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Osmotic and elastic adjustments in cold desert shrubs differing in rooting depth: coping with drought and subzero temperatures

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Abstract Physiological adjustments to enhance tolerance or avoidance of summer drought and winter freezing were studied in shallow- to deep-rooted Patagonian cold desert shrubs. We measured leaf water potential (Ψ_L), osmotic potential, tissue elasticity, stem hydraulic characteristics, and stomatal conductance (g_S) across species throughout the year, and assessed tissue damage by subzero temperatures during winter. Species behavior was highly dependent on rooting depth. Substantial osmotic adjustment (up to 1.2 MPa) was observed in deep-rooted species exhibiting relatively small seasonal variations in Ψ_L and with access to a more stable water source, but having a large difference between predawn and midday Ψ_L. On the other hand, shallow-rooted species exposed to large seasonal changes in Ψ_L showed limited osmotic adjustment and incomplete stomatal closure, resulting in turgor loss during periods of drought. The bulk leaf tissue elastic modulus (ε) was lower in species with relatively shallow roots. Daily variation in g_S was larger in shallow-rooted species (more than 50 % of its maximum) and was negatively associated with the difference between Ψ_L at the turgor loss point and minimum Ψ_L (safety margin for turgor maintenance). All species increased ε by about 10 MPa during winter. Species with rigid tissue walls exhibited low leaf tissue damage at −20 °C. Our results suggest that osmotic adjustment was the main water relationship adaptation to cope with drought during summer and spring, particularly in deep-rooted plants, and that adjustments in cell wall rigidity during the winter helped to enhance freezing tolerance.

Keywords Elastic modulus · Hydraulic conductivity · Stomatal conductance · Tissue injury · Water relationships

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Introduction

Cold and drought are the two most important environmental stresses that affect growth, productivity, and distribution of plants worldwide (Levitt 1980; Boyer 1982). In deserts at high latitudes (cold deserts) or high elevations, both stresses occur in the same ecosystem type but usually at different times of the year. Gradual exposure of plants to increasingly unfavorable growing conditions might trigger physiological and biochemical adjustments that protect them from injury when environmental stresses become more severe. Leaf tissue water relationship parameters have been used to assess the potential resistance of a species to drought (Bowman and Roberts 1985; Abrams 1988;

Kubiske and Abrams 1994; White et al. 1996; Ngugi et al. 2003). However, some leaf physiological attributes, such as tissue water content, membrane permeability, adjustment of cell solute concentration, and cell wall elasticity characteristics that help plants cope with drought may also help them cope with freezing damage (Levitt 1980; Loik and Nobel 1991; Nilsen 1991; Callister et al. 2008). Plants might exhibit partly overlapping responses to freezing and drought stress because freezing-induced injury in plants is usually the result of cellular dehydration (Xin and Browse 2000), particularly in species that tolerate extracellular ice formation.

Osmotic adjustment is generally thought to be the major mechanism that helps to maintain cell turgor in evergreen plant species when the water potential decreases, sustaining water uptake and plant metabolic activity during drought (Turner and Jones 1980). Osmotic adjustment occurs when the osmotic potential of cells is lowered through active solute accumulation rather than passively as a result of tissue dehydration. Under cold conditions, this is believed to decrease the freezing temperature of the cell sap reducing the probability of ice formation. Additionally, increases in compatible solutes such as proline, other osmotically active amino acids, and soluble sugars protect the structural integrity of cell membranes from cellular dehydration during extracellular freezing (Thomas and James 1993; Xin and Browse 2000; Kosová et al. 2007).

Changes in tissue elasticity, which modify the relationship between turgor pressure and cell volume, also play an important role in the cell resistance to dehydration. Similar to the drought effect, freezing-induced cell dehydration results in the collapse of the cell wall around the shrinking protoplasm and, depending on the properties of cell components, may lead to different degrees of frost injuries (Levitt 1980). When the tissue elasticity is high, the cell wall deforms readily, and thus a loss of water will cause a large change in volume but a small change in turgor, because the cell wall shrinks and continue to squeeze the cytoplasm. On the other hand, when the tissues are rigid, a large loss of water causes little change in the volume of the cell, but a rapid decrease in turgor that allows the cell to avoid further water loss. In addition to its effects on dehydration avoidance, the cell wall mechanical properties contribute to the formation of an effective barrier against the propagation of extracellular ice in cell walls and to the avoidance of intracellular freezing (Solecka et al. 2008). Deposition of extensin, a glycoprotein, and other structural components on cell wall during cold acclimatization is known to provide greater structural rigidity, preventing cell contraction and collapse during freezing (Weiser et al. 1990; Cavender-Bares 2005).

Cold desert species are exposed to low soil water availability and high vapor pressure deficits during summer and to sub-zero temperatures during winter. In the Patagonian steppe, the largest cold desert in South America (150–300 mm of annual precipitation), winter precipitation is accumulated in the soil (sometimes in the form of snow) for spring use, while higher temperatures and strong winds in the summer cause rapid water loss from plants and soil. Soil water availability is also highly variable with depth with shallow soil layers having lower water content than deep layers (Bucci et al. 2009). Physiological processes in plants of the Patagonian steppe are influenced by low soil water availability in the upper soil layers during periods when temperatures are favorable for growth (Sala et al. 1989; Golluscio and Oesterheld 2007; Bucci et al. 2009; Durante et al. 2011; Iogna et al. 2011) and by low temperatures during winter. The physiological and morphological mechanisms that contribute to drought and freezing resistance in Patagonian steppe species are unknown. The aim of this study was to assess the water relationship and hydraulic characteristics of seven Patagonian woody species with different maximum rooting depths. In particular, we focused on seasonal variations in bulk leaf osmotic potential and the bulk elastic modulus. We sought to determine whether osmotic adjustment and increases in cell wall rigidity occur in response to summer drought and subzero temperatures, respectively, and if these responses depend on species-specific patterns of soil water uptake. We hypothesized that species with shallow root systems and therefore exposed to more extreme summer drought exhibit greater osmotic adjustment and more rigid cell walls than deep-rooted species with access to a more stable water source. We also hypothesized that the leaf bulk modulus of elasticity increases during the winter with the resulting increase in tissue rigidity serving as a mechanism to protect cell metabolism from the effects of extracellular freezing.

