

## CARBON ISOTOPE DISCRIMINATION IN THE C<sub>4</sub> SHRUB *ATRIPLEX CONFERTIFOLIA* ALONG A SALINITY GRADIENT

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**ABSTRACT**—Carbon isotope discrimination ( $\Delta$ ) was measured for leaves of *Atriplex confertifolia* along a salinity gradient in northern Utah. Over this gradient, the variation of  $\Delta$  values was high for a C<sub>4</sub> species, and the  $\Delta$  values were positively correlated with salinity in both years of the study. Of the possible explanations for this pattern, the  $\Delta$  results are consistent with the notion that salinity induces an increase in the bundle sheath leakiness of these C<sub>4</sub> plants.

*Key words:* carbon isotope ratio, salt stress, bundle sheath leakiness, halophyte, desert ecology.

The analysis of carbon isotope ratios (<sup>13</sup>C/<sup>12</sup>C) has become a useful tool for understanding various integrated aspects of plant metabolism, including numerous investigations of plant-environment interactions. The impact of environmental factors on carbon isotope discrimination ( $\Delta$ ) by plants with C<sub>3</sub> photosynthesis has been well studied; however, only a limited number of studies have examined variation of  $\Delta$  values in C<sub>4</sub> plants (O'Leary 1988, Farquhar et al. 1989, Peisker and Henderson 1992). In part, this disparity stems from C<sub>4</sub> plants having much smaller variation of  $\Delta$  values than C<sub>3</sub> plants. Additionally,  $\Delta$  values in C<sub>3</sub> plants have been correlated with water-use efficiency, and this has led to an emphasis on applying carbon isotope analyses to breeding programs (Farquhar et al. 1989, Ehleringer et al. 1993). However, a few recent studies have demonstrated that variation of  $\Delta$  values in C<sub>4</sub> plants may reflect environmental influences on physiological function (Bowman et al. 1989, Meinzer et al. 1994). In this study we examined variation of  $\Delta$  values in a C<sub>4</sub> perennial shrub, *Atriplex confertifolia* (Torr. & Frem.) Wats., and its relationship to natural conditions of soil salinity.

The  $\Delta$  value of a C<sub>4</sub> plant integrates two factors that can impact productivity: (1) the ratio of intercellular to ambient CO<sub>2</sub> concentration ( $c_i/c_a$ ), which can reduce photosynthetic activity when low, and (2) bundle sheath leakiness ( $\phi$ ), which reduces photosynthetic efficiency when high. Farquhar (1983) modeled the relationship between these factors

and carbon isotope discrimination in C<sub>4</sub> plants as

$$\Delta = a + (b_4 + b_3\phi - a) c_i/c_a, \quad (1)$$

where  $a$  (4.4‰) is discrimination against the heavier <sup>13</sup>CO<sub>2</sub> molecule relative to the lighter <sup>12</sup>CO<sub>2</sub> based on differential rates of diffusion,  $b_3$  (29‰) is the discrimination due to a greater affinity for <sup>12</sup>CO<sub>2</sub> relative to <sup>13</sup>CO<sub>2</sub> by ribulose biphosphate carboxylase (Rubisco), and  $b_4$  (typically  $\approx -5.2\%$ ) is discrimination based on the steps leading to, and including, CO<sub>2</sub> fixation by phosphoenol pyruvate carboxylase (PEPC) after atmospheric CO<sub>2</sub> enters the leaf. The  $b_4$  term varies slightly as a function of temperature and is negative (greater proportion of <sup>13</sup>CO<sub>2</sub>) due to fractionation associated with the hydration of CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup> (Mook et al. 1974). The discrimination terms of Equation 1 ( $a$ ,  $b_3$ , and  $b_4$ ) are constants, for the most part, and thus differences among  $\Delta$  values are the result of changes in  $\phi$  and/or  $c_i/c_a$  during CO<sub>2</sub> assimilation.

In C<sub>4</sub> plants, CO<sub>2</sub> is initially fixed by PEPC in the mesophyll cells, transported and decarboxylated in the bundle sheath cells, and then refixed by Rubisco. However, before the assimilation by Rubisco a fraction of the CO<sub>2</sub> may diffuse out through apoplastic portions of the bundle sheath cells. This is known as "leakiness" and is thought to be reduced by suberization of bundle sheath surfaces (Farquhar 1983). This leakiness, however, may be increased by environmental stresses, such as salinity (Bowman et al. 1989), and an increase

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in leakiness represents an energetic cost to the plant as a result of incomplete carbon assimilation or overcycling (Ehleringer and Pearcy 1983, Jenkins et al. 1989, Henderson et al. 1992).

