NOTE

Effects of environmental salinity on carbon isotope discrimination and stomatal conductance in Spartina grasses

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ABSTRACT: High salinity levels have long been recognized as an important environmental factor influencing plant growth and performance. Salt stress and concomitant water stress are likely to affect photosynthesis by reductions in stomatal conductance necessary to prevent loss of water vapor. Despite inundation with saline water, salt marsh communities exhibit high primary productivity. This study tested the effects of 0 to 40% salinity on leaf carbon isotope discrimination (Δ), leaf stomatal conductance (g_s), and leaf conductance to CO_2 (g_{CO_2}) in the emergent estuarine C_4 grasses *Spartina alterniflora*, *S. anglica*, and *S. patens*. Δ increased with increasing salinity in greenhouse and growth chamber experiments. g_s and g_{CO_2} peaked at moderate salinity levels (10 to 20%) but significantly decreased at higher salinities in all species.

KEY WORDS: Salt stress · Photosynthesis · Spartina · δ^{13} C · Stable isotopes

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INTRODUCTION

The high and fluctuating salinity levels that characterize coastal salt marshes can strongly affect plant productivity (Drake 1989). Cordgrasses of the genus *Spartina* (Poaceae) flourish in many estuarine marsh communities and are apparently highly tolerant of saline conditions. *Spartina* grasses are the dominant component of many salt marsh communities (Teal & Teal 1969) and are important contributors to estuarine food webs in native ranges (Peterson et al. 1985). The importance of *Spartina* species in estuarine communities makes understanding the effects of salinity on *Spartina* relevant in considerations of marsh health and production.

Saline soils decrease a plant's ability to take up water by increasing the osmotic strength of the soil. This is similar mechanistically to drought stress; both parameters are often incorporated with measures of water potential (Ψ). Elevated soil salinity causes low Ψ

and concomitant water stress to plants (Jennings 1976). Stomatal aperture is sensitive to changes in Ψ , so stomatal conductance is often reduced when environmental Ψ is lowered (Willmer 1983). Therefore stomatal closure usually accompanies drought and salt stress. A decrease in stomatal conductance can reduce incoming CO_2 and thus reduce photosynthetic rates.

Stable isotope techniques are useful in studying carbon metabolism involved with photosynthesis (Farquhar et al. 1989) and plant water-use efficiency (WUE; Farquhar & Richards 1984). Past studies have found that carbon isotope discrimination (Δ) and WUE are tightly related because both parameters depend on stomatal aperture (Farquhar et al. 1982a). This relationship is easily demonstrated in C3 species (Farquhar & Richards 1984) because of large carbon discriminations resulting from Rubisco fractionation (O'Leary 1981). This Δ -WUE relationship is also present in C4 species, but to a lesser degree than in C3 plants because of limited Rubisco discrimination and smaller

variations in internal CO_2 concentrations (Farquhar 1983, Henderson et al. 1998). Despite the importance of salinity in the salt marsh environment, the effect of salinity on Δ and WUE in estuarine grasses is presently unknown.

Leaf stable carbon isotope values can be used to determine the degree to which stomatal conductance is affected by salinity-induced water stress. Previous studies have shown a decrease in ¹³C discrimination with increasing salinity in C₃ plants (e.g. Farguhar et al. 1982b, Neales et al. 1983, Brugnoli & Lauteri 1991, van Groenigen & van Kessel 2002). The opposite trend has been observed in a few salt-stressed terrestrial C₄ species (Bowman et al. 1989, Meinzer et al. 1994, Sandquist & Ehleringer 1995, Zhu & Meinzer 1999), but has not previously been demonstrated in estuarine species. In C₄ plants, a negative fractionation involved with CO₂ hydration (O'Leary 1981) leads to increasing discrimination against ¹³C in response to increasing salt levels, leading to more negative $\delta^{13}C$ in plant tissues.