Materials and methods

Site and species description

The research was carried out at La Dora Ranch in northwest Santa Cruz, Argentina (46°31′S, 71°03′W), at an elevation of 400–420 m a.s.l. The study sites are located in an area characterized by small rolling hills where the vertical distance between the top of the hills and the lower part of the topography is less than 10 m. Mean annual rainfall is 188 mm falling mostly in the fall and winter (April to September), sometimes in the form of snow, and the mean annual air temperature is 9.0 °C. Average summer (December–February) and winter (June–August) temperatures are 14 and 3 °C, respectively. Soils are generally either gravelly sandy loams or gravelly loamy sands

(Douglas and Bockheim 2006). There is a calcareous stony layer at 80–150 cm below the soil surface that some roots can penetrate. Water release curves indicate that these soils have high water availability (water potential close to zero) at low volumetric water content (between 8 and 12 %) (Paruelo et al. 1988).

The vegetation is typical of a Patagonian shrubby steppe characterized by tussock grasses and shrubs. The dominant shrub species are *Mulinum spinosum* (Cav.) Pers, *Adesmia boronioides* J. D. Hooker, *Senecio filaginoides* De Candolle, and *Colliguaja integerrima* Gilles et Hooker ex Hooker, and the most conspicuous grass species are *Stipa speciosa* Trinius et Ruprecht, *Stipa humilis* Cav., and *Poa ligularis* Nees. Grasses are active most of the year and exhibit rapid leaf expansion in the early spring. Shrubs show a clear-cut seasonal pattern of growth; most of them decrease metabolic activity during winter and exhibit an active growth phase during the spring and summer. Seven dominant shrub species were selected (n = 3–5 plants per species): *A. boronioides*, *Berberis microphylla* Jussieu Lam; *C. integerrima*, *Schinus johnstonii* Barkley, *Lycium chilense* Miers ex Bertero, *M. spinosum*, and *S. filaginoides*. Species were selected to also encompass a wide range of maximum rooting depth (Bucci et al. 2009). The study species were assigned to three groups according to their maximum rooting depth (Bucci et al. 2009): <1, 1–2, and >2 m (Table 1), hereafter referred to as shallow-, intermediate-, and deep-rooted species.

Environmental variables and soil water content

Relative humidity and air temperature were monitored continuously with sensors connected to data loggers (HOBOs pro series; Onset Computer, Pocasset, MA, USA). Air saturation deficit (D) was calculated as the difference between saturation vapor pressure at the air temperature and ambient vapor pressure.

Soil samples for gravimetric water content were collected with a Dutch Auger monthly from February 2008 until February 2009. Samples were obtained at 5, 10, 40, 60, and 100 cm depth. Soil samples were obtained from three profiles per sampling date. Dry weights were obtained after placing the soil samples in an oven at 105 °C for 72 h. Gravimetric water content at 200 cm depth was calculated using ECH_2O probes (Decagon Devices), which estimate volumetric water content and soil bulk density values at that depth.

Leaf water potential and pressure–volume curves

Predawn and minimum leaf water potential (Ψ_L) were measured monthly with a pressure chamber (PMS system; Corvallis, OR, USA) from March 2008 to March 2009. Ten leafy twigs from a different individual per species were obtained before dawn and at 1400 hours. Predawn Ψ_L was measured on uncovered leaves (freely transpiring) because previous studies revealed that leaves of those species had negligible nocturnal transpiration, allowing Ψ_L to attain nocturnal equilibrium with soil water potential (Bucci et al. 2009, 2011).

We developed pressure–volume (P–V) relationships by branch dehydration to estimate bulk leaf water relationships on a seasonal basis for all five evergreen species (winter and spring 2008 and summer 2009). Measurements were performed on exposed, expanded, terminal shoots. Five shoots per species were sampled at predawn and transferred to the laboratory where the stems were recut under distilled water. Samples were non-hydrated to avoid alteration in water relationship characteristics as observed in species from arid ecosystems (e.g., Meinzer et al. 1986) and in preliminary experiments with these species (Bucci, unpublished results). The shoots were first weighed to the nearest 0.001 mg to obtain the initial fresh weight and immediately placed in the pressure chamber (PMS system; Corvallis) to obtain the initial water potential. The procedure was repeated many times while the shoot was allowed to dehydrate under ambient conditions (20–25 °C). Finally, shoots were dried in an oven at 80 °C for 72 h and their dry

Table 1 Family, life form, height, leaf phenology, and rooting depth for all studied species

Species	Family	Life form	Leaf phenology	Mean height (cm)	Rooting depth (m)
M. spinosum	Apiaceae	Cushion	Deciduous	40 ± 6	<1
S. filaginoides	Asteraceae	Small shrub	Evergreen	55 ± 4	<1
A. boronioides	Fabaceae	Tall shrub	Evergreen	90 ± 7	1–2
C. integerrima	Euphorbiaceae	Tall shrub	Evergreen	105 ± 11	1–2
L. chilense	Solanaceae	Tall shrub	Deciduous	88 ± 5	1–2
B. microphylla	Berberidaceae	Tall shrub	Evergreen	170 ± 9	>2
S. johnstonii	Anacardiaceae	Tall shrub	Evergreen	210 ± 15	>2

Values are mean ± 1SE (n = 5)

Information about maximum rooting depth was extracted from Bucci et al. (2009)

weights were recorded. Saturated weights of non-hydrated samples were estimated by determining hydrated/dry weight ratios for parallel samples obtained from the same individual on the same date. The tissue water relationship parameters calculated from moisture release curves were osmotic potential at full turgor (π^{100}) and at the turgor loss point (π^{0}), relative water content at turgor loss point (RWC_{TLP}), symplastic fraction (SWF), bulk modulus of elasticity (ε), and solute content. The symplastic solute content per unit dry mass was determined as follows: tissue dry mass was subtracted from tissue fresh mass to obtain tissue water content which was then multiplied by SWF to estimate the symplastic water volume. Saturated osmotic potential was converted to osmolality by multiplying saturated osmotic potential by 410 milliosmol MPa^{-1}. Osmolality was then multiplied by the symplastic water volume and divided by the dry mass of the sample (Tyree et al. 1978).