Leakiness affects  $\Delta$  because it causes the bundle sheath cell to become an open system and therefore allows expression of discrimination by Rubisco ( $b_3$ ). The proportion of  $\text{CO}_2$  that leaks out of the bundle sheath cell ( $\phi$ ) modifies the degree to which  $b_3$  is expressed and thereby determines the relationship between  $\Delta$  and  $c_i/c_a$  (Eq. 1). At low  $\phi$  values the relationship between  $\Delta$  and  $c_i/c_a$  is negative, at high  $\phi$  the relationship is positive, and at  $\phi \approx 0.32$ ,  $\Delta$  is constant at 4.4‰ regardless of  $c_i/c_a$ . Equation 1 also predicts that for any given  $c_i/c_a$ , an increase in  $\phi$  results in an increase in  $\Delta$ . Given these relationships, variation of  $\Delta$  values in  $\text{C}_4$  plants can provide an indication of bundle sheath leakiness and its relationship to environmental stresses.

To date, much work investigating variation of  $\Delta$  in  $\text{C}_4$  plants has come from either laboratory gas exchange studies (Evans et al. 1986, Bowman et al. 1989, Henderson et al. 1992) or theoretical models (Peisker 1982, Farquhar 1983, Peisker and Henderson 1992). There is little direct information on environmental stresses that influence  $\Delta$  in natural populations of  $\text{C}_4$  plants (except see Walker and Sinclair 1992). Here we report on changes in  $\Delta$  values for the  $\text{C}_4$  species *Atriplex confertifolia* found along a natural salinity gradient in Utah. The purpose of this study was to determine if  $\Delta$  values changed in relation to soil salinity under field conditions, and if these changes corresponded to variation in  $\phi$  values. Two previous laboratory studies have shown that higher soil salinity does increase  $\Delta$  values in  $\text{C}_4$  plants and that this change is a result of greater  $\phi$  (Bowman et al. 1989, Meinzer et al. 1994). For *A. confertifolia*, we hypothesized that the same trend would be found over a transect of naturally increasing soil salinity.

## METHODS

### Study Sites

Four study sites of increasing salinity were chosen along a south-to-north transect in the northern end of Skull Valley (Tooele County, UT) flanking the western slope of the Stans-

bury Mountain Range. The four sites range in elevation from 1366 m to 1286 m (Fig. 1). Site 1 (1366 m) is dominated by sagebrush (*Artemisia tridentata*) with low densities of *Atriplex confertifolia*, *Juniperus osteosperma*, and *Tetradymia spinosa*. Weedy grasses and annual species of the Chenopodiaceae are also found within disturbed areas of this and all other sites. Greasewood (*Sarcobatus vermiculatus*) is the dominant species at sites 2 (1317 m) and 3 (1294 m) with *A. confertifolia* co-occurring in low frequency. Site 4 (1286 m), along the margins of the salt flats, is a heterogeneous site with a mixed community of salt-tolerant species. *S. vermiculatus* is the dominant species with moderate densities of *Allenrolfea occidentalis*, *Atriplex gardneri*, *A. confertifolia*, *Chrysothamnus viscidiflorus*, *Kochia americana*, and *Suaeda torreyana*.

Weather data for this transect are taken from the Grantsville weather station (Grantsville, Tooele County, UT, 1307 m) located 17.3 km E and 8.2 km S from the center of our study transect.

### Leaf and Soil Samples

Leaves of *Atriplex confertifolia* and soil samples were collected from each of the four transect sites in October 1991 and 1992, with the help of the 1991 and 1992 Plant Ecology classes from the University of Utah. Recently matured leaves of *A. confertifolia* were collected from five to eight individuals per site in 1991 and three per site in 1992. Leaf samples were oven-dried (70°C, 7 d), ground with mortar and pestle, and analyzed for carbon isotopic composition (Windy Ike, Delta S mass ratio spectrometer, Finnigan-MAT, San Jose, CA) relative to the Pee Dee Belemnite standard. Analyses were done at the Stable Isotope Ratio Facility for Environmental Research (SIRFER, University of Utah, Salt Lake City, UT). Carbon isotope ratio values ( $\delta$ ) were transformed to discrimination ( $\Delta$ ) values as

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p), \quad (2)$$

where  $\delta_p$  is the measured carbon isotope ratio of the plant, and  $\delta_a$  is the carbon isotope ratio of  $\text{CO}_2$  in the atmosphere (-0.008 or -8‰; Farquhar et al. 1989). The standard per mil (‰) notation is used throughout for ease of presentation, and the overall, long-term error