Salt marsh communities occur at coastal locations where fresh- and saltwater mix. Soil salinities in wellmixed areas will normally be 15 to 25% (e.g. Bertness & Ellison 1987), but can be greater in high marsh areas where evaporative stresses are high (Pennings & Bertness 2001), sometimes up to a marine level of 35% or slightly higher. Since halotolerant salt marsh grasses encounter chronic (and fluctuating) salinity stress, it is likely that the increased ¹³C discrimination signal will be present in leaves, and that this signal will provide an integrated measure of salinity stress during plant growth. In this study, leaf stomatal conductance (g_s) , leaf conductance to CO_2 (g_{CO_2}), and carbon isotope discrimination (Δ) were measured in the emergent estuarine C₄ grasses Spartina alterniflora Loisel., S. anglica C.E. Hubbard, and S. patens (Aiton) Muhl. grown under 0 to 40% salinity in greenhouse and growth chamber studies. Differences in these parameters were anticipated from salinity-induced changes in photosynthetic properties.

MATERIALS AND METHODS

Plants were collected for experimentation from field sites and were maintained under greenhouse and growth chamber conditions. *Spartina alterniflora* was collected in Willapa Bay, Washington (46° 35′ N, 124° 01′ W), *S. anglica* was collected in northern Puget Sound, Washington (48° 15′ N, 122° 26′ W), and *S. patens* was obtained from the Gulf of Mexico near Panacea, Florida (30° 02′ N, 84° 23′ W).

Tillers from all plants were potted individually in a 50/50 (v/v) sand/potting soil mixture and were

watered to saturation twice weekly with modified Hoagland nutrient solution (Epstein 1972). Plants were allowed 30 d to acclimate to these conditions before initiating the experiment. Due to space constraints, plants were separated into 2 populations: plants were either grown in a greenhouse or in a walk-in growth chamber. The greenhouse was well ventilated and conditions consisted of natural lighting (average irradiance was 400 μmol quanta m⁻² s⁻¹ during daylight hours, with peaks near 1500 μmol m⁻² s⁻¹ on sunny days) with 26°C daytime temperatures and 18°C at night. Growth chamber conditions were a 14 h photoperiod with 26°C days and 18°C nights. Light was provided by 400 W metal halide lamps with irradiance about 300 μmol quanta m⁻² s⁻¹ at bench level.

Plants were randomized between treatments and were placed into large plastic tubs (12 pots per tub in an unbalanced block design; 4 to 6 tubs per treatment) and flooded with enough water to submerge plants to a level 2 cm above the soil surface (about 12 l per tub). The water was completely replaced weekly. During the acclimation period, salinity levels were increased 10% wk⁻¹ until treatments included 0, 10, 20, 30, and 40% salt in greenhouse plants and 0, 15, and 30% salt in growth chamber plants (Instant Ocean salts; Aquarium Systems). After proper salinity levels were reached, plants grew at least 30 d in their respective treatments before measurements were made.

 $g_{\rm s}$ was measured in greenhouse plants with an LI-1600 steady state porometer (LI-COR). Measures of $g_{\rm s}$ were made on each plant during mid-day hours on clear days between 23 May and 4 June 2005. Total leaf vapor conductance $(g_{\rm v})$ is a more inclusive measure that also accounts for boundary layer conductance $(g_{\rm a})$, and is therefore relevant to measures of CO₂ assimilation $(g_{\rm CO_2}=0.625g_{\rm v},~{\rm Salisbury}~{\rm \&~Ross}~1992).~g_{\rm v}$ was calculated after Campbell & Norman (1998) as:

$$g_{\rm v} \; (\text{mol m}^{-2} \; \text{s}^{-1}) = \frac{1}{\frac{1}{g_{\rm s}} + \frac{1}{g_{\rm a}}}$$
 (1)

 $g_{\rm a}$ was calculated after Campbell & Norman (1998) as:

$$g_a \text{ (mol m}^{-2} \text{ s}^{-1}\text{)} = (0.147)\sqrt{\frac{u}{d}}$$
 (2)

where u is wind speed (m s⁻¹), measured with a Traceable® hot wire anemometer (Control Company), and d is the characteristic dimension of the leaf, found by multiplying 0.72 by the maximum leaf width (Campbell & Norman 1998). Measures of $g_{\rm CO_2}$ allow researchers to calculate ${\rm CO_2}$ assimilation rates (A) with simple models, e.g. $A = g_{\rm CO_2}(C_{\rm a} - C_{\rm i})$, where $C_{\rm a}$ and $C_{\rm i}$ are ambient and intercellular concentrations of ${\rm CO_2}$

(Salisbury & Ross 1992). Since CO_2 levels within the greenhouse were likely not equal to ambient, we present only conductances and not A.

