of nitrogen on organic solute levels in the seedlings were similar to those observed for the plants derived from cuttings (Table 1; Fig. 3).

Selected solutes in S. alterniflora leaf blades collected from the South San Francisco Bay

Na\(^+\) was the major solute in leaf blades of S. alterniflora collected from the field (Table 2). K\(^+\) was also a major solute, although K\(^+\) levels were only 19–43% of the values for Na\(^+\). Glycinebetaine was a major organic solute in all leaf blades and, in some instances, it was present at a level similar to that of K\(^+\) (Table 2). Sucrose was also present in high amounts and during spring its level was similar to that of glycinebetaine. DMSP levels were 25–39% of the values for glycinebetaine, while those of proline were only 9–24% of the values for glycinebetaine. Asparagine levels were high only in the younger plants from the autumn sampling.

Comparison of the solutes in the youngest and oldest leaf blades of field-grown S. alterniflora showed that the youngest leaf blade contained higher levels of K\(^+\), glycinebetaine, glucose, proline, asparagine, and DMSP, but lower Na\(^+\) and sucrose (Table 3; \(P<0.05\)). These results indicate that organic solutes, together with K\(^+\), may play particularly important roles as osmotica in the youngest leaf blade.

Discussion

Glycinebetaine, proline, DMSP, sucrose, glucose, and asparagine were present at high levels in Spartina alterniflora leaves collected from different marshes along the east (Cavalieri and Huang, 1981; Dacey et al., 1987; Otte and Morris, 1994) and west coasts (Tables 2, 3) of North America. Except for DMSP, the levels of these solutes increased in response to high NaCl treatments in our greenhouse experiments (Fig. 3), a finding consistent with their putative role in osmotic adjustment.

The high NaCl treatment did not increase DMSP levels, suggesting DMSP was not involved in the osmotic adjustment of S. alterniflora leaf blades. This finding is consistent with studies of DMSP in S. anglica (van Diggelen et al., 1986) and in S. alterniflora in the field (Otte and Morris, 1994), both of which found no relationship between leaf DMSP and root zone salinity levels. These findings differ from a previous conclusion (Dacey et al., 1987) that DMSP was involved in the osmotic adjustment of S. alterniflora leaves. This conclusion was derived from a positive correlation between leaf DMSP and soil salinity levels at field sites (Dacey et al., 1987). The correlation, however, was low and it is possible that factors other than salinity, for example, nitrogen which strongly affects DMSP levels (Fig. 3D; Dacey et al., 1987; Otte and Morris, 1994) may have contributed to the variations among field sites.

The high levels of DMSP in S. alterniflora leaf blades may function as a constitutive organic osmoticum. Information regarding the cellular compartmentation of DMSP is required to elucidate its possible role in this regard. Constitutive osmotica should be of adaptive value to organisms that inhabit saline environments. Nevertheless, since marsh salinity can vary both spatially and temporally (Webb, 1983), organic solutes (e.g. glycinebetaine) whose levels can respond to changes in salinity would be required for osmotic adjustment.

The solute composition of leaf blades can vary greatly depending on their age or position on the main stem (Colmer et al., 1995). Comparison of solute levels in the oldest and youngest leaf blades of S. alterniflora showed that K\(^+\), glycinebetaine, proline, asparagine, and glucose may play important roles as osmotica in the youngest leaf, while Na\(^+\) and sucrose may serve this role in the oldest leaf (Table 3). In contrast, leaf tissue differences for DMSP level were far less marked than those of the

### Table 2. Levels of selected solutes in Spartina alterniflora leaf blades collected from the South San Francisco Bay during two seasons

<table>
<thead>
<tr>
<th>Solutes (µmol g(^{-1}) dry wt.)</th>
<th>Season and plant category</th>
<th>Younger</th>
<th>Older</th>
<th>Younger</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autumn 1993</td>
<td>942±38</td>
<td>831±19</td>
<td>1075±21</td>
<td>1242±27</td>
</tr>
<tr>
<td>Na(^+)</td>
<td></td>
<td>406±17</td>
<td>231±21</td>
<td>206±10</td>
<td>292±19</td>
</tr>
<tr>
<td>K(^+)</td>
<td></td>
<td>264±20</td>
<td>179±12</td>
<td>237±8</td>
<td>179±14</td>
</tr>
<tr>
<td>Glycinebetaine</td>
<td></td>
<td>37±6</td>
<td>19±4</td>
<td>28±6</td>
<td>15±4</td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td>48±8</td>
<td>1±0.2</td>
<td>3±1</td>
<td>5±1</td>
</tr>
<tr>
<td>Asparagine</td>
<td></td>
<td>102±6</td>
<td>45±3</td>
<td>60±7</td>
<td>63±5</td>
</tr>
<tr>
<td>DMSP</td>
<td></td>
<td>85±0</td>
<td>68±12</td>
<td>207±22</td>
<td>243±18</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>9±1</td>
<td>7±1</td>
<td>13±2</td>
<td>20±2</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>59±1</td>
<td>44±1</td>
<td>118±5</td>
<td>124±7</td>
</tr>
</tbody>
</table>

For plants raised from seed and grown at 500 mol m\(^{-3}\) Na\(^+\), a 1000-fold increase in external sulphate concentration had no effect on the levels of DMSP or the other organic solutes (Table 1). However, an increase in nitrogen supply from 0.5 to 10 mol m\(^{-3}\) reduced the levels of DMSP by about 50%, but increased those of glycinebetaine by 70–80%, of proline by 2.7–3.9 times, and of asparagine by 4.9–12.4 times (Table 1; \(P<0.05\)). These effects of nitrogen on organic solute levels in the seedlings were similar to those observed for the plants derived from cuttings (Table 1; Fig. 3).