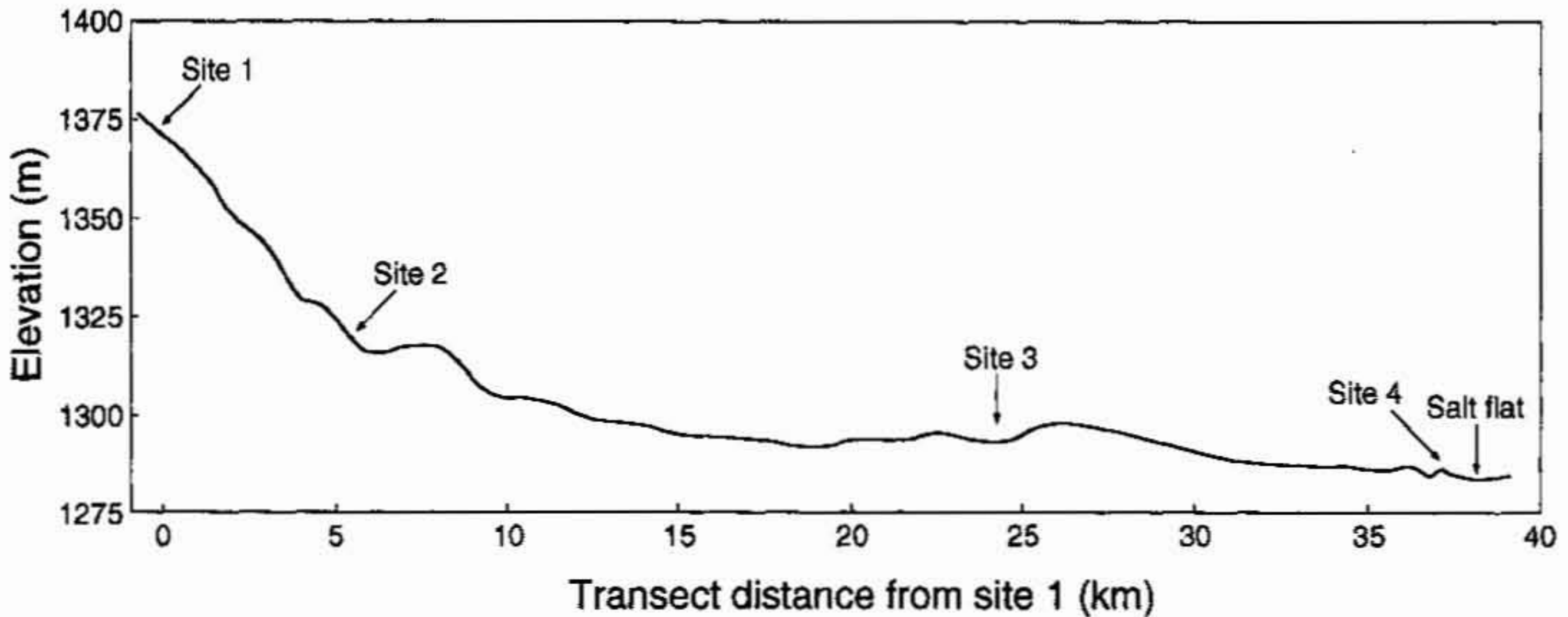


Fig. 1. Study transect in cross section. Shown is the topography over the transect and localities of each study site based on the approximate linear distance from site 1.

associated with carbon isotope determination is  $\pm 0.11\%$ .

Soil samples were collected from two depths (15–20 cm and 40–60 cm) in two to six excavation pits at each site. Approximately 200 g of freshly extracted soil from each hole and depth was placed immediately into soil canisters, sealed, and kept cool until analysis in the laboratory. In the lab one subsample per canister was removed for salinity analyses. The remaining soil was used for gravimetric water content determination based on the difference between soil fresh (wet) weight and dry weight (i.e., water content) relative to the soil dry weight. Soils were dried at 70°C for 7 d.

In 1991 the soil salinity analysis was based on electrical conductivity (EC) of an aqueous solution extracted from a 1:2 soil:deionized water mixture, and in 1992 from a 1:5 soil:deionized water mixture. There was no evidence that the 1:2 mixture was ion saturated; thus, to standardize these ratios, the ECs of samples using a 1:2 solution were extrapolated to EC based on a 1:5 ratio assuming a linear dilution relationship. Tests confirmed that this extrapolation was valid even for EC values higher than those found in actual field samples.

Although a more standard procedure for salinity determination is the "soil paste" method, the 1:5 ratio method we used is recommended as a simpler technique to determine relative salinity contents (Rhoades 1982) and is suitable for the purposes of this study (i.e., standardized comparison of relative salinities among sites). Additionally, the ECs of 1:5

ratio extracts are highly correlated with soil paste ECs for soils within and near our transect (D. G. Williams unpublished data). Electrical conductivity is reported in  $\mu\text{mhos cm}^{-1}$  ( $1 \mu\text{mhos cm}^{-1} = 0.1 \text{ mS m}^{-1} = 0.502 \text{ mM NaCl}$ ), and the data were log transformed for statistical analyses. Interannual comparisons of means for each soil trait were done by *t* tests, and correlations between soil trait and plant carbon isotope discrimination means were determined by Pearson product-moment correlation.

## RESULTS

### Transect Characterization

Salinity increased across the gradient in both the 1991 and 1992 samples; electrical conductivity increased by two orders of magnitude over the entire transect (Table 1). Site 1 was the least saline, and salinity progressively increased toward the highly saline site 4.