Bulk modulus of elasticity (ε) was calculated over the full range of positive turgor as described by Evans et al. (1990):

$$\varepsilon = (\Delta\Psi p/\Delta RWC)$$

where $\Delta\Psi p$ is the change in pressure potential and ΔRWC is the change in relative water content. Bulk elastic modulus over the full range of turgor was used to better represent tissue elastic properties across the full range of turgor values including the wilting point.

Stomatal conductance

Diurnal measurements of stomatal conductance to water vapor (g_S) were performed during the summer using a steady-state porometer (LI-1600; LICOR Inc., Lincoln, NE, USA). Three to five fully expanded and exposed leaves from each species (derived from 3 to 5 individuals per species) were marked in the morning and measurements were done on these leaves at 0900 hours and early afternoon (1400 hours). Leaves *of L. chilense* were too small to get reliable g_S with our instrument despite using a small aperture (0.5 cm^2). Measurements of g_S in *M. spinosum* were performed on the basal portion of the spiny trisect leaves.

Leaf tissue damage

The electrolyte leakage method was used to assess the influence of low temperatures on leaf tissue damage (Wilner 1960). Mature leaves were collected from the field in the early morning, kept in plastic bags with moist paper towels (to prevent water loss), and then transported to the laboratory immediately for measurements. Leaf samples (whole leaves; around 0.2 g) were placed into sealed tubes and incubated in a freezer. The freezer was then cooled down at a rate of 7 °C h^{-1} from room temperature to −20 °C. After maintaining the samples at −20 °C for 15 min, the samples were taken out of the freezer and thawed at 4 °C for 2 h, and then 10 ml of deionized water was added to each tube. The solutions with leaf samples were held at 4 °C for 24 h with occasional mixing and shaking. Electrical conductivity (EC) of the solution was then measured with an electrical conductance/resistance meter (Hanna HI 98311; Hanna Instruments). After EC measurements, the tubes were put into an autoclave. Electrical conductivity of the solution with leaf samples was measured again after 24 h with occasional mixing and shaking. The relative EC, as an indicator of membrane damage or ion leakage was calculated for each sample as a percentage:

$$\text{Relative EC} = (\text{EC after the temperature treatment/EC autoclave}) \times 100.$$

Statistical analysis

The SPSS 11.5 statistical package (SPSS, Chicago, IL, USA) was used for statistical analysis. A two-way analysis of variance (ANOVA) was used to test the data for differences among rooting depth group, for season differences and for interactions. Variables within a species were analyzed for normal distribution using the Kolmogorov–Smirnov test, and one-way ANOVA was applied to test differences in means of seasons. Once it was determined that differences existed among the means, Tukey's test was used to compare the significance of seasons for each species. For *M. spinosum* and *L. chilense* where variables were only measured during summer and spring a Student's *t* test was performed. The same treatment was applied to compare morning and afternoon g_S within a species. In order to evaluate of maximum rooting depth effect on g_S, a one-way ANOVA was performed.

Results

Mean monthly temperature at the site ranged from 1.3 °C in July to 19 °C in January (Fig. 1a). Absolute minimum temperature was −15 °C in July and the absolute maximum temperature was 37 °C in February of 2008. Mean monthly air saturation deficit (*D*) varied from 1.9 kPa in the summer (November) to 0.2 kPa in the winter (July) (Fig. 1a).

Soil water content varied seasonally from close to 0 % at 5 cm depth to 8 % at 60 cm depth (Fig. 1b). Soil water content within the upper 100 cm tended to be higher during winter (June and July). For the deepest soil layer (200 cm),

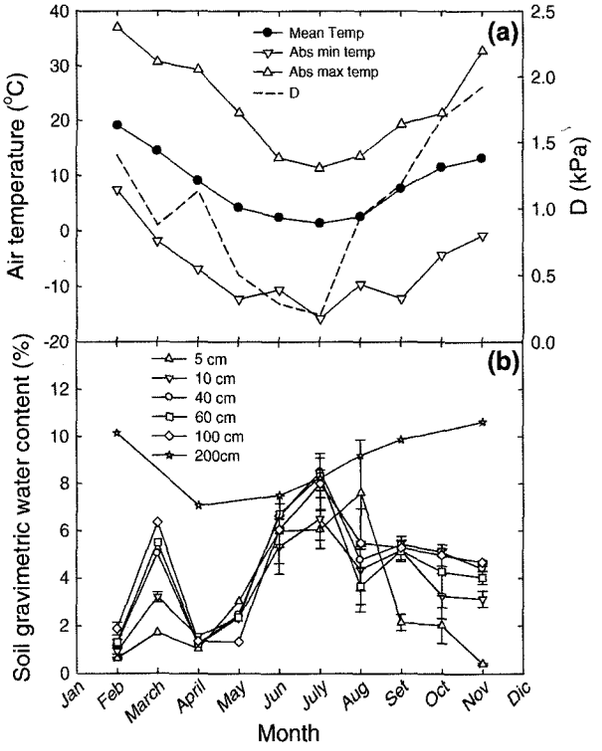

Fig. 1 **a** Seasonal variation in mean monthly air temperature, absolute minimum and maximum air temperature and mean monthly air saturation deficit (*D*) and **b** soil gravimetric water content to 5, 10, 40, 60, 100, and 200 cm depth from February 2008 to November 2009. *Points* in (**b**) correspond to mean values ± 1SE ($n = 3$)

the soil water content was always higher than 7 %, reaching 11 % in the spring and summer (Fig. 1b). Seasonal changes were relatively small at 200 cm, suggesting that at this depth there was more water available for plants.