Selected solutes in S. alterniflora leaf blades collected from the South San Francisco Bay

Na\(^+\) was the major solute in leaf blades of S. alterniflora collected from the field (Table 2). K\(^+\) was also a major solute, although K\(^+\) levels were only 19–43% of the values for Na\(^+\). Glycinebetaine was a major organic solute in all leaf blades and, in some instances, it was present at a level similar to that of K\(^+\) (Table 2). Sucrose was also present in high amounts and during spring its level was similar to that of glycinebetaine. DMSP levels were 25–39% of the values for glycinebetaine, while those of proline were only 9–24% of the values for glycinebetaine. Asparagine levels were high only in the younger plants from the autumn sampling.

Comparison of the solutes in the youngest and oldest leaf blades of field-grown S. alterniflora showed that the youngest leaf blade contained higher levels of K\(^+\), glycinebetaine, glucose, proline, asparagine, and DMSP, but lower Na\(^+\) and sucrose (Table 3; \(P<0.05\)). These results indicate that organic solutes, together with K\(^+\), may play particularly important roles as osmotica in the youngest leaf blade.

Discussion

Glycinebetaine, proline, DMSP, sucrose, glucose, and asparagine were present at high levels in Spartina alterniflora leaves collected from different marshes along the east (Cavalieri and Huang, 1981; Dacey et al., 1987; Otte and Morris, 1994) and west coasts (Tables 2, 3) of North America. Except for DMSP, the levels of these solutes increased in response to high NaCl treatments in our greenhouse experiments (Fig. 3), a finding consistent with their putative role in osmotic adjustment.

The high NaCl treatment did not increase DMSP levels, suggesting DMSP was not involved in the osmotic adjustment of S. alterniflora leaf blades. This finding is consistent with studies of DMSP in S. anglica (van Diggelen et al., 1986) and in S. alterniflora in the field (Otte and Morris, 1994), both of which found no relationship between leaf DMSP and root zone salinity levels. These findings differ from a previous conclusion (Dacey et al., 1987) that DMSP was involved in the osmotic adjustment of S. alterniflora leaves. This conclusion was derived from a positive correlation between leaf DMSP and soil salinity levels at field sites (Dacey et al., 1987). The correlation, however, was low and it is possible that factors other than salinity, for example, nitrogen which strongly affects DMSP levels (Fig. 3D; Dacey et al., 1987; Otte and Morris, 1994) may have contributed to the variations among field sites.

The high levels of DMSP in S. alterniflora leaf blades may function as a constitutive organic osmoticum. Information regarding the cellular compartmentation of DMSP is required to elucidate its possible role in this regard. Constitutive osmotica should be of adaptive value to organisms that inhabit saline environments. Nevertheless, since marsh salinity can vary both spatially and temporally (Webb, 1983), organic solutes (e.g. glycinebetaine) whose levels can respond to changes in salinity would be required for osmotic adjustment.

The solute composition of leaf blades can vary greatly depending on their age or position on the main stem (Colmer et al., 1995). Comparison of solute levels in the oldest and youngest leaf blades of S. alterniflora showed that K\(^+\), glycinebetaine, proline, asparagine, and glucose may play important roles as osmotica in the youngest leaf, while Na\(^+\) and sucrose may serve this role in the oldest leaf (Table 3). In contrast, leaf tissue differences for DMSP level were far less marked than those of the
other solutes. The relatively similar levels of DMSP in different-age leaf blades is consistent with its possible role as a constitutive osmoticum.

Nitrogen is a limiting nutrient in many ecosystems, including S. alterniflora salt marshes (Patrick and Delaune, 1976). One advantage of accumulating DMSP may be that nitrogen allocation to compatible solutes can be reduced (Rhodes and Hanson, 1993). The opposite nitrogen dose–response of DMSP to that of the amino acids suggests that it may substitute for them, as an osmoticum, when nitrogen availability is low. In addition, nitrogen use would also be improved if nitrogen was preferentially allocated to glycinebetaine (1 nitrogen per molecule) rather than to asparagine (2 nitrogens per molecule). The percentages of total leaf blade nitrogen allocated to asparagine and glycinebetaine were 9% and 12.5%, respectively, for saltized plants supplied with 10 mol m⁻³ nitrogen; but 0.2% and 10.5% for those with 0.1 mol m⁻³ nitrogen (data not shown). These data support the notion of preferential nitrogen allocation to glycinebetaine, when nitrogen availability is low. Furthermore, the accumulation of asparagine under the high nitrogen treatments may function in nitrogen storage (Jeffries, 1980; Rabe, 1990), as well as playing a role in osmotic adjustment.

In summary, the failure of DMSP to accumulate in S. alterniflora (present findings) and S. anglica (van Diggelen et al., 1986) in response to high NaCl treatments, suggests it is not involved in the osmotic adjustment of Spartina leaves. Furthermore, there was no effect of high external sulphate on leaf blade DMSP levels, suggesting it does not play a role in sulphate detoxification. DMSP may, however, function as a constitutive organic osmoticum since it was always present at relatively high levels (namely 45–130 μmol g⁻¹ dry wt.).

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