There were few differences between years in soil electrical conductivity. Significant differences were found at only two sites and at only one depth per site. Furthermore, sites gave opposite results: soils of site 3 at the 15–20-cm depth had greater conductivity in 1991 than 1992 ( $t = 4.33$ ,  $P < .01$ ), and soils from site 1 at the 40–60-cm depth had higher conductivity in 1992 than in 1991 ( $t = 4.60$ ,  $P < .01$ ).

Gravimetric water content also increased over the transect from site 1 to site 4 (Table 1). Soil water content was somewhat greater in

TABLE 1. Soil properties at two depths for sites 1–4 along the study transect ( $n$  = number of pits; one sample for each depth per pit). Soil water content was measured as gravimetric water content, and electrical conductivity is of an aqueous extract from 1:5 soil:water mixture (extrapolated for 1991 from 1:2 ratio; see text).

	Soil water content (%) @15-20 cm			Soil water content (%) @40-60 cm			Electrical conductivity ( $\mu\text{mhos/cm}$ ) @15-20 cm			Electrical conductivity ( $\mu\text{mhos/cm}$ ) @40-60 cm		
	Mean	SE	$n$	Mean	SE	$n$	Mean	SE	$n$	Mean	SE	$n$
OCTOBER 1991												
Site 1	4.66	0.300	4	5.34	0.234	4	89	15.7	4	70	3.2	4
Site 2	4.15	0.687	4	7.23	0.360	4	91	7.6	4	324	81.1	4
Site 3	11.79	1.446	4	17.24	0.892	4	2309	114.1	4	2066	657.7	4
Site 4	24.84	7.578	6	39.41	7.841	6	3596	587.6	6	3382	530.7	6
OCTOBER 1992												
Site 1	2.89	0.454	2	3.81	0.402	3	84	6.3	3	93	3.7	3
Site 2	4.79	0.226	2	5.56	0.499	3	144	29.2	3	324	111.0	3
Site 3	2.46	0.270	2	10.26	3.672	3	546	459.3	3	984	858.5	3
Site 4	10.66	0.950	2	NA	—	—	1640	1440.0	2	3250	350.0	2

1991 than in 1992, but significant differences at both depths were found only at site 1 (15–20-cm depth,  $t = 3.34$ ,  $P < .05$ ; 40–60-cm depth,  $t = 3.52$ ,  $P < .05$ ). Rainfall over the 10-wk period prior to sampling in 1991 was much greater than that of 1992 (82.5 mm vs. 18.8 mm), which likely accounts for the trend of greater water content in the soils during the 1991 sample period.

#### Carbon Isotope Discrimination

Along the transect the carbon isotope discrimination for *Atriplex confertifolia* ranged from a low of  $4.74 \pm 0.96\text{‰}$  at site 1 in 1992, to a high of  $6.55 \pm 0.11\text{‰}$  at site 3 in 1991 (Fig. 2). This range of nearly 2‰ is high for  $C_4$  plants (Farquhar et al. 1989). The mean  $\Delta$  value was always greater than 4.4‰, and for only a single sample was the individual shrub value less than 4.4‰. These high  $\Delta$  values indicate that the mean  $\phi$  values were always greater than 0.32 (Eq. 1).

With respect to the environmental parameters examined along the transect, mean leaf  $\Delta$  was not significantly correlated with water content during any observation, but was positively correlated with log EC (Fig. 2). Inclusion of the notably low  $\Delta$  value of site 4 in 1991 resulted in a nonsignificant, positive trend (but when excluded,  $\Delta$  was significantly correlated with log EC in 1991 at the deeper soil depth,  $R = 1.0$ ,  $P < .01$ ). In 1992 there was a highly significant, positive correlation of  $\Delta$  and log EC for both the shallow soils ( $R = .978$ ,  $P < .05$ ) and deeper soil depths ( $R = .999$ ,  $P < .001$ ) (Fig. 2).

#### DISCUSSION

Variation in carbon isotope discrimination values of  $C_4$  plants is, in part, dependent upon the proportion of  $\text{CO}_2$  that is initially fixed by PEPC and ultimately diffuses out of the bundle sheath cells without being refixed (i.e., the leakiness,  $\phi$ ). Leakiness might be influenced by environmental stresses, such as salinity (Bowman et al. 1989, Meinzer et al. 1994), because such stresses could disrupt membrane properties or the biochemical coordination between the  $C_4$  and  $C_3$  cycles operating in the mesophyll and bundle sheath cells, respectively (Peisker and Henderson 1992). The other component influencing variation of  $\Delta$  in  $C_4$  plants is  $c_i/c_a$ . Figure 3 illustrates how the relationship between  $\Delta$  and  $c_i/c_a$  depends upon the value of  $\phi$  (from Eq. 1), and provides a model for how changes in  $\phi$  and  $c_i/c_a$  can account for the changes in  $\Delta$  values we observed.