A two-way ANOVA considering the maximum rooting depth, season, and their interaction as main factors showed significant effects on species water relationship characteristics: predawn and midday Ψ_L, π^{100}, π^0, ε, and osmotically active solute content (Table 2). There were no significant effects for season and interaction for solute content (Table 2). Seasonal patterns of predawn Ψ_L varied between species according to their rooting depth (Fig. 2; Table 2). Species with shallow root systems (*M. spinosum* and *S. filaginoides*) exhibited larger fluctuations in predawn Ψ_L between summer and winter (up to 4.5 MPa) than species with deep roots (*B. microphylla* and *S. johnstonii* (up to 0.8 MPa). Similar seasonal behavior was observed in minimum Ψ_L with up to 4.5 and 2.5 MPa differences between summer and winter, respectively, when comparing shallow- and deep-rooted plants. Both predawn and minimum Ψ_L were higher during winter and spring (June–November) compared to summer (Fig. 2). Consistent with this pattern was a decrease in π^{100} and π^0 during the summer for most of the species (Fig. 3). The seasonal changes in π^{100} and π^0 were too small (~0.1 MPa) in *M. spinosum*, *S. filaginoides*, and *L. chilense* (Fig. 3) to prevent summertime turgor loss (cf. Figs. 2, 3). In the remaining species, π^{100} and the π^0 decreased by 0.5–1.2 and 0.7–2.0 MPa, respectively, between winter and summer. No significant relationship between minimum Ψ_L and π^0 was observed when all species were considered (Fig. 4), but a significant linear relationship ($P < 0.001$), with a slope not significantly different from that of the 1:1 relationship existed among the species capable of maintaining positive turgor. All these species maintained a substantial safety margin for turgor maintenance ($|\pi^0 - \Psi_{min}|$) throughout the seasons, ranging from 0.8 to 1.5 MPa (Fig. 4).

Stomatal conductance measured during summer (g_S) varied significantly between species with different maximum rooting depth (one-way ANOVA, $F = 50.9$; $P < 0.001$). Species with deep roots had higher maximum g_S (observed in the morning) and minimum g_S (observed in the afternoon) compared to species with intermediate and shallow roots ($P < 0.001$) (Fig. 5). Maximum g_S during summer ranged from 110 mmol m^{-2} s^{-1} in *B. microphylla* to close to 50 mmol m^{-2} s^{-1} in the shallow-rooted species *S. filaginoides* (Fig. 5). The percent of decrease of g_S between morning (0900 hours) and early afternoon (1400 hours) was higher (between 40 and 60 %) for species that lost turgor (positive values for the difference between π^0 and minimum Ψ_L) than for species that were able to maintain positive turgor throughout the day (between 18 and 30 %; Fig. 6a). The percentage of decrease of g_S was also positively correlated with the species-specific minimum Ψ_L (Fig. 6b). We used the percentage of decrease in g_S instead of absolute amount of variation in g_S to normalize for the natural variation in maximum g_S across species.

Species-specific symplastic fraction (SWF) varied from 33 % in *S. johnstonii* to 88 % in *C. integerrima* during the

Table 2 Two-way ANOVA of predawn and minimum leaf water potential (Ψ_L), osmotic potential at full turgor π^{100}, osmotic potential at zero turgor (π^0), solute content and bulk elastic modulus (ε) per maximum rooting depth and season, and their interaction ($R \times S$)

Dependent variable	Factors		
	Rooting depth	Season	R × S
Predawn Ψ_L	32.3 (0.000)	39 (0.000)	9.9 (0.000)
Minimum Ψ_L	3.3 (0.045)	156 (0.000)	6.8 (0.000)
π^{100}	12.3 (0.000)	4.3 (0.018)	3.8 (0.019)
π^0	9.13 (0.000)	7.24 (0.006)	4.35 (0.004)
Solutes	5.3 (0.008)	0.88 (0.42)	0.2 (0.91)
ε	84.6 (0.000)	22.3 (0.000)	3.7 (0.01)

Values are *F* values, and values between parentheses are *P* values

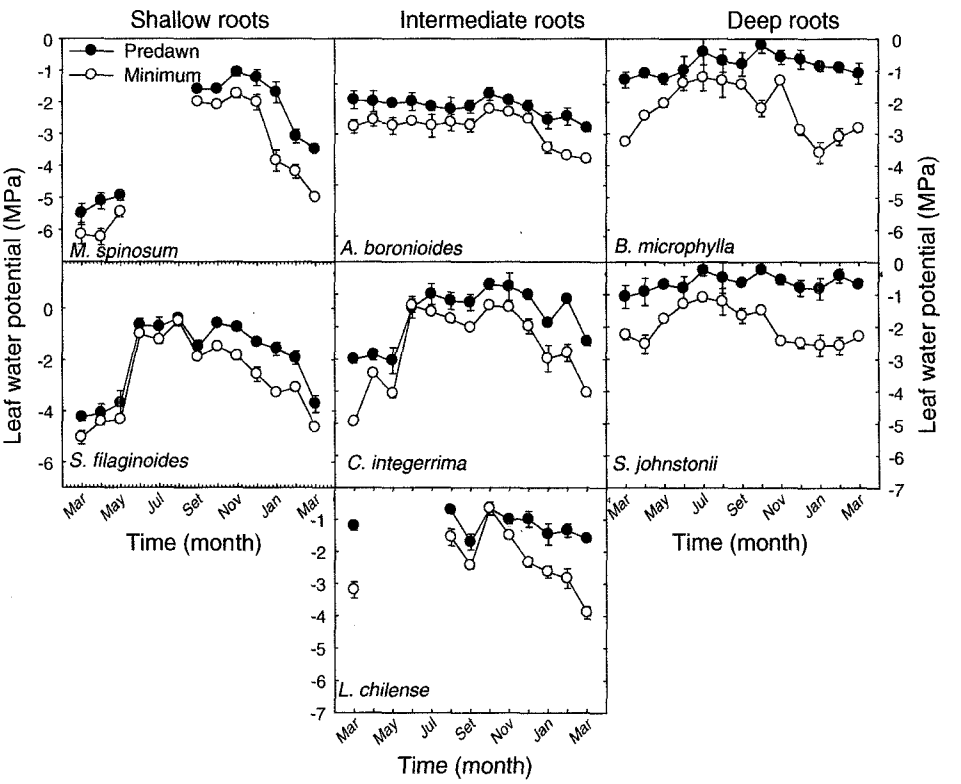

Fig. 2 Seasonal patterns of predawn and midday leaf water potential of seven Patagonian shrub species from March 2008 to March 2009. Each *point* corresponds to a mean value ± 1SE per species (n = 3–5). *Panels* are grouped by maximum rooting depth categories

