Journal of Experimental Botany, Vol. 57, No. 1, pp. 139–147, 2006
doi:10.1093/jxb/erj016 Advance Access publication 29 November, 2005

Journal of Experimental Botany

RESEARCH PAPER

High apoplastic solute concentrations in leaves alter water relations of the halophytic shrub, *Sarcobatus vermiculatus*

J. J. James[1,*], N. N. Alder[1,†], K. H. Mühling[1,‡], A. E. Läuchli[1], K. A. Shackel[2], L. A. Donovan[3] and J. H. Richards[1]

[1] *Department of Land, Air, and Water Resources, University of California, Davis, CA 95616-8627, USA*

[2] *Plant Sciences, University of California, Davis, CA 95616, USA*

[3] *Plant Biology, University of Georgia, Athens, GA 30602, USA*

Received 29 June 2005; Accepted 10 October 2005

Abstract

Predawn plant water potential (Ψ_w) is used to estimate soil moisture available to plants because plants are expected to equilibrate with the root-zone Ψ_w. Although this equilibrium assumption provides the basis for interpreting many physiological and ecological parameters, much work suggests predawn plant Ψ_w is often more negative than root-zone soil Ψ_w. For many halophytes even when soils are well-watered and night-time shoot and root water loss eliminated, predawn disequilibrium (PDD) between leaf and soil Ψ_w can exceed 0.5 MPa. A model halophyte, *Sarcobatus vermiculatus*, was used to test the predictions that low predawn solute potential (Ψ_s) in the leaf apoplast is a major mechanism driving PDD and that low Ψ_s is due to high Na^+ and K^+ concentrations in the leaf apoplast. Measurements of leaf cell turgor (Ψ_p) and solute potential (Ψ_s) of plants grown under a range of soil salinities demonstrated that predawn symplast Ψ_w was 1.7 to 2.1 MPa more negative than predawn xylem Ψ_w, indicating a significant negative apoplastic Ψ_s. Measurements on isolated apoplastic fluid indicated that Na^+ concentrations in the leaf apoplast ranged from 80 to 230 mM, depending on salinity, while apoplastic K^+ remained around 50 mM. The water relations measurements suggest that without a low apoplastic Ψ_s, predawn Ψ_p may reach pressures that could cause cell damage. It is proposed that low predawn apoplastic Ψ_s may be an efficient way to regulate Ψ_p in plants that accumulate high concentrations of osmotica or when plants are subject to fluctuating patterns of soil water availability.

Key words: Apoplast, Great Basin, nanolitre osmometer, predawn water potential, pressure probe, salinity, solute potential, turgor.

Introduction

Predawn plant water potential is often considered to reflect soil moisture availability to plants and as such is used to interpret a range of physiological and ecological parameters such as maximum stomatal conductance and transpiration (Reich and Hinckley, 1989; Améglio and Archer, 1996; Mediavilla and Escudero, 2003), growth (Mitchell *et al*., 1993), and differences in rooting depth, stress tolerance, and habitat partitioning between different species or life stages (Davis and Mooney, 1986; Donovan and Ehleringer, 1994; Peuke *et al*., 2002; Filella and Peñuelas, 2003). The use and interpretation of Ψ_w follows from classical water relations models, based on thermodynamics and the Ohm's law analogy, that predict that predawn plant Ψ_w will equilibrate with soil Ψ_w in the rooting zone (Slatyer, 1967). However, there is a growing body of evidence suggesting that predawn plant Ψ_w can be substantially more negative than root-zone soil Ψ_w in many species. For example, Donovan *et al*. (2001) documented significant predawn disequilibrium between plant–soil Ψ_w (hereafter predawn disequilibrium, PDD; *sensu* Donovan *et al*., 1999) in 15 out of 15 species surveyed under controlled

* Present address and to whom correspondence should be sent: USDA-ARS, Eastern Oregon Agricultural Research Center, 67826-A Hwy 205, Burns, OR 97720, USA. E-mail: jeremy.james@oregonstate.edu
† Present address: Medical Biochemistry and Genetics, Texas A&M University Health Science Center, College Station, TX 77840, USA.
‡ Present address: Institute of Plant Nutrition, Justus-Liebig-University, Ludwigstraße 23, D-35390 Giessen, Germany.

environmental conditions. For seven of these species PDD ranged from 0.5 to 2.3 MPa. The limited number of field experiments addressing PDD largely support the observations made in controlled environments. In Great Basin, Mojave, and Tunisian desert shrubs PDD ranged from 1.1 to 2.7 MPa (Ourcival *et al*., 1994; Donovan *et al*., 2003; James *et al*., 2005). Comparably large PDD in field populations have also been reported for a range of crop and tree species (see review in Donovan *et al*., 2001; Bucci *et al*., 2004). While these observations change thinking about how plants may interact with the soil and atmospheric moisture environment, better understanding of mechanisms driving PDD is needed to evaluate the adaptive significance and physiological and ecological tradeoffs of PDD.

A number of mechanisms that prevent overnight equilibration between plant and soil Ψ_w have been recognized, including night-time water loss through the canopy or root system, low hydraulic conductivity, high capacitance, and soil moisture heterogeneity (Blake and Ferrell, 1977; Richards and Caldwell, 1987; Ourcival and Berger, 1995; Sellin, 1999). For many halophytes and moderately salt-tolerant desert species, however, even when soils are well-watered and night-time water loss eliminated, PDD can still be significant (0.2–1.6 MPa), suggesting that other mechanisms linked to ion physiology may contribute to PDD in these and other arid-land species (Donovan *et al*., 2001, 2003).

One putative mechanism that could contribute to large PDD in halophytes is high solute concentration in the leaf apoplast. Although classical water relations assume that Ψ_s in the leaf apoplast and stem xylem lumen are similar and low (−0.01 MPa) (Ritchie and Hinckley, 1975; Passioura, 1991; Boyer, 1995), experimental observations of predawn leaf Ψ_w significantly lower than xylem Ψ_p in halophytes suggests that, in these species, predawn Ψ_s in the leaf apoplast could be substantial (Donovan *et al*., 2001, 2003). To understand how this mechanism could contribute to PDD, it is important to recognize that the leaf apoplast is spatially distant from the stem xylem lumen but that these regions are not separated by a membrane. Water movement between the symplast and apoplast involves the plasmalemma, and hence is determined by the sum of pressure and osmotic components, whereas water movement through the apoplast (xylem conduits and cell wall space) is driven solely by pressure differences.