We found that  $\Delta$  values of *A. confertifolia* increased by 2‰ in concordance with increasing salinity (Fig 2). These  $\Delta$  values were always greater than 4.4‰; therefore the  $\phi$  values must be greater than 0.32 (cf. Fig. 3). A 2‰ increase in  $\Delta$  values, at  $\phi > 0.32$ , cannot be explained solely by changes in  $c_i/c_a$  given the typical range of  $c_i/c_a$  values for  $C_4$  plants under ambient conditions (0.20–0.40; Percy and Ehleringer 1984). To do so would require either extreme leakiness values ( $\phi \geq 0.6$ ) or an increase of  $c_i/c_a$  with increasing salinity since  $\Delta$  and  $c_i/c_a$  are positively related when  $\phi > 0.32$ . Leakiness values greater than 0.6 have

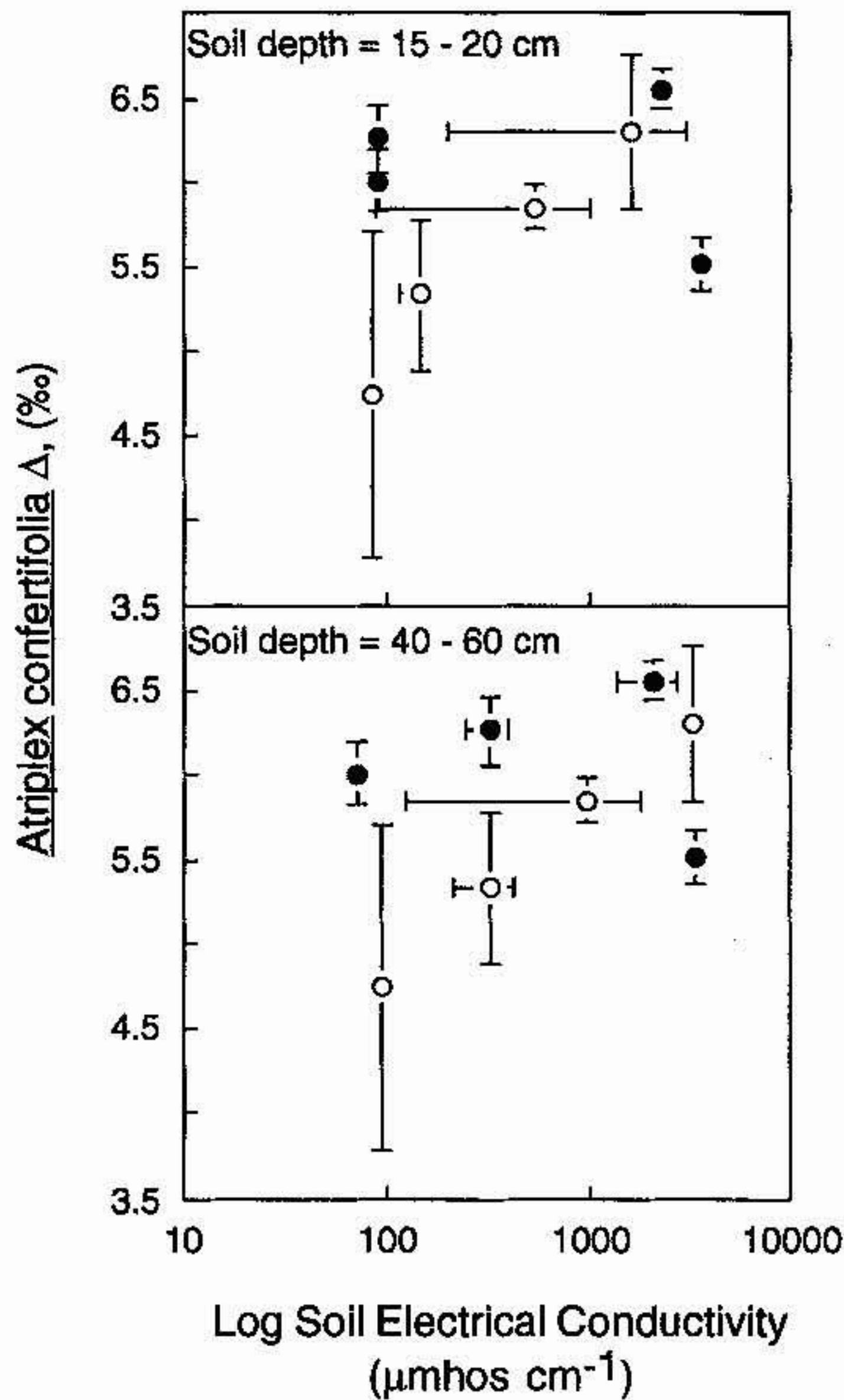


Fig. 2. Relationship between carbon isotope discrimination ( $\Delta$ ) of *Atriplex confertifolia* leaves and log electrical conductivity (log EC) of soil at two depths, 15–20 cm and 40–60 cm, for sites 1–4 along the transect. Closed symbols (●) are study site means for 1991, and open symbols (○) are those for 1992. Error bars are  $\pm$  1 SE.

never been reported, and the latter explanation is unlikely since salt stress typically decreases or does not change  $c_i/c_a$  (Long and Baker 1986, Flanagan and Jefferies 1988). A simpler explanation for the change in  $\Delta$  values is that  $\phi$  increases with higher salinity. A 2‰ increase based on changes in  $\phi$  values can be easily accommodated within the limits of  $c_i/c_a$  found for  $C_4$  plants (Fig. 3). Thus, changes in  $\Delta$  values for *A. confertifolia* are more likely due to an increase of  $\phi$  associated with a change in salinity; consequently, the presence of a significant relationship between  $\Delta$  values and EC (Fig. 2).