summer, and the values of SWF were significantly lower in summer than in winter for *A. boronioides*, *C. integerrima*, *B. microphylla*, and *S. johnstonii* (data not shown). The modulus of elasticity increased significantly during the winter season across species (Fig. 7). Seasonal increases in ε between summer and winter (cell walls became more rigid) were similar for all species (about 10 MPa; Fig. 7). The shallow-rooted species *M. spinosum* and *S. filaginoides* had maximum ε values of 4 MPa (more elastic cell walls), compared to species with deep root systems (ε values up to 26 MPa).

Species-specific osmotic potential at full turgor became more negative with increases in the driving force for water uptake ($\Delta\Psi_{predawn-minimum}$) during summer (Fig. 8a). Summer native leaf-specific hydraulic conductivity (k_L; data taken from Bucci et al. 2009) was negatively correlated with $\Delta\Psi_{predawn-minimum}$ (Fig. 8b). The species with the lowest (more negative) osmotic potential at full turgor (*B. microphylla*) exhibited the lowest k_L and the largest $\Delta\Psi_{predawn-minimum}$ (2.2 MPa).

The species-specific bulk modulus of elasticity measured in the summer was correlated with predawn Ψ_L during the summer, a surrogate of rooting depth for these species (Bucci et al. 2009) (Fig. 9). The species with relatively high predawn Ψ_L and deep roots such as *S. johnstonii*, had more rigid cell walls (higher ε) compared to species with more negative predawn Ψ_L and shallow roots such as *S. filaginoides* which have more elastic cell walls.

The percentage of leaf tissue injury at −20 °C during winter (minimum air temperature observed) varied according to species, from 25 % in *S. filaginoides* to only 1 % in *B. microphylla* (Fig. 10), suggesting high species-specific variation in leaf freezing resistance. This variability in percentage of leaf damage was linearly correlated to the species-specific variation in ε during the winter (Fig. 10). Species with higher ε (more rigid tissue walls) exhibited less damage at −20 °C compared to species with lower ε.

Discussion

Seasonal changes in water potentials

During the study period, the plants experienced environmental conditions typical of the Patagonian steppe: a dry summer with very low soil water content in the 0–100 cm layer and a cold winter with temperatures falling well below 0 °C and higher soil moisture. Soil water content was higher and more stable throughout the year at 200 cm depth. Soil water potentials at 25–100 cm depth estimated from soil moisture release curves ranged from −2.5 to −4.0 MPa during the summer (unpublished information), while soil water potentials at 200 cm depth remained close to 0 MPa throughout the year. These environmental characteristics were reflected in the water relationship, stomatal conductance, and hydraulic traits of the study species.

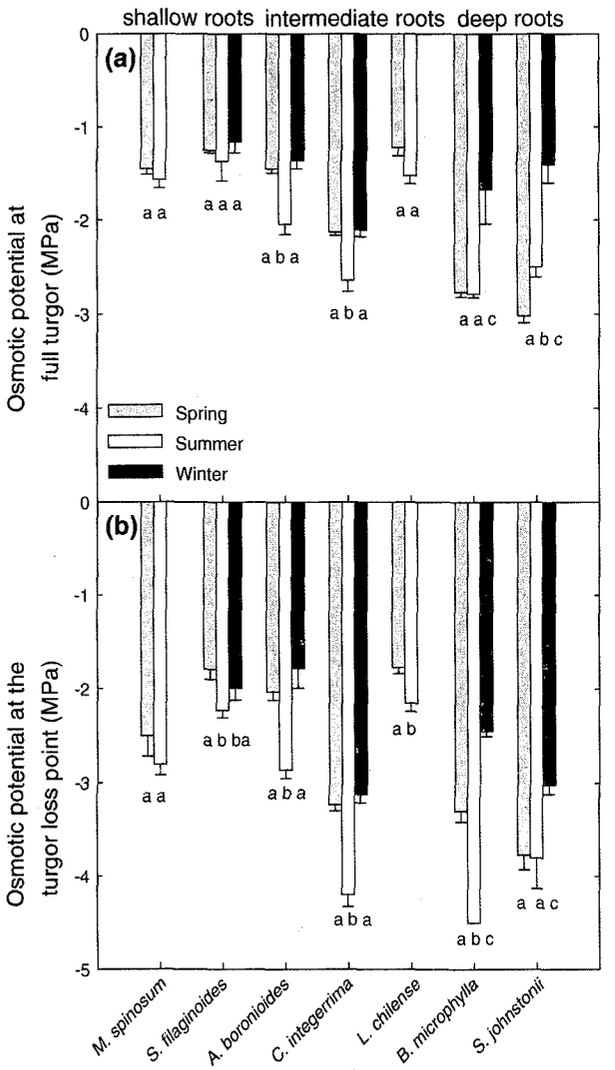

Fig. 3 Osmotic potential at **a** full turgor and **b** at the turgor loss point during spring, summer and winter. Each *bar* is the mean value + 1SE ($n = 5$) per species. *Bars* within a species with different *letters* indicate significant differences ($P \leq 0.05$) between seasons