Therefore, in non-transpiring, well-watered plants, equilibration between xylem and apoplast Ψ_p and between symplast and apoplast Ψ_w is expected. High solute concentration in the leaf apoplast would lower leaf apoplastic Ψ_w to a value below that of xylem Ψ_p. High solute concentrations in the leaf apoplast would not be expected to dissipate overnight into the xylem lumen, and thus reduce stem xylem Ψ_s, because of relatively low permeability in unstirred layers of intercellular and cell wall spaces and large diffusional distances between the leaf apoplast and stem xylem lumen (Carpita, 1982; Grignon and Sentenac, 1991; Jungk, 1991). If the leaf symplast Ψ_w is in equilibrium with leaf apoplast Ψ_w, then leaf Ψ_w would be significantly lower than stem Ψ_p, thus contributing to the observed PDD between leaf Ψ_w and stem Ψ_p and between leaf and soil Ψ_w (Donovan *et al*., 1999). Under these conditions the Ψ_w difference between the stem xylem and the leaf apoplast would not drive water flow because the pressure difference remains zero. The apoplastic solute accumulation mechanism may be of high magnitude for halophytes growing in saline soils, with Na^+ accumulating as the major solute in the leaf apoplast, but a similar mechanism could explain smaller predawn differences that have been observed between stem xylem Ψ_p and leaf Ψ_w in glycophytes and halophytes growing in non-saline soils (Donovan *et al*., 2001). Here K^+ might be expected to be the major solute in the leaf apoplast.

While solute accumulation in the leaf apoplast during daytime transpiration has been documented in both glycophytes and halophytes (Meinzer and Moore, 1988; Flowers *et al*., 1991; Canny, 1993), it is not known if high predawn concentrations of leaf apoplastic solutes contribute to the large differences (0.5–2.3 MPa) between predawn leaf and soil Ψ_w observed in halophytes (Donovan *et al*., 2001). A better understanding of predawn water and ionic relations in the leaf apoplast is essential for refining predictions of current water relations models. In addition, quantifying the potential magnitude of apoplastic solute concentration and how it may vary with soil salinity could improve understanding of turgor regulation and nutrient relations of halophytes.

The main objective of this study was to determine if high solute accumulation in the leaf apoplast could be a driver of significant PDD documented in well-watered, non-transpiring halophytes. The halophyte *Sarcobatus vermiculatus* (Hook.) Torrey (Chenopodiaceae) was used as a model because both greenhouse and field experiments have demonstrated large PDD in this species even when other mechanisms known to contribute to PDD have been minimized. In field experiments, *Sarcobatus* predawn leaf Ψ_w was 0.9 MPa more negative than stem xylem Ψ_p. In greenhouse experiments, predawn difference between leaf Ψ_w and xylem Ψ_p was 1.22 MPa in plants growing in soils watered with 100 mM NaCl but lower, around 0.5 MPa, for plants growing in non-saline soils (Donovan *et al*., 1999, 2003). Based on these measurements it was predicted that: (i) predawn solute potential in the leaf apoplast would be substantial, resulting in a significantly lower apoplastic Ψ_s than stem xylem Ψ_s; (ii) the major solute contributing to differences in predawn leaf apoplast and stem xylem Ψ_s would be Na^+; and (iii) leaf apoplastic Na^+ concentration and corresponding magnitude of leaf apoplastic Ψ_s, however, would be a function of soil salinity.

The estimation of water relations and the ionic composition of the leaf apoplast is notoriously difficult (Cosgrove

and Cleland, 1983; Yu *et al*., 2000) so two experimental approaches were used to address these objectives. In the first experiment the magnitude of apoplastic Ψ_s was estimated by calculating the difference between leaf symplast and stem xylem Ψ_w (Murphy and Smith, 1994). For these calculations, leaf cell Ψ_p was measured directly with a cell pressure probe and stem xylem Ψ_p with a pressure chamber. Leaf cell Ψ_s and stem xylem Ψ_s were measured with a cryoscopic osmometer. In the second experiment, apoplastic fluid was isolated and concentrations of major apoplastic cations were quantified (Lohaus *et al*., 2001), allowing estimates of ion contributions to apoplastic Ψ_s.

Materials and methods

Experiment 1: Leaf symplast and xylem water relations

Plant materials and treatments: *Sarcobatus* is a phreatophytic, C_3 shrub that readily establishes on saline soils throughout the Great Basin Desert of North America. Naturally established seedlings of *Sarcoabtus* were collected from the Mono Basin Ecosystem Research Site north of Mono Lake, California, USA, (38° 5′ N, 118° 56′ W, 1958 m elevation) (Toft, 1995; Donovan *et al*., 1996; Drenovsky and Richards, 2005) and transplanted individually into 4.0 l, 35 cm deep, Tree Pots™ (Stuewe and Sons, Corvallis, OR) with a 3:1 v:v fritted clay:sand medium. Seedlings were grown in a greenhouse at the University of California, Davis campus and received quarter-strength modified Hoagland's solution (Epstein, 1972) four or five times each week. Two months after transplanting, experimental plants were randomly assigned to receive one of three levels of NaCl (0, 100, 300 mM) added to the nutrient solution. The 450 mM NaCl treatment used in Experiment 2 (see below), was not included in the water relations measurements due to logistical constraints associated with the amount of time needed to measure each water relations parameter. Background Na^+ levels in the greenhouse watering system were <0.5 mM. Plants were watered with the corresponding salinity treatment for 3 months before measurements were initiated.

Water potential measurements: The evening before measurements were made, plant canopies were bagged during the night to prevent night-time transpiration completely and soils were watered to field capacity (Donovan *et al*., 1999, 2003; Snyder *et al*., 2003). All measurements were made on mature leaves that had emerged and expanded during the salinity treatments. Predawn measurements were made between 04.30 h and 05.00 h. Midday measurements were made between 13.30 h and 14.30 h.