The trend of increasing  $\Delta$  values with increasing salinity held in all but one site in the two-year study (site 4 in 1991). This devia-

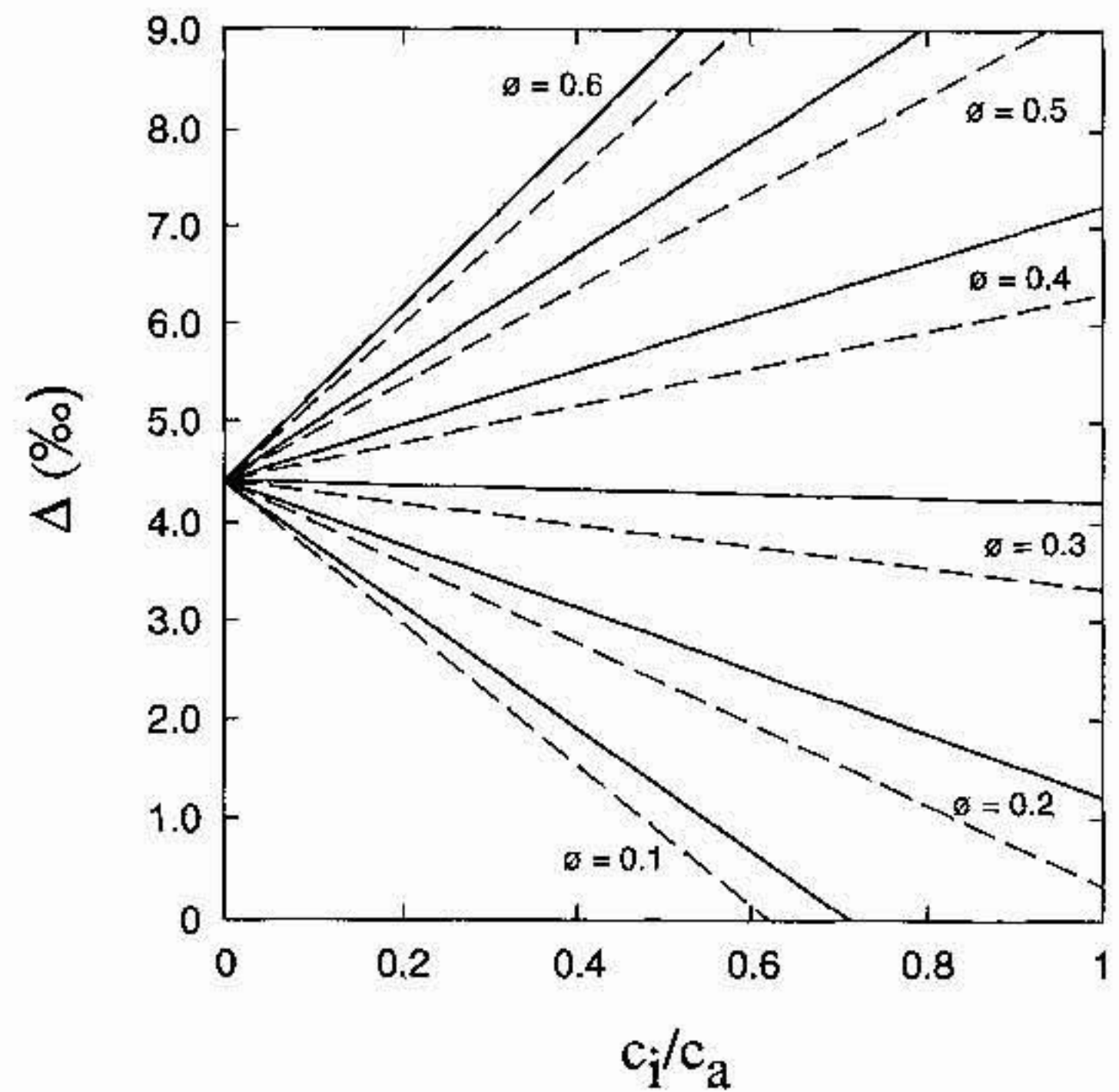


Fig. 3. Model for the relationship between carbon isotope discrimination ( $\Delta$ ) and  $c_i/c_a$  (ratio of intercellular to ambient  $CO_2$ ) based on Equation 1 and for  $\phi$  values ranging from 0.1 to 0.6. Dashed and solid lines represent the range of  $\Delta$  values for each  $\phi$  value depicted, based on a high leaf temperature ( $34^\circ C$ ) where  $b_4 = -4.8\text{‰}$  (solid line) and a lower leaf temperature ( $25^\circ C$ ) where  $b_4 = -5.7\text{‰}$  (dashed line).