Plants were maintained for 60 to 80 d in their respective salinity treatments. During this time, plants increased their fresh weight biomass from 250 to 800%. Therefore, carbon isotope measurements on all but the oldest leaves represented carbon fixed during the experimental growth period. At harvest, 2 complete middle-aged leaves were sampled from similar-sized shoots from all plants for subsequent isotope analysis. The leaves were dried at 65°C for 2 to 3 d, then ground to powder with a mortar and pestle. Stable carbon isotope composition was determined using a Eurovector elemental analyzer interfaced with a continuous flow Micromass Isoprime isotope ratio mass spectrometer. Routine precision for the instrument was ±0.06% for δ^{13} C. Average δ^{13} C values for source CO₂ were obtained by sampling mid-day air in growing locations with evacuated gas-sampling vials. The absolute $\delta^{13}C$ values of plants grown in the growth chamber were slightly lower than δ^{13} C values of greenhouse plants due to differences in source CO₂. The growth chamber used in this study received air originating in the building ventilation system that was lighter ($\delta^{13}C = -10.7\%$) than CO₂ measured in the greenhouse (δ^{13} C = -9.5%). For this reason, absolute δ^{13} C values of greenhouse or growth chamber plants reflect source CO2 while differences among salinity treatments reflect physiological isotope effects. Therefore, discrimination (Δ) values were calculated so that both groups of plants could be analyzed together. Δ was calculated as:

$$\Delta = \delta^{13} C_{\text{source}} - \delta^{13} C_{\text{product}}$$
 (3)

where $\delta^{13}C_{source}$ is the $\delta^{13}C$ value of ambient CO_2 and $\delta^{13}C_{product}$ represents the $\delta^{13}C$ of carbon fixed into plant tissue (O'Leary et al. 1992). The effect of salinity on Δ was measured for each species using analysis of variance. In these models, treatments were blocked by growing location. Multiple individuals were averaged within each tub. g_s and g_{CO_2} measures were compared between species and treatments using analysis of variance; in these models, treatments were blocked by tub (SAS v8.0; α = 0.05).

RESULTS

A significant increase in Δ was observed with increasing soil salinity in all *Spartina* spp. (Fig. 1). Unlike many other studies, we observed very little mortality in higher salinity treatments because plants were slowly acclimated to increased salinity conditions. Leaf Δ values became more positive as a function of increasing salinity (ANOVA, p < 0.042). Overall Δ

shifts for Spartina spp. were nearly 2% across the 0 to 40% salinity gradient.

Leaf δ^{13} C ranged from -12.1 to -15.6% in greenhouse plants and from -13.6 to -17.7% in growth chamber plants. These δ^{13} C values are slightly more negative than the -13.5% typically found in C_4 leaves (O'Leary 1981), reflecting the difference in δ^{13} C of source CO_2 between ambient (δ^{13} C = -8%), greenhouse (δ^{13} C = -9.5%), and growth chamber (δ^{13} C = -10.7%). Differences in Δ represent physiological responses to salinity and were uniform between greenhouse and growth chamber plants.

Mean g_s ranged from 0.02 to 0.23 mol m⁻² s⁻¹ across species and treatments (Fig. 2). g_s peaked at moderate salinities (10 to 20%) but was significantly decreased

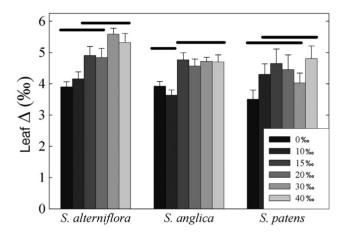


Fig. 1. Spartina spp. Carbon isotope discrimination (‰) in leaf tissues of flooded-treatment plants grown under salinities ranging from 0 to 40‰. Shown is the least squares mean of 3 to 5 individuals \pm SE. Lines over the bars represent significant differences at $\alpha=0.05$ (Scheffe adjustment)