Leaf cell turgor (Ψ_p) was measured using a cell pressure probe (Husken *et al*., 1978). Glass microcapillary tips were prepared as described in Shackel *et al*. (1987). Briefly, borosilicate glass was pulled on a pipette puller (Model 750, Kopf, Tujunga, CA) and bevelled using a modified jet stream microbeveller (Ogden *et al*., 1978). Tips viewed through a microscope (×100) were aligned in a stream of 0.05 μm alumina grinding compound solution for a total of 1–2 min with pressurized air (~0.7 MPa) applied to the non-bevelled end to prevent entry of grinding compound into the open tip. Tips were washed in distilled water to remove contaminants adhering to the glass surface. Prior to measurements, pipettes were filled with silicone oil (SF 96/50, Thomas Scientific, Swedesboro, NJ), and attached to a piezo-controlled micromanipulator (Leica Microsystems AG, Wetzlar, Germany) set to a motor speed of 25 μm s^{-1} and the smallest possible step size (0.5 μm). The micropipette tip and leaf cells were viewed through a microscope (×600) equipped with a vertical illuminator (BHMJ System, Olympus Corp., Melville, NY) and a long working distance (11 mm, ×20 objective, 1-LM546, Olympus Corp.). A video monitor interfaced with the microscope facilitated manipulation of the micropipette and adjustment of the meniscus. Measurements were made on both epidermal and mesophyll cells; results were analysed separately but were pooled for leaf-level water relations comparisons because differences between cell types were small and non-significant under the conditions of these experiments (see below and Results). Cells were penetrated to a distance of 10–15 μm and the oil pressure in the micropipette was adjusted to bring the oil/cell sap meniscus to within ~5 μm of the cell surface. For mesophyll cell measurement, the micropipette was slowly inserted through the epidermus with the oil under pressure (~0.1 MPa) until a meniscus was re-established. Ψ_p was recorded only when the probe pressure had stabilized for 10 min, indicating that cell membranes remained intact (Shackel *et al*., 1987). Measurements of predawn and midday leaf cell Ψ_p were made on 3–6 plants for each salinity treatment. Within each replicate, 3–4 cells each from epidermis and mesophyll, were measured to assess and account for cell–cell heterogeneity.

Leaf cell solute potential (Ψ_s) measurements were made using a direct-reading nanolitre osmometer (Clifton Technical Physics, Hartford, NY). Cell sap from both epidermal and mesophyll cells was collected by aspiration into glass micropipettes using the pressure probe apparatus described above. Samples were discharged into the middle of a reservoir on a silver platform containing immersion oil such that the diameter of the globular sample was typically half that of the reservoir diameter. The platform was pressed onto a cooling stage using thermal grease, and the freezing point was determined by viewing the sample through a microscope. Measurements of predawn and midday leaf cell Ψ_s were made on 4–7 plants for each salinity treatment. Cell sap samples of mesophyll and epidermal cells for each replicate plant were composited separately from 4–10 cells of each type.

Bulk leaf Ψ_s was measured on leaves following measurements of cell Ψ_p and Ψ_s. Each leaf was excised from the stem, rinsed with distilled water, and blotted dry. The leaf was then ground in a piston press, and expressed sap was drawn into a syringe. The sap was immediately loaded onto paper discs, and the bulk leaf Ψ_s was measured using a Wescor 5500 vapour pressure osmometer (Wescor Inc., Logan, UT). Measurements of predawn and midday bulk leaf Ψ_s were made on 3–7 plants for each salinity treatment.

Stem xylem pressure (Ψ_p) was measured using a pressure chamber (PMS Inc., Corvallis, OR). Measurements of predawn xylem Ψ_p were made on 4–7 plants for each salinity treatment. Xylem solute potential (Ψ_s) was measured by expressing xylem sap (approximately 5−10 μl, depending on stem size) from the cut end of a stem in the pressure chamber, collecting the sap on paper discs, and measuring Ψ_s using the vapour pressure osmometer. Standard precautionary steps to minimize contamination and errors in the xylem Ψ_p and Ψ_s measurements were followed (Turner, 1988; Boyer, 1995) which included removal of phloem around the cut surface before measurements were made. Predawn xylem Ψ_s was measured on 4–7 plants for each salinity treatment. Soil water potential (Ψ_w) was measured with individually calibrated screen-cage thermocouple psychrometers installed in the centre of the pots and monitored hourly with a data logger (Richards and Caldwell, 1987).

Experiment 2: Leaf apoplastic solutes

Plant materials and treatments: Seedlings of *Sarcobatus* were collected from the same location and transplanted using pots and soil media as the plants in Experiment 1. After establishment in an unheated greenhouse at the University of California Davis, plants were randomly grouped into five experimental blocks. Because extraction of apoplastic fluid was only conducted during predawn hours (04.00–06.00 h; see below) it was not possible to harvest all

experimental plants at the same time. Blocks were therefore used to account for variation that occurred through time as samples were harvested successively through a 25 week period. Within each block, 40–60 plants were randomly assigned to receive one of four salinity levels (0, 100, 300, 450 mM NaCl) and 6–12 plants per treatment per block were harvested.

The initiation of the salinity treatments for each block was staggered by approximately 3 weeks to allow sufficient time to harvest each block under predawn conditions and to ensure that all plants were exposed to salinity treatments for a similar duration. For the salinity treatments, all plants within a block were moved from the greenhouse to a controlled environment facility. Plants were exposed to an average day/night temperature of 30/8 °C and a photoperiod of 14 h (800 μmol m^{-2} s^{-1} PPFD) for 6 weeks. Plants were watered twice a week with the assigned salinity treatment. Essential nutrients were supplied every other week as one-quarter strength modified Hoagland's solution (Epstein, 1972).

Collection and analysis of apoplastic fluid: The vacuum infiltration technique, slightly modified from the method of Mühling and Sattelmacher (1995) and Lohaus *et al*. (2001) was used to isolate leaf apoplastic fluid. Extraction of apoplastic fluid began after the replicates within a block had been exposed to salinity treatments for 6 weeks. The apoplastic fluid from all replicates within a block was extracted within a week between 04.00–06.00 h. Approximately 4 g FW of fully expanded leaves were cut at the base of the petiole from the plant with a razor blade, rinsed with deionized water, blotted dry, and were then vacuum infiltrated for 5 min with either 250 mM sorbitol to quantify concentrations of unbound apoplastic cations or 100 mM $BaCl_2$ to quantify concentrations of bound and unbound apoplastic cations. The difference in apoplastic cation concentration between the two infiltration media provides an estimate of bound apoplastic cations. The infiltrated leaves were gently blotted dry, divided into four subsamples, and leaves in each subsample were placed, petiole up, into a 15 ml centrifuge tube. The centrifugation was carried out at 600 *g* at 4 °C for 6 min. The apoplastic fluid was stored at −25 °C until analysis. Contamination of apoplastic fluid with symplastic fluid was assessed by comparing total soluble protein concentration in samples centrifuged at 600 *g* and 6000 *g* (Wimmer *et al*., 2003). Total soluble protein concentration in samples centrifuged at 600 *g* was less than 5% of the total protein concentration in samples centrifuged at 6000 *g* (18±7.1 and 382±30.1 μg ml^{-1}, respectively; mean ±SE, *n*=30) suggesting that cytoplasmic contamination of apoplastic fluid was minimal with the centrifugation force applied.