tion could be due simply to the high degree of edaphic variability at site 4; this location had the greatest topographic variability, highest species diversity, and greatest overall variance for soil conductivity and water content (Table 1). Site 4 was also extremely wet in 1991 (near 40% water content at 40–60-cm depth), which may have diluted the salinity of these soils, thereby reducing the salinity experienced by the plants. Without a more detailed study, however, this deviation remains unexplained.

Previous studies have found contrasting patterns of the relationship between  $\Delta$  and salinity. In a laboratory study with 11  $C_4$  species, Henderson et al. (1992) found that  $\phi$  values were invariable and low, remaining at  $\phi \sim 0.21$ , thereby resulting in a negative relationship between  $\Delta$  and  $c_i/c_a$  (Fig. 3). The small variation they observed in  $\Delta$  values was attributed to changes in  $c_i/c_a$  values. However, in an earlier study with the  $C_4$  monocots *Zea mays* and *Andropogon glomeratus*, Bowman et al. (1989) found that  $\Delta$  values of salt-stressed plants were more dramatically influenced by changes in  $c_i/c_a$  than were control plants. The increase of  $\Delta$  values with salinity was explained by a changing relationship between  $\Delta$

and  $c_i/c_a$  due to increasing  $\phi$  values as the water status of salt-stressed plants declined through the day (Bowman et al. 1989).

Recently, Meinzer et al. (1994) also observed that increasing salinity resulted in increases of  $\Delta$  values. Using two sugarcane cultivars, they showed that change in  $\Delta$  value could be ascribed to greater  $\phi$  values as salinity increased, and that variability of  $c_i/c_a$  had much less impact on the increase of  $\Delta$  values. In contrast, Walker and Sinclair (1992) reported that  $\Delta$  values of two Australian  $C_4$  *Atriplex* species *decreased* at sites with increased salinity. The  $\Delta$  values of these Australian *Atriplex* leaves were greater than 4.4‰, which could have been achieved only with a bundle sheath leakage greater than 0.32 (Fig. 3). Since the relationship between  $\Delta$  and  $c_i/c_a$  is positive at  $\phi > 0.32$  (Fig. 3), the Walker and Sinclair data suggest that salinity affected a decrease of  $c_i/c_a$  and, therefore, a decrease of  $\Delta$ .

Our findings of a positive correlation between  $\Delta$  values of *Atriplex confertifolia* and salinity are in contrast to findings of Walker and Sinclair (1992). Our observations, like those of Bowman et al. (1989) and Meinzer et al. (1994), suggest that changes in leaf carbon isotope discrimination result from an increased bundle sheath leakage when plants are exposed to a salinity stress. The mechanism of change in  $\phi$  values is likely to be associated with physical changes in the bundle sheath permeability to  $CO_2$  (or to  $HCO_3^-$ ) and/or biochemical changes in the coupling of Rubisco and PEPC activity. Such biochemical changes due to salinity have been previously found. Guy and Reid (1986) have shown that salinity may reduce Rubisco activity in  $C_3$  plants without a concomitant decrease in PEPC activity. Increased salinity (NaCl) has also been shown to increase PEPC activity in some  $C_4$  halophytes (Shomer-Ilan et al. 1985). Any such increase in the activities of  $C_4$  carboxylation enzymes relative to those of  $C_3$  carboxylation enzymes in  $C_4$  plants should increase  $\phi$  values (Peisker and Henderson 1992). Thus, under natural conditions it appears that salinity could increase  $\Delta$  values of *A. confertifolia* by influencing an increase in  $\phi$  values.

The relationship between salt stress and  $\phi$  of  $C_4$  plants may be species specific or even population specific and may account for discrepancies among different studies of  $\Delta$  values in  $C_4$  plants. For example, there is high vari-

ability among previous studies of carbon isotope discrimination in *Atriplex confertifolia*; mean  $\Delta$  values range from 4.4‰ (Marino et al. 1992) to 6.9‰ (Troughton et al. 1974). Yet, each of these observations is consistent with the notion that  $\phi$  values exceed 0.32 and are therefore high compared to nonhalophytic  $C_4$  species (Henderson et al. 1992).

In the present study we have shown that salinity may be one factor that significantly influences variation of  $\Delta$  values in  $C_4$  plants, most likely through an effect on bundle sheath leakiness. While variation in  $\Delta$  values of  $C_4$  plants may provide new insights into plant-salinity dynamics along environmental gradients, results also suggest that caution is necessary when using  $\Delta$  values of  $C_4$  plants to interpret historical changes in atmospheric  $CO_2$  concentrations and  $^{13}C$  values, as has been proposed by Marino et al. (1992).