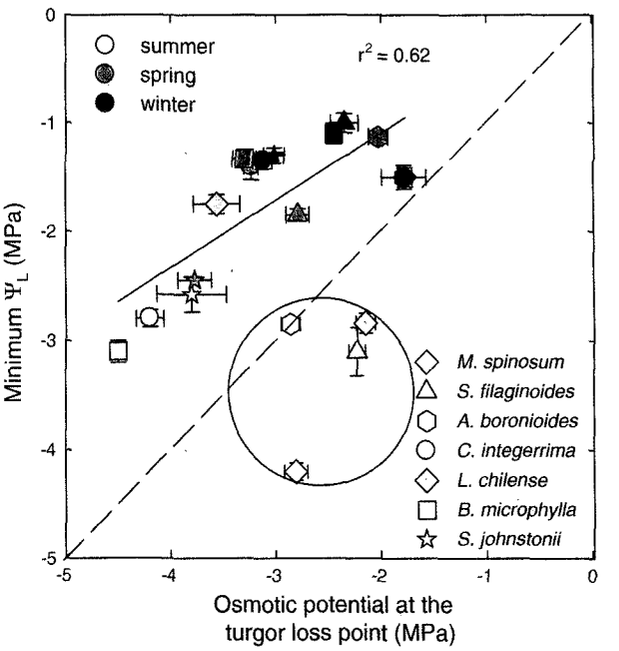

Fig. 4 Relationship between minimum leaf water potential (minimum Ψ_L) and osmotic potential at the turgor loss point during spring, summer and winter. *Symbols* correspond to mean values ± 1SE ($n = 5$) per species. The *solid line* indicates the linear regression fitted to points above the *dashed line* (1:1 relationship): $y = 0.15 + 0.62x$; $P < 0.05$. The *large circle* encompasses the species which lost cell turgor during the measurement period

Variation in the seasonal patterns of Ψ_L and in the P–V relationships across species was partially explained by the species-specific differences in rooting depth. At each depth, the soil water availability was different, increasing from shallow to deep soil depth. Consistent with the rooting depth and with the soil water content of the different layers, predawn and minimum Ψ_L of deep-rooted species did exhibit small seasonal variations, suggesting that these species effectively had access to soil layers with a relatively stable water source. In contrast, the large seasonal fluctuations in Ψ_L observed in shallow-rooted species suggest that they are subjected to large seasonal changes in soil water availability. These species also had more negative predawn Ψ_L during the dry period compared with the predawn Ψ_L of species with intermediate and deep rooting depth. Predawn Ψ_L can be a proxy for soil water potential when nocturnal transpiration is negligible (Bucci et al. 2004). In view of previous studies showing that nocturnal

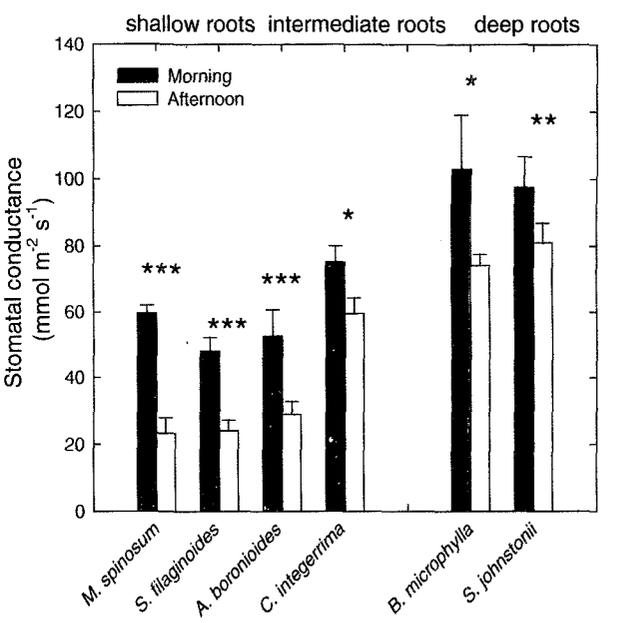

Fig. 5 Summer stomatal conductance (g_s) measured in the morning (9 h) and in the afternoon (14 h). *Bar* corresponds to the mean value + 1SE ($n = 3$ to 5) per species. Significant differences within a species: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

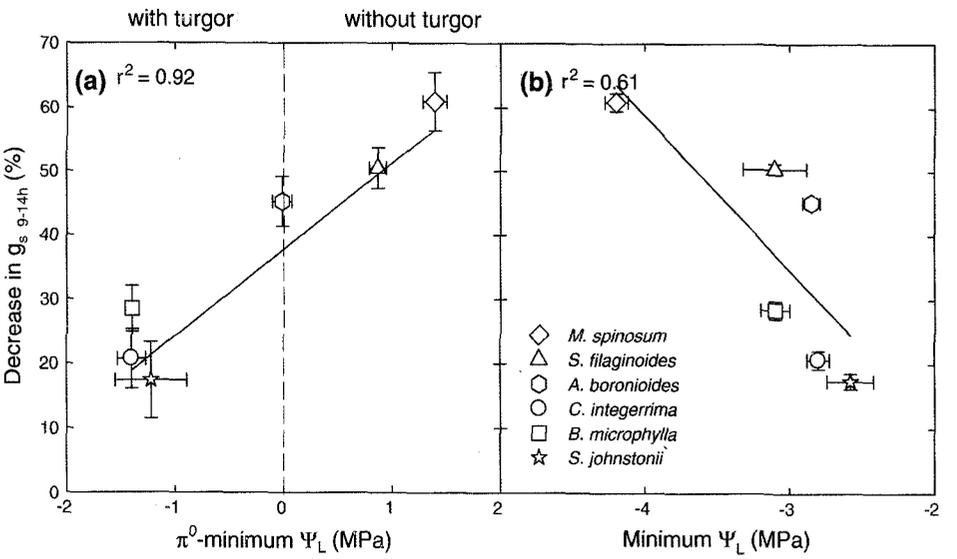

Fig. 6 Percentage decrease in stomatal conductance (g_s) between 0900 and 1400 hours in relation to **a** the difference between turgor loss point and minimum leaf water potential (π^0-minimum Ψ_L) and **b** minimum Ψ_L for six Patagonian shrubs during summer. Each *symbol* represents the mean value ± 1SE (n = 3–5) per species. The *solid lines* represent linear regressions fitted to the data: **a** $y = 41 + 14x$, $P < 0.01$; **b** $y = -38-24x$, $P < 0.05$

water loss is negligible in these species and in other Patagonian shrubs (Bucci et al. 2011; Iogna et al. 2011), the summer predawn Ψ_L measured in this study should be representative of effective rooting depth and water availability of the soil layers explored by roots.