Concentrations of Na^+, K^+, and Ca^{2+} in the apoplast and leaf sap were analysed with an ion chromatograph (Model DX4500i, Dionex, Sunnyvale, CA) using a cation exchange column (AS12, Dionex) connected to a conductivity detector. Isocratic elution with methanesulphonic acid (20 mmol l^{-1}, 1 ml min^{-1}) was used for the separation of cations. To prevent interference with organic components samples were first filtered through a 0.2 μm membrane filter. Calculation of the ion concentration in the apoplastic water space was determined by multiplying the ion concentration of the fluid extracted with sorbitol with a dilution factor (Lohaus *et al*., 2001) which was determined using the silicone oil method (Cosgrove and Cleland, 1983) and the indigo carmine method (Husted and Schjoerring, 1995) for apoplastic air and water, respectively.

Statistical analysis

Effects of soil salinity on leaf and xylem water relations and leaf apoplastic Na^+, K^+, and Ca^{2+} concentrations were analysed using ANOVA. Assumptions of ANOVA were evaluated using Shapiro–Wilk test for normality and Levene's test for homogeneity of variance. When these assumptions were violated, ANOVA models were weighted by the inverse of the variance (Neter *et al*., 1990). Following ANOVA, differences between treatments were determined using the Ryan–Einot–Gabriel–Welsch multiple range test (SAS, 2001).

Results and discussion

Experiment 1: Leaf symplast and xylem water relations

In all treatments *Sarcobatus* exhibited significant predawn disequilibria of large magnitude. Under well-watered conditions with canopies bagged during the night to eliminate night-time canopy water loss completely, *Sarcobatus* predawn leaf symplast Ψ_w was 1.70–2.37 MPa more negative than predawn soil Ψ_w with PDD between leaf and soil water potential increasing with higher substrate salinity (Table 1). These results are largely consistent with field and greenhouse studies that have demonstrated large PDD based on discrepancies between psychrometric measurements of predawn soil and bulk leaf Ψ_w. For example, under controlled environmental conditions *Sarcobatus* bulk

Table 1. *Predawn leaf symplast (cell measurements) and stem xylem water (Ψ_w), solute (Ψ_s) and pressure (Ψ_p) potential (MPa) for* Sarcobatus *growing in three salinity treatments (mean ±SE, see methods for sample size)*

Symplast Ψ_s and Ψ_p include measurements of both epidermal and mesophyll cells (see Materials and methods for experimental approach and measurement techniques; see also Table 2). All plant canopies were bagged during the night period before predawn measurements to minimize night-time shoot water loss. Different letters indicate significantly different predawn Ψ_w between leaf symplast, stem xylem, and soil within a salinity treatment (P <0.05). Based on water relations theory, leaf apoplastic Ψ_w was assumed to be in equilibrium with leaf symplast Ψ_w (indicated by = = =). Likewise, leaf apoplastic Ψ_p also was assumed to be in equilibrium with stem xylem Ψ_p (indicated by = = =). Based on these equilibrium assumptions which match experimental conditions, the contribution of apoplastic solute potential Ψ_s to apoplastic water potential was estimated (shown in parentheses).

Treatment (mM NaCl)		Leaf symplast (MPa)	Leaf apoplast (MPa)	Stem xylem (MPa)	Soil (MPa)
0	Ψ_w	−1.71±0.07 a = = = = =	−1.71	−1.07±0.08 b	−0.01±0.01 c
	Ψ_s	−1.95±0.07	(−0.75)	−0.16±0.02	
	Ψ_p	0.32±0.03	−0.96 = = = = = = =	−0.96±0.08	
100	Ψ_w	−2.61±0.11 a = = = = =	−2.61	−1.23±0.06 b	−0.45±0.01 c
	Ψ_s	−3.03±0.09	(−1.57)	−0.20±0.05	
	Ψ_p	0.39±0.01	−1.03 = = = = = = =	−1.03±0.06	
300	Ψ_w	−3.65±0.11 a = = = = =	−3.65	−1.74±0.69 b	−1.28±0.16 c
	Ψ_s	−3.93±0.08	(−2.12)	−0.24±0.05	
	Ψ_p	0.34±0.01	−1.53 = = = = = = =	−1.53±0.07	

leaf predawn Ψ_w was around 1.4 MPa more negative than soil Ψ_w, but in the field was up to 2.7 MPa more negative than soil Ψ_w (Donovan *et al*., 1999, 2003). Similarly, under controlled environmental conditions bulk leaf predawn water potentials were 2 and 1 MPa more negative than soil Ψ_w for the Tunisian desert shrubs *Artemisia herba-alba* and *Anthyllis henoniana*, respectively (Ourcival and Berger, 1995). This work extends these findings by demonstrating that large PDD exists between individual leaf cell symplastic Ψ_w and stem xylem Ψ_w under well-watered, uniform soil conditions and when shoot water loss is eliminated.

Predawn leaf symplast Ψ_w also was significantly more negative than predawn xylem Ψ_w in all treatments (Table 1). Under experimental conditions that eliminated night-time shoot and root water loss, Ψ_w of the leaf symplast and adjacent leaf apoplast should equilibrate overnight. Similarly, equilibration between the leaf apoplast Ψ_p and xylem Ψ_p is assumed because these areas are not separated by a membrane. Under these experimental conditions and equilibrium assumptions, the large discrepancy between predawn xylem Ψ_p and leaf symplast Ψ_w suggests a predawn leaf apoplastic Ψ_s ranging from -0.75 to -2.12 MPa (Table 1). These calculated predawn leaf apoplastic Ψ_s values are more negative with increasing soil salinity. Further, these predawn leaf apoplastic Ψ_s values are substantially more negative than the xylem Ψ_s of *Sarcobatus* observed in this experiment (Table 1) and measured in the field (Donovan *et al*., 1996). Equilibration between leaf apoplast and xylem Ψ_s is not necessarily expected because of the relatively large diffusional distance between these areas and the low diffusion rates of ions in the leaf apoplast (Carpita, 1982; Jungk, 1991; Canny, 1995). Previous studies on actively growing tissues of crop plants have demonstrated that apoplastic Ψ_s can be as much as 0.3 MPa lower than xylem Ψ_s during the daytime when transpiration rates are high (Cosgrove and Cleland, 1983; Meinzer and Moore, 1988). These results suggest that even for mature tissue under minimal transpiration conditions, predawn leaf apoplast Ψ_s can be very low and that predawn leaf apoplast Ψ_s does not equilibrate with predawn xylem Ψ_s. As a consequence, low leaf apoplast Ψ_s reduces predawn leaf apoplastic Ψ_w to a value significantly lower than predawn xylem Ψ_w.