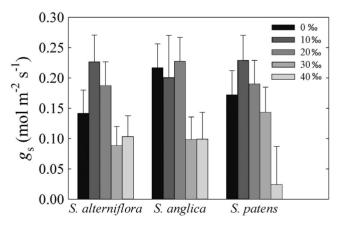


Fig. 2. Spartina spp. Effect of increasing salinity on leaf stomatal conductance (g_s) given as the least squares mean \pm SE of 3 to 5 individuals

at higher salinities across species. There were no significant differences between species (ANOVA, p = 0.503) or the species \times treatment interaction (p = 0.380), indicating all species responded similarly to increasing salinity. g_s in the 10 and 20% treatments was significantly greater than in the 30 and 40% treatments (p \leq 0.046; Scheffe adjustment). $g_{\rm CO_2}$ ranged from 0.03 to 0.14 mol m⁻² s⁻¹ (data not shown). Air currents in the greenhouse were enough to make q_a almost negligible, making q_s the primary contributor to $g_{
m v}$ and hence $g_{{
m CO}_2}$. $g_{{
m CO}_2}$ closely paralleled $g_{
m s}$ (correlation, r = 0.957). g_{CO_2} also peaked at moderate salinities (10 to 20%) and significantly decreased at higher salinities across species (ANOVA, $p \le 0.002$). If one assumed ambient CO₂ concentrations of 370 ppm and $C_{\rm i}/C_{\rm a}$ ratios near 0.36 (Henderson et al. 1998), ${\rm CO_2}$

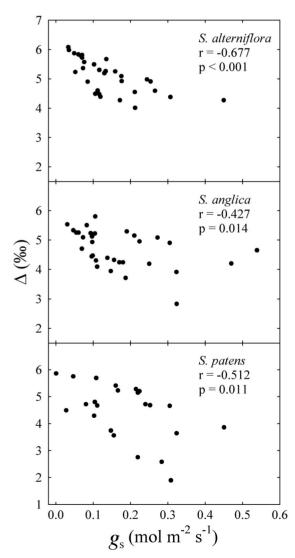


Fig. 3. Spartina spp. Relationship of discrimination (Δ) to leaf stomatal conductance (g_s)

assimilation values in the present study would be similar to previously published measures for CO_2 uptake by *Spartina pectinata* under salinity stress (Heckathorn & DeLucia 1991).

Increasing salinity led to increases in Δ and decreases of g_s across species. There was a significant, indirect relationship between g_s and Δ in greenhouse plants in all 3 species (Fig. 3; correlation, p \leq 0.014).

DISCUSSION

High salinity is an important factor influencing plant growth in estuaries. Most halophytes are facultative (Zhu 2001); there may be no obligate halophytes among the Angiosperms (Barbour 1970). Therefore salt is a burden for all plants. Understanding the physiological effects of salt on photosynthetic parameters of a dominant salt marsh species may provide insights into carbon fixation and estuarine production.

The *Spartina* spp. in this study showed a Δ increase of up to 2% with increasing salinity. This change in Δ is similar to results presented for *Atriplex lentiformis* (Zhu & Meinzer 1999) in response to a similar salinity gradient. Similar-sized increases in Δ have been measured under smaller salinity increases in other C_4 species, including *Atriplex confertifolia* (Sandquist & Ehleringer 1995), *Saccharum* spp. hybrids (Meinzer et al. 1994), *Zea mays*, and *Andropogon glomeratus* (Bowman et al. 1989). However, the trend observed in the present study was opposite the results presented by Walker & Sinclair (1992) for *Atriplex vesicaria* and *A. stipitata* (also see discussion by Sandquist & Ehleringer 1995).

Farquhar (1983, modified by Henderson et al. 1992) proposed the following model to predict 13 C discrimination in C_4 plants:

$$\Delta = a + [b_4 + \Phi(b_3 - s) - a](C_i/C_a) \tag{4}$$

In this model, a is the carbon fractionation due to diffusion of CO_2 in air (4.4%), b_4 is the fractionation by PEP carboxylase and from dissolution of CO_2 to HCO_3^- (about -5.7% at $25^{\circ}C$), b_3 is fractionation by Rubisco (29%), Φ is the proportion of CO_2 that leaks out of the bundle sheath cells, s is the fractionation of CO_2 during leakage from bundle sheath cells (1.8%), and C_i/C_a is the ratio of internal to ambient partial pressures of CO_2 . Under physiological conditions, all factors will remain constant except Φ and C_i/C_a (Farquhar 1983). Therefore, variations in Δ in C_4 plants will reflect changes in Φ or C_i/C_a .