None of the shrub species studied exhibited isohydric behavior of Ψ_L. The changes in both midday and predawn Ψ_L were strongly coupled to variation in soil water potential in the upper part of the soil profile for the shallow-rooted species. On the other hand, we expected that deep-rooted species would exhibit strong isohydric behavior when comparing minimum Ψ_L between the wet and dry seasons due to their access to relatively stable water sources. However, seasonal stability of Ψ_L was not observed in these species. Longer pathways for water transport up to the leaves and strong seasonal variations in temperature may explain the lack of isohydric behavior in these deep-rooted plants. Soil temperatures close to 0 °C increase the resistance to water uptake and transport by roots, reducing the rate of water supply to the plant (Goldstein and Nobel 1991; Graefe et al. 2011). Species in ecosystems with a marked dry season can exhibit isohydric behavior as a result of a strong stomatal control, a reduction of total leaf area per plant, or an increase in hydraulic conductance (Barradas et al. 2004; Bucci et al. 2005; Fisher et al. 2006). A strong stomatal control of water loss was not observed in most species in this study. Although the potential advantages of aniso hydric behavior associated with relatively unrestricted stomatal opening during the dry summer (despite the potential increases in water deficits) are not evident, this behavior could allow higher gas exchange rates (Franks et al. 2007) during the season when the temperatures in Patagonia are more favorable for growth.

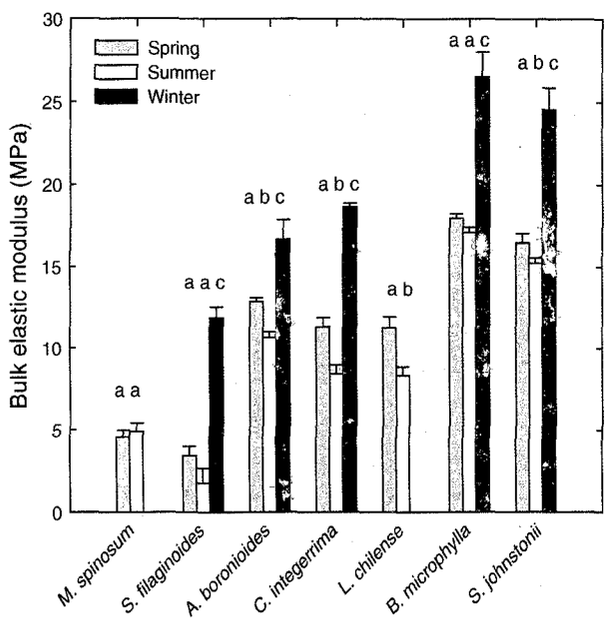

Fig. 7 Bulk leaf tissue elastic modulus during spring, summer and winter. Each *bar* represents the mean value ± 1SE (n = 5) per species. *Columns* within a species with different *letters* indicate significant differences between seasons (one-way ANOVA, $P < 0.01$)

Stomatal conductance and tissue osmotic and elastic adjustments during summer

Both deep- and shallow-rooted species showed osmotic adjustment, changes in tissue elasticity, diurnal stomatal closure, and other compensatory responses to low soil water availability during summer. However, and contrary to our hypothesis, these morpho-physiological changes were not sufficient to allow leaves of some species to maintain turgor as the dry season intensified. The two shallow-rooted species studied lost turgor during the summer. Comparing π^0 with predawn and minimum Ψ_L indicated that turgor in leaves of these species were above

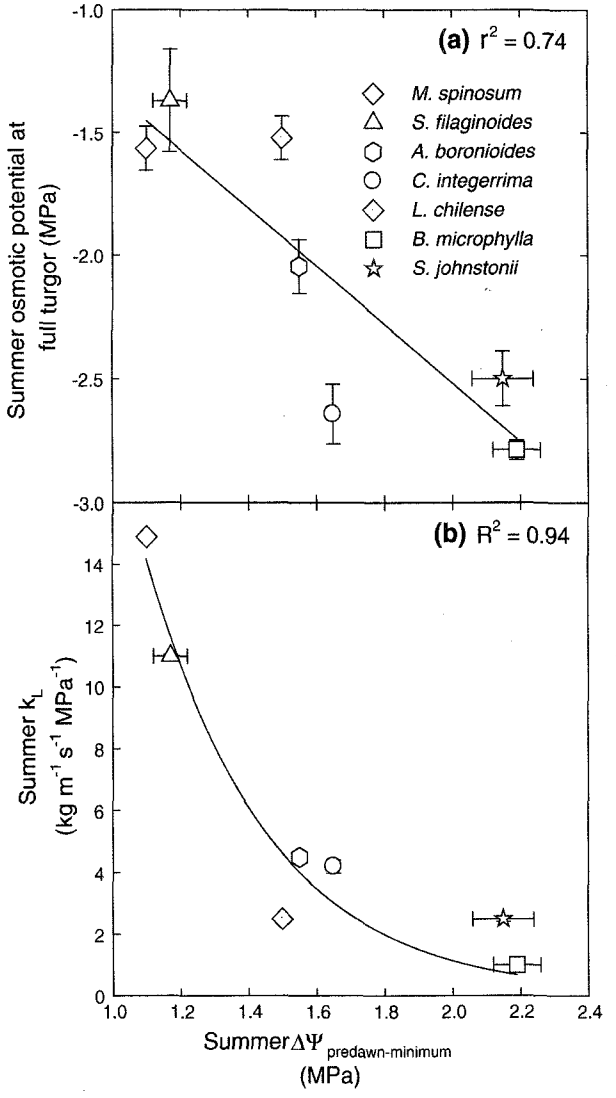

Fig. 8 Summer **a** osmotic potential at full turgor and **b** native leaf specific hydraulic conductivity (k_L) in relation to the difference between minimum and predawn leaf water potential ($\Delta\Psi_{predawn-minimum}$) for seven Patagonian shrub species. Data of k_L were taken from Bucci et al. (2009). Each *symbol* represents the mean value ± 1SE ($n = 5$) per species. *Solid line* in (**a**) indicates the linear regression fitted to the data: $y = -0.15-0.18x$, $P < 0.01$, and in (**b**) indicates the exponential decay function fitted to the data $y = 309\ \hat{e}-2.8x$, $P < 0.005$