As expected, predawn leaf symplast Ψ_w decreased with increasing salinity (Table 1). The effect of salinity on cell water relations, however, was similar between mesophyll and epidermal cells under the minimal transpiration conditions of these experiments (Table 2). Although cell Ψ_p declined during the day with transpirational water loss, cell Ψ_p remained remarkably consistent between cell types and substrate salinities. As a result, for both cell types the decline in cell Ψ_w with increasing salinity was largely due to a decline in Ψ_s but not cell Ψ_p. Fricke *et al*. (1994) and Fricke (1997) demonstrated that Ψ_p in barley leaves was largely unaffected by substrate salinity and that, while cell Ψ_s declined with increasing salinity, changes in Ψ_s were similar between epidermal and mesophyll cells. Also consistent with these observations, Clipson *et al*. (1985) demonstrated that cell Ψ_p in the halophyte *Suaeda maritima* was not affected by soil salinity ranging from 0 to 400 mM NaCl despite large changes in cell Ψ_s. Although spatial gradients in Ψ_p and Ψ_s in leaf tissue are commonly reported during daytime measurements, the uniformity in epidermal and mesophyll Ψ_p, as well as the close agreement between bulk leaf Ψ_s and the Ψ_s of individual cells during predawn measurement conditions (Table 2), indicates that leaf cell Ψ_w is relatively homogenous within these mature leaves and that this estimate of leaf cell Ψ_w is a good measure of bulk leaf Ψ_w.

Accounting for the entire difference between predawn soil and leaf Ψ_w still requires the large predawn discrepancy between soil and xylem Ψ_w to be explained. In this experiment, predawn xylem Ψ_w was about 1 MPa more negative than soil Ψ_w (Table 1). This study's experimental conditions of bagging and homogeneous soil moisture around the entire root system eliminated night-time transpiration and hydraulic redistribution. Yet pressure chamber readings indicated tension remaining in the stem xylem. While similar observations of large PDD between soil and xylem Ψ_w have been observed in a range of species (Donovan *et al*., 2001) the mechanisms driving this disequilibrium remain unknown. One possibility is that this disequilibrium is caused by high concentrations of apoplastic solutes in the root intercellular spaces (Stirzaker and Passioura, 1996; Donovan *et al*., 1999). Data on root water potential components were not quantified in this study. Such data are needed, however, to account for the soil–xylem discrepancy that was observed and to evaluate mechanisms contributing to this difference.

Experiment 2: Leaf apoplastic solutes

Predawn leaf apoplastic Na^+ and K^+ concentrations were substantial in all treatments (Fig. 1). These leaf apoplastic Na^+ and K^+ concentrations were 25- and 5-fold greater, respectively, than *Sarcobatus* xylem ion concentrations reported previously (Donovan *et al*., 1996). Leaf apoplastic Na^+ significantly increased in *Sarcobatus* plants receiving additional NaCl in the watering solution (P=0.007), but this increase did not significantly differ among the 100, 300, and 450 mM salinity treatments (Fig. 1). Leaf apoplastic K^+ was not significantly affected by soil salinity (P >0.05). Apoplastic Na^+ and K^+ did not increase significantly when leaves were infiltrated with $BaCl_2$ compared with when leaves were infiltrated with sorbitol (Fig. 2; P >0.05) whereas Ca^{2+} concentrations did increase (P <0.05), suggesting that the majority of Na^+ and K^+ in the apoplast is soluble while the majority of Ca^{2+} is tightly sorbed to fixed anions on the cell walls (Mühling and Sattelmacher, 1995; Mühling and Läuchli, 2002).

Table 2. *Predawn and midday leaf epidermal and mesophyll cell pressure (Ψ_p) and solute (Ψ_s) potentials (mean ±SE) for* Sarcobatus *growing under three salinity treatments*

Measurements for cell Ψ_p and Ψ_s were made using a cell pressure probe and nanoliter osmometer, respectively. Bulk leaf tissue was sampled for Ψ_s to compare with individual cell measurements but bulk leaf tissue Ψ_p was not measured (NM).

Treatment (mM NaCl)	Cell type or bulk	Predawn		Midday	
		Ψ_p	Ψ_s	Ψ_p	Ψ_s
0	Epidermal	0.31±0.04	−2.05±0.15	0.25±0.05	−2.48±0.18
	Mesophyll	0.33±0.05	−1.91±0.08	0.34±0.08	−2.16±0.14
	Bulk	NM	−1.88±0.14	NM	−2.32±0.21
100	Epidermal	0.38±0.03	−2.94±0.24	0.25±0.08	−3.02±0.29
	Mesophyll	0.40±0.01	−3.05±0.03	0.21±0.07	−2.82±0.23
	Bulk	NM	−3.11±0.16	NM	−3.14±0.23
300	Epidermal	0.35±0.02	−4.09±0.06	0.20±0.02	−3.95±0.17
	Mesophyll	0.33±0.03	−3.88±0.23	0.22±0.03	−3.88±0.24
	Bulk	NM	−3.27±0.10	NM	−3.68±0.11

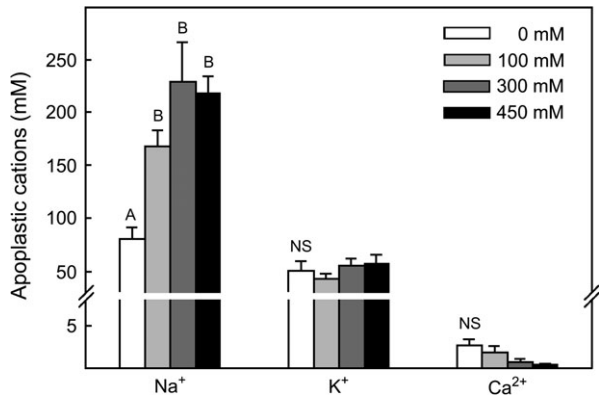

Fig. 1. Concentration of soluble Na^+, K^+, and Ca^{2+} in the leaf apoplast of *Sarcobatus* growing in four salinity treatments (mean ±SE, *n*=30–55). Each replicate measurement was made on approximately 4 g FW of leaves infiltrated with 250 mM sorbitol and centrifuged at 600 *g* for 6 min. Letters indicate significant differences in cation concentration (P <0.05) between salinity treatments. Note scale change across *y*-axis break.