The C_4 grasses in this study showed increases in carbon isotope discrimination (Δ) with increasing salinity. According to the above model, decreases in C_i/C_a or increases in Φ will lead to increased Δ (when Φ

< 0.32, Sandquist & Ehleringer 1995). In this study, all species showed a significant decrease in stomatal conductance with increasing salinity (Fig. 2). Whereas decreasing q_s may suggest decreasing C_i/C_a , previous studies have shown that C_i/C_a values did not change with increasing salinity in C₄ plants (Bowman et al. 1989, Meinzer et al. 1994). In the present study, a significant relationship was found between Δ and g_s (Fig. 3). This may suggest a role of q_s in determining Δ in these marsh halophytes. However, quantum efficiency measures on Distichlis spicata and numerous Spartina spp. indicate Φ increases with salinity (Long & Baker 1986, B. R. Maricle et al. unpubl.). Additionally, C_i/C_a values in C_4 plants depend on more than just q_s . An increase of Φ with unchanging C_i/C_a suggests salinity may act to increase internal cycling of CO2 in salt-stressed C₄ species. Perhaps one physiological feature contributing to salt tolerance in C4 monocots is an ability to cycle CO₂ internally as an outlet for excess light energy when g_s is low.

Plant sensitivity to salt may be indicated by the magnitude with which Δ changes over increasing salinity. Only one other study (involving a single terrestrial salt flat species), has investigated Δ changes in response to salinities as high as full strength seawater (Zhu & Meinzer 1999). The 2% change in Δ observed over a gradient of freshwater to 40% salt (~600 mM) in *Spartina* spp. is comparable to the 1.5 to 3% change in Δ observed over much narrower NaCl gradients $(\leq 85 \text{ mM})$ in Saccharum spp. (Meinzer et al. 1994), Zea mays, or Andropogon glomeratus (Bowman et al. 1989). This would indicate that stomatal conductance and Φ are much less sensitive to salinity in *Spartina* spp. grasses compared with these species. In terrestrial Atriplex species, the effect of salinity on Δ appears to be variable. A range of salt concentrations up to ~52 mM resulted in a 2% change in Δ in the salt tolerant Atriplex confertifolia (Sandquist & Ehleringer 1995), whereas in A. lentiformis exposure to 0 to 600 mM NaCl was required to induce a 2% change in Δ (Zhu & Meinzer 1999).

The relationship between salinity and Δ may be used to make inferences about primary production of marsh grasses during a growing season. If atmospheric $\delta^{13}C$ values are monitored seasonally, one can determine amounts of growth that occurred under various salinity levels by determining how much leaf carbon falls into established categories of Δ . This relationship also would allow researchers to reconstruct changes in the seasonal salinity status if plant growth rates are known. Conversely, if seasonal changes in salinity are known, one can calculate growth rates of plants, providing one knows the patterns of $\delta^{13}C$ occurring over the length of grass blades (Sasakawa et al. 1989, Meinzer & Saliendra 1997). Fluctuations in leaf $\delta^{13}C$

have been observed along the length of field collected Spartina spp. blades. Over a growing season, leaf δ^{13} C values in field-collected *S. alterniflora* plants increased (Δ decreased) by 1 to 1.5%, moving toward the younger tissue near the intercalary meristem at the base of the leaf (B. R. Maricle & R. W Lee unpubl. data). This pattern has been attributed to changes in the ratio of PEP carboxylase to Rubisco activity in maize leaves (Sasakawa et al. 1989) and to changes in stomatal conductance and photosynthetic rates in sugarcane leaves (Meinzer & Saliendra 1997). If this slope is corrected for, researchers can monitor changes in plant δ^{13} C against changes in atmospheric δ^{13} C, producing Δ values that may correlate with environmental factors such as salinity. Since grass blades grow from a basal meristem, these variations may reflect environmental changes during the growth season in a manner analogous to tree rings (Helliker & Ehleringer 2002).

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