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#### LITERATURE CITED

- BOWMAN, W. D., K. T. HUBICK, S. VON CAEMMERER, AND G. D. FARQUHAR. 1989. Short-term changes in leaf carbon isotope discrimination in salt- and water-stressed  $C_4$  grasses. *Plant Physiology* 90: 162-166.
- EHLERINGER, J., AND R. W. PEARCY. 1983. Variation in quantum yield for  $CO_2$  uptake among  $C_3$  and  $C_4$  plants. *Plant Physiology* 73: 555-559.
- EHLERINGER, J. R., A. E. HALL, AND G. D. FARQUHAR. 1993. Stable isotopes and plant carbon-water relations. Academic Press, San Diego, CA. 555 pp.
- EVANS, J. R., T. D. SHARKEY, J. A. BERRY, AND G. D. FARQUHAR. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate  $CO_2$  diffusion in leaves of higher plants. *Australian Journal of Plant Physiology* 13: 281-292.
- FARQUHAR, G. D. 1983. On the nature of carbon isotope discrimination in  $C_4$  species. *Australian Journal of Plant Physiology* 10: 205-226.
- FARQUHAR, G. D., J. R. EHLERINGER, AND K. T. HUBICK. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Molecular Biology* 40: 503-537.
- FLANAGAN, L. B., AND R. L. JEFFERIES. 1988. Stomatal limitation of photosynthesis and reduced growth of

- the halophyte, *Plantago maritima* L., at high salinity. *Plant, Cell and Environment* 11: 239–245.
- GUY, R. D., AND D. M. REID. 1986. Photosynthesis and the influence of CO<sub>2</sub> enrichment on  $\delta^{13}\text{C}$  values in a C<sub>3</sub> halophyte. *Plant, Cell and Environment* 9: 65–72.
- HENDERSON, S. A., S. VON CAEMMERER, AND G. D. FARQUHAR. 1992. Short-term measurements of carbon isotope discrimination in several C<sub>4</sub> species. *Australian Journal of Plant Physiology* 19: 263–285.
- JENKINS, C. L. D., R. T. FURBANK, AND M. D. HATCH. 1989. Mechanism of C<sub>4</sub> photosynthesis. A model describing the inorganic carbon pool in bundle sheath cells. *Plant Physiology* 91: 1372–1381.
- LONG, S. P., AND N. R. BAKER. 1986. Saline terrestrial environments. Pages 63–102 in N. R. Baker and S. P. Long, editors, *Photosynthesis in contrasting environments*. Elsevier Scientific Publishers, New York, NY.
- MARINO, B. D., M. B. McELROY, R. J. SALAWITCH, AND W. G. SPAULDING. 1992. Glacial-to-interglacial variations in the carbon isotopic composition of atmospheric CO<sub>2</sub>. *Nature* 357: 461–466.
- MEINZER, F. C., Z. PLAUT, AND N. Z. SALIENDRA. 1994. Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. *Plant Physiology* 104: 521–526.
- MOOK, W. G., J. C. BOMMERSON, AND W. H. STAVERMAN. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth and Planetary Science Letters* 22: 169–176.
- O'LEARY, M. H. 1988. Carbon isotopes in photosynthesis. *BioScience* 38: 325–336.
- PEARCY, R. W., AND J. EHLERINGER. 1984. Comparative ecophysiology of C<sub>3</sub> and C<sub>4</sub> plants. *Plant, Cell and Environment* 7: 1–13.
- PEISKER, M. 1982. The effect of CO<sub>2</sub> leakage from bundle sheath cells on carbon isotope discrimination in C<sub>4</sub> plants. *Photosynthetica* 16: 533–541.
- PEISKER, M., AND S. A. HENDERSON. 1992. Carbon: terrestrial C<sub>4</sub> plants. *Plant, Cell and Environment* 15: 987–1004.
- RHOADES, J. D. 1982. Soluble salts. Pages 167–179 in *Methods of soil analysis, part 2. Chemical and microbiological properties*. ASA-SSSA, Madison, WI.
- SHOMER-ILAN, A., D. MOUALEM-BENO, AND Y. WAISEL. 1985. Effects of NaCl on the properties of phosphoenolpyruvate carboxylase from *Suaeda monoica* and *Chloris gayana*. *Physiologia Plantarum* 65: 72–78.
- TROUGHTON, J. H., P. V. WELLS, AND H. A. MOONEY. 1974. Photosynthetic mechanisms and paleoecology from carbon isotope ratios in ancient specimens of C<sub>4</sub> and CAM plants. *Science* 185: 610–612.
- WALKER, C. D., AND R. SINCLAIR. 1992. Soil salinity is correlated with a decline in  $^{13}\text{C}$  discrimination in leaves of *Atriplex* species. *Australian Journal of Ecology* 17: 83–88.

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