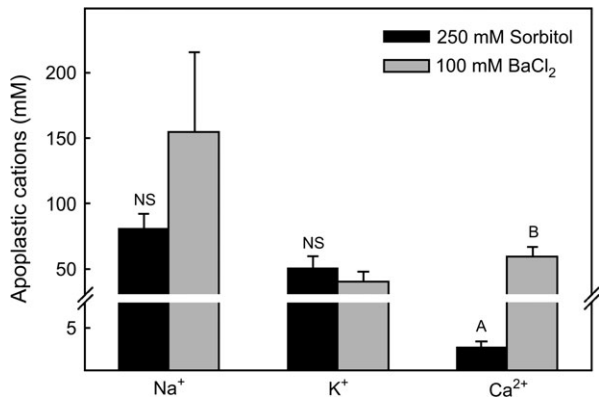

Fig. 2. Concentration of soluble (250 mM sorbitol infiltration solution) and exchangeable plus soluble (100 mM $BaCl_2$ infiltration solution) Na^+, K^+, and Ca^{2+} in the leaf apoplast of *Sarcobatus* (mean ±SE, *n*=44–86). Values for the two different infiltration solutions were averaged across the four salinity treatments. Letters indicate significant differences in cation concentration (P <0.05) between infiltration solutions. Note scale change across *y*-axis break.

The relatively high leaf apoplastic K^+ and Na^+ under low salinity and the substantial increase in apoplastic Na^+ at high salinity strongly suggests that these ions are major drivers of the low apoplastic Ψ_s of *Sarcobatus* observed in the first experiment and contribute to the large PDD observed in this species in previous greenhouse and field studies. Using the van't Hoff relation, and assuming that Na^+ and K^+ completely dissociate with Cl^- in the apoplast, the estimated combined Ψ_s of these ions is about −0.6 MPa in the 0 mM NaCl treatment and as low as −1.4 MPa with 300 mM NaCl. While other apoplastic solutes such as sugars and amino acids probably further reduce Ψ_s of the leaf apoplast in *Sarcobatus* (Lohaus *et al.*, 2001), the contribution of Na^+ and K^+ alone corresponds to 80% and 65% of the predawn apoplastic Ψ_s calculated in Experiment 1 for plants grown in the 0 and 300 mM salinity treatments, respectively. While a similar pattern of apoplastic ion concentrations may be expected for other halophytes, low predawn apoplastic Ψ_s observed in glycophytes, which tend to exclude Na^+ at the root surface and sequester what Na^+ is absorbed in roots and stems (Läuchli and Epstein, 1990), are probably due to high K^+ or other solute (e.g. sugars) levels.

While the concentration of leaf apoplastic Na^+ and K^+ observed in *Sarcobatus* under predawn conditions were much higher than values typically found in crop plants during daytime conditions (Lohaus *et al*., 2001; Mühling and Läuchli, 2002; Wimmer *et al*., 2003), high apoplastic solute concentrations have been reported during daytime measurements in some systems. For example, when rice (*Orzya sativa*) and *Suaeda maritima* are grown under salinity stress, apoplastic Na^+ can reach concentrations exceeding 500 mM as a result of the evaporative separation of water and Na^+ in the leaf apoplast during daytime transpiration (Flowers and Yeo, 1986; Flowers *et al*., 1991). Similarly, Canny (1995) documented leaf apoplastic K^+ levels ranging from 20 to 200 mM in *Helianthus annuus* during the daytime. In *Helianthus*, however, K^+ did not appear to accumulate in the transpirational stream. Instead,

high apoplastic K^+ levels appeared to be a result of rapid symplastic influx and efflux of K^+ and the recirculation of K^+ between the leaf symplast and phloem.

These results suggest that high apoplastic ion concentrations in *Sarcobatus* could result from either residual build-up from daytime transpiration or ion transport between the symplast and apoplast. In the field, however, the difference between predawn leaf and xylem Ψ_w was similar between *Sarcobatus* plants that had canopies bagged overnight to eliminate night-time transpiration and plants where canopies were left unbagged, indicating similar predawn leaf apoplastic Ψ_s (Donovan *et al*., 2003). This suggests that the high apoplastic Na^+ and K^+ levels in *Sarcobatus* are not a simple function of transpiration rate but may be highly regulated by ion transport between the symplast and apoplast. If ion concentrations in this compartment are regulated, then the logical question remains whether there is a functional role for high apoplastic solute concentrations or if this trait is a simple by-product related to some other aspect of ion physiology or metabolism.

Regulation of Na^+ and K^+ concentrations in the leaf apoplast might be a mechanism allowing desert shrubs and halophytes to regulate Ψ_p in the leaf symplast (Tomos, 1988; Tomos *et al*., 1992). While much work has focused on understanding how plant cells increase or maintain Ψ_p during drought or high soil salinity by accumulation of compatible solutes and/or ions in the symplast, there has been little recognition of how high apoplastic solute concentrations might prevent the occurrence of excessive Ψ_p if leaves accumulate substantial quantities of osmotica. This may be the case for *Sarcobatus*. For example, even under low-to-moderate salinity, Na^+ concentrations in leaves of *Sarcobatus* can exceed 8–10% in field- and greenhouse-grown plants (Richards, 1994; Donovan *et al*., 1997). In this experiment, this high Na^+ accumulation resulted in predawn leaf Ψ_s ranging from -1.95 to -3.93 MPa (Table 1). If the leaf mesophyll and epidermal cells equilibrated with the Ψ_w of the xylem without the contribution of leaf apoplastic solutes, cell Ψ_p would be expected to increase from 0.32 MPa to 0.88 MPa under 0 mM NaCl while cell Ψ_p would increase from 0.34 MPa to 2.2 MPa under 300 mM NaCl. Turgor pressure of well-watered plant cells generally ranges from 0.3 to 1.0 MPa (Tomos, 1988). In this experiment, Ψ_p in mesophyll and epidermal cells averaged about 0.35 MPa and remained remarkably constant over a range of external salinities despite large decreases in cell Ψ_s. This suggests that, even under moderate soil salinity, the predawn Ψ_p that *Sarcobatus* would have to achieve to equilibrate with xylem Ψ_w without the contribution of apoplastic solutes would probably be too high for mature leaf cell function and could cause cell damage or inhibit cell–cell transport processes (Oparka and Dam, 1992; Moreshet *et al*., 1999). Because of the small volume of the apoplast relative to the symplast, regulation of solute concentration in this compartment may be a very efficient means to regulate Ψ_p. Similarly, Matthews and Shackel (2005) proposed that apoplastic solutes are important in preventing the occurrence of excessive Ψ_p in fleshy fruits, which accumulate substantial concentrations of sugars (–3.5 MPa for grapes) as part of normal development, even under irrigated conditions.