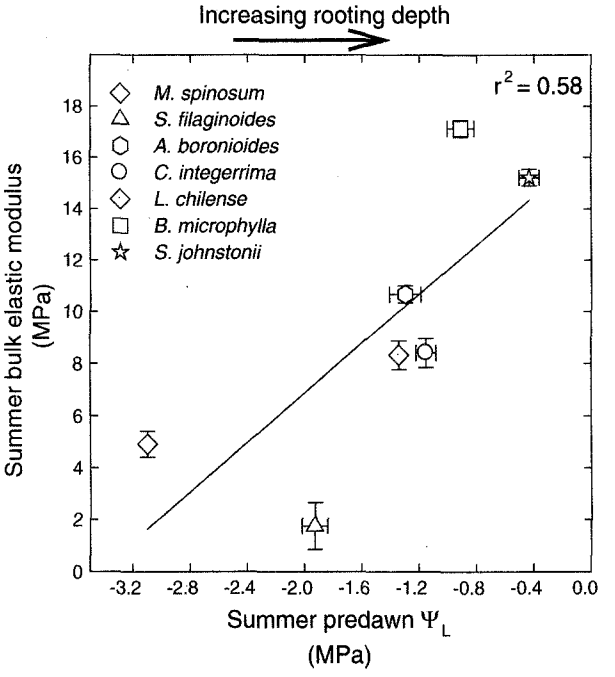

Fig. 9 Relationship between the leaf tissue bulk elastic modulus measured in the summer and summer predawn Ψ_L. Each *symbol* represents the mean value ± 1SE ($n = 5$). *Solid line* indicates the linear regression fitted to the data $y = 9.0-2.4x$, $P < 0.05$

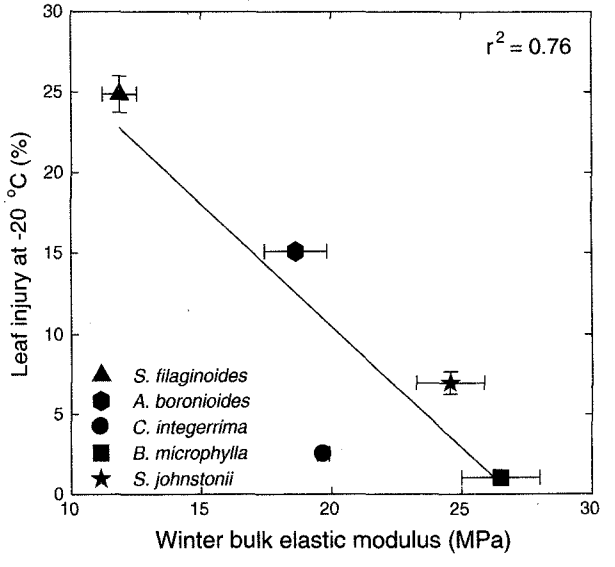

Fig. 10 Relationship between the leaf tissue bulk elastic modulus during the winter and the percentage of leaf injury at −20 °C for species that retain their leaves during the cold period. Each *symbol* represents the mean value ± 1SE ($n = 5$) per species. The *solid line* indicates the linear regression fitted to the data $y = 40.7-1.5x$, $P < 0.05$

zero turgor only throughout the winter and spring, whereas in *A. boronioides* and *L. chilense* (species with intermediate-rooting depth), cell turgor was maintained at least during the morning throughout the year. *Mulinum spinosum* and *L. chilense* are two drought deciduous species that minimize water loss through leaf senescence during late summer (Campanella and Bertiller 2008; Damascos et al. 2008) rather than adjusting leaf tissue physiological characteristics, and consequently turgor loss may not have a large impact on growth in these two species.

Stomatal closure is a common response to increasing water deficits (Mitchell et al. 2008; Bucci et al. 2005, 2008), but the extent and velocity of this response may also

differ among species. Variability in the species-specific decrease of g_s and its relationship with minimum Ψ_L in this study suggests that the species differed in the degree of stomatal sensitivity to the daily decrease of Ψ_L. Species with shallow roots such as *M. spinosum* were approximately five times more responsive to changes in Ψ_L (Fig. 6b) than species with deep roots such as *B. heterophylla*. The strong stomatal control in shallow-rooted species allows them to have a strong isohydrodynamic behavior sensu Franks et al. (2007), and consequently they were able to maintain a constant but relatively low water potential gradient between soil and leaves. Although this implies a lower driving force to transport a given amount of water to the leaves, these species have a compensatory mechanism consisting of a relatively high k_L (Bucci et al. 2009; Fig. 8b). Durante et al. (2011) compared the theoretical hydraulic efficiency and resistance of *M. spinosum* and *L. chilense*, and found that the former (the species with shallow roots) is more efficient but less hydraulically safe than the latter with intermediate-rooting depth when exposed to water deficits.

Osmotic adjustment, in addition to improving soil water uptake under dry conditions (Tyree and Jarvis 1982), allows the maintenance of open stomata at lower values of Ψ_L (Myers and Landsberg 1989). However, in this study, species which lost turgor were able to maintain 40–60 % of stomatal aperture with respect to its maximum value during summer days. Similar results have been found in sagebrush (*Artemisia tridentata*), a dominant species in the cold deserts of North America, where transpiration (a proxy of stomatal conductance) continued at relatively high values, despite the apparent loss of turgor (Kolb and Sperry 1999), perhaps because guard cell turgor was not closely coupled with that of the bulk leaf tissue (Turner and Jones 1980). Additionally, species with higher stomatal sensitivity to water deficits had lower ε (data not shown; $R^2 = 0.64$; $P < 0.05$). This behavior seems surprising since it appears to contradict a common assumption that, because stomatal closure is triggered by the loss of cell turgor, stomata should be more sensitive to water deficits in species with more rigid leaf cell walls (Corcuera et