Conclusion

Taken together, these experiments demonstrate that ion concentrations in the leaf apoplast can be substantial in a model halophyte, greatly reducing Ψ_s in the leaf apoplast and creating a large disequilibrium between predawn leaf and xylem Ψ_w, which contrasts with classical expectations of predawn plant–soil water relations. Regulating ion concentrations in the leaf apoplast appears to be an important turgor regulation mechanism in halophytes and arid-land plants that accumulate high concentrations of osmotica in the symplast to maintain Ψ_p and high stomatal conductance during the daytime (Flowers *et al*., 1977; Romo and Haferkamp, 1989) but then need to prevent excess Ψ_p from occurring during the night-time when transpiration decreases and xylem Ψ_w increases. In addition to diurnal regulation of Ψ_p, rapid regulation of symplastic Ψ_p through apoplastic osmotic adjustments may be particularly important in ecological situations when plants that have developed high levels of compatible solutes in leaves experience rapid increases in soil Ψ_w. This would happen, for example, when roots of salt-accumulating arid-land riparian (e.g. *Tamarisk* spp.) and phreatophytic plants (e.g. *Sarcobatus*) access relatively fresh groundwater or experience large pulses of summer rain. This mechanism of Ψ_p regulation is also predicted to be important in salt-marsh species exposed to variable fresh water and salt water inputs. Further work is need, however, to understand the extent that apoplastic solutes prevent excess Ψ_p when water status recovers overnight and how this can vary between species and environmental conditions.

Acknowledgements

We thank R Drenovsky, R O'Dell, and B Richards for help with plant collection and laboratory work. This research was supported by NSF grants IBN-9816670 to LA Donovan and IBN-9903004 and IBN-0416581 to JH Richards. Additional support was provided by the California Agricultural Experiment Station and an NSF Graduate Fellowship to NN Alder. JH Richards also thanks the UC Valentine Eastern Sierra Reserve for support.

References

Améglio T, Archer P. 1996. Représentativité du potentiel de base sur sols à humidité hètèrogéne. *Agronomie* **16,** 493–503.

Blake J, Ferrell WK. 1977. Association between soil and xylem water potential, leaf resistance, and abscisic acid content in

droughted seedlings of Douglas-Fir (*Pseudotsuga menziesii*). *Physiologia Plantarum* **39,** 106–109.

Boyer JS. 1995. *Measuring the water status of plants and soils.* San Diego: Academic Press.

Bucci SJ, Scholz FG, Guillermo G, Meinzer FG, Hinojosa JA, Hoffmann WA, Agusto CF. 2004. Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiology* **24,** 1119–1127.

Canny MJ. 1993. The transpiration stream in the leaf apoplast: water and solutes. *Philosophical Transactions of the Royal Society of London, Series B-Biological Sciences* **341,** 87–100.

Canny MJ. 1995. Apoplastic water and solute movement: new rules for an old space. *Annual Review of Plant Physiology and Plant Molecular Biology* **46,** 215–236.

Carpita NC. 1982. Limiting diameters of pores and the surface structure of plant cell walls. *Science* **218,** 813–814.

Clipson NJW, Tomos AD, Flowers TJ, Jones RGW. 1985. Salt tolerance in the halophyte *Suaeda maritima* L Dum: the maintenance of turgor pressure and water potential gradients in plants growing at different salinities. *Planta* **165,** 392–396.

Cosgrove DJ, Cleland RE. 1983. Solutes in the free space of growing stem tissues. *Plant Physiology* **72,** 326–331.

Davis SD, Mooney HA. 1986. Water use patterns of four co-occurring chaparral shrubs. *Oecologia* **70,** 172–177.

Donovan LA, Ehleringer JR. 1994. Water stress and use of summer precipitation in a Great Basin shrub community. *Functional Ecology* **8,** 289–297.

Donovan LA, Grise DJ, West JB, Pappert RA, Alder NN, Richards JH. 1999. Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. *Oecologia* **120,** 209–217.

Donovan LA, Linton MJ, Richards JH. 2001. Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. *Oecologia* **129,** 328–335.

Donovan LA, Richards JH, Muller MW. 1996. Water relations and leaf chemistry of *Chrysothamnus nauseosus* ssp *consimilis* (Asteraceae) and *Sarcobatus vermiculatus* (Chenopodiaceae). *American Journal of Botany* **83,** 1637–1646.

Donovan LA, Richards JH, Linton MJ. 2003. Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials. *Ecology* **84,** 463–470.

Donovan LA, Richards JH, Schaber EJ. 1997. Nutrient relations of the halophytic shrub, *Sarcobatus vermiculatus*, along a soil salinity gradient. *Plant and Soil* **190,** 105–117.

Drenovsky RE, Richards JH. 2005. Low N and P resorption correlates with nutrient limitation in two desert shrubs. *Plant Ecology* (in press).

Epstein E. 1972. *Mineral nutrition of plants: principles and perspectives.* New York: Wiley.

Filella I, Peñuelas J. 2003. Partitioning of water and nitrogen in co-occurring Mediterranean woody shrub species of different evolutionary history. *Oecologia* **137,** 51–61.

Flowers TJ, Hajibagheri MA, Yeo AR. 1991. Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for the Oertli hypothesis. *Plant, Cell and Environment* **14,** 319–325.

Flowers TJ, Troke PF, Yeo AR. 1997. The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology* **28,** 89–121.

Flowers TJ, Yeo AR. 1986. Ion relations of plants under drought and salinity. *Australian Journal of Plant Physiology* **13,** 75–91.

Fricke W. 1997. Cell turgor, osmotic pressure and water potential in the upper epidermis of barley leaves in relation to cell location and in response to NaCl and air humidity. *Journal of Experimental Botany* **48,** 45–58.

Fricke W, Leigh RA, Tomos AD. 1994. Concentrations of inorganic and organic solutes in extracts from individual epidermal, mesophyll and bundle sheath cells of barley leaves. *Planta* **192,** 310–316.

Grignon C, Sentenac H. 1991. pH and ionic conditions in the apoplast. *Annual Review of Plant Physiology and Plant Molecular Biology* **42,** 103–128.

Husken D, Steudle E, Zimmermann U. 1978. Pressure probe technique for measuring water relations of cells in higher plants. *Plant Physiology* **61,** 158–163.

Husted S, Schjoerring JK. 1995. Apoplastic pH and ammonium concentration in leaves of *Brassica napus* L. *Plant Physiology* **109,** 1453–1460.

James JJ, Tiller RL, Richards JH. 2005. Multiple resources limit plant growth and function in a saline-alkaline desert community. *Journal of Ecology* **93,** 113–126.

Jungk AO. 1991. Dynamics of nutrient movement at the soil–root interface. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots, the hidden half.* New York: Marcel Dekker, 455–481.

Läuchli A, Epstein E. 1990. Plant response to saline and sodic conditions. In: Tanji KK, ed. *Agricultural salinity assessment and management.* New York: American Society of Civil Engineers, 113–137.

Lohaus G, Pennewiss K, Sattelmacher B, Hussmann M, Mühling KH. 2001. Is the infiltration-centrifugation technique appropriate for the isolation of apoplastic fluid? A critical evaluation with different plant species. *Physiologia Plantarum* **111,** 457–465.

Matthews MA, Shackel KA. 2005. Growth and water transport in fleshy fruits. In: Holbrook NM, Zwieniecki MA, eds. *Vascular transport in plants.* Boston: Elsevier Academic Press, 189–197.

Mediavilla S, Escudero A. 2003. Mature trees versus seedlings: differences in leaf traits and gas exchange patterns in three co-occurring Mediterranean oaks. *Annals of Forest Science* **60,** 455–460.

Meinzer FC, Moore PH. 1988. Effect of apoplastic solutes on water potential in elongating sugarcane leaves. *Plant Physiology* **86,** 873–879.

Mitchell RJ, Zutter BR, Green TH, Perry MA, Gjerstad DH, Glover GR. 1993. Spatial and temporal variation in competitive effects on soil moisture and pine response. *Ecological Applications* **3,** 167–174.

Moreshet S, Yao C, Aloni B, Karni L, Fuchs M, Stanghellini C. 1999. Environmental factors affecting the cracking of greenhouse-grown bell pepper fruit. *Journal of Horticultural Science and Biotechnology* **74,** 6–12.

Mühling KH, Läuchli A. 2002. Effect of salt stress on growth and cation compartmentation in leaves of two plant species differing in salt tolerance. *Journal of Plant Physiology* **159,** 137–146.

Mühling KH, Sattelmacher B. 1995. Apoplastic ion concentration of intact leaves of field bean (*Vicia faba*) as influenced by ammonium and nitrate nutrition. *Journal of Plant Physiology* **147,** 81–86.

Murphy R, Smith JAC. 1994. A critical comparison of the pressure probe and pressure chamber techniques for estimating leaf cell turgor pressure in *Kalanchoë daigremontiana*. *Plant, Cell and Environment* **17,** 15–29.

Neter J, Wasserman W, Kutner MH. 1990. *Applied linear statistical models: regression, analysis of variance, and experimental design*, 3rd edn. Homewood: Irwin.

Ogden TE, Citron MC, Pierantoni R. 1978. The jet stream microbeveler: an inexpensive way to bevel ultrafine glass micropipets. *Science* **201,** 469–470.

Oparka KJ, Dam P. 1992. Direct evidence for pressure-generated closure of plasmodesmata. *The Plant Journal* **2,** 241–750.

Ourcival JM, Berger A. 1995. Equilibrium between soil-water potential and predawn water potential of 2 pre-Saharan shrub species of Tunisia. *Journal of Arid Environments* **30,** 175–183.

Ourcival JM, Floret C, Lefloch E, Pontanier R. 1994. Water relations between two perennial species in the steppes of southern Tunisia. *Journal of Arid Environments* **28,** 333–350.

Passioura JB. 1991. An impasse in plant water relations. *Botanica Acta* **104,** 405–411.

Peuke AD, Schraml C, Hartung W, Rennenberg H. 2002. Identification of drought-sensitive beech ecotypes by physiological parameters. *New Phytologist* **154,** 373–387.

Reich PB, Hinckley TM. 1989. Influence of predawn water potential and soil to leaf hydraulic conductance on maximum daily leaf diffusive conductance in two oak species. *Functional Ecology* **3,** 719–726.

Richards JH. 1994. *Physiological limits of plants in desert playa environments.* Land, Air, and Water Resources Paper 100026. University of California, Davis.

Richards JH, Caldwell MM. 1987. Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia* **73,** 486–489.

Ritchie GA, Hinckley TM. 1975. The pressure chamber as an instrument for ecological research. *Advances in Ecological Research* **9,** 165–254.

Romo JT, Haferkamp MR. 1989. Water relations of *Artemisia tridentata* ssp. *wyomingensis* and *Sarcobatus vermiculatus* in the steppe of southeastern Oregon. *American Midland Naturalist* **121,** 155–164.

SAS. 2001. *SAS/STAT user's guide.* Version 8. Cary: SAS Institute.

Sellin A. 1999. Does pre-dawn water potential reflect conditions of equilibrium in plant and soil water status? *Acta Oecologica* **20,** 51–59.

Shackel KA, Matthews MA, Morrison JC. 1987. Dynamic relation between expansion and cellular turgor in growing grape (*Vitis vinifera* L.) leaves. *Plant Physiology* **84,** 1166–1171.

Slatyer RO. 1967. *Plant–water relationships.* New York: Academic Press.

Snyder KA, Richards JH, Donovan LA. 2003. Night-time conductance in C_3 and C_4 species: do plants lose water at night? *Journal of Experimental Botany* **54,** 861–865.

Stirzaker RJ, Passioura JB. 1996. The water relations of the root–soil interface. *Plant, Cell and Environment* **19,** 201–208.

Toft CA. 1995. A 10-year demographic study of rabbitbrush (*Chrysothamnus nauseosus*): growth, survival and water limitation. *Oecologia* **101,** 1–12.

Tomos AD. 1988. Cellular water relations in plants. In: Franks F, ed. *Water science reviews*, Vol. 3. Cambridge: Cambridge University Press, 186–277.

Tomos AD, Leigh RA, Palta JA, Williams JHH. 1992. Sucrose and cell water relations. In: Pollock CJ, Farrar JF, Gordon AJ, eds. *Carbon partitioning within and between organisms.* Oxford: Bios Scientific Publishers Limited, 71–89.

Turner NC. 1988. Measurement of plant water status by the pressure chamber technique. *Irrigation Science* **9,** 289–308.

Wimmer MA, Mühling KH, Läuchli A, Brown PH, Goldbach HE. 2003. The interaction between salinity and boron toxicity affects the subcellular distribution of ions and proteins in wheat leaves. *Plant, Cell and Environment* **26,** 1267–1274.

Yu Q, Tang C, Kuo J. 2000. A critical review on methods to measure apoplastic pH in plants. *Plant and Soil* **219,** 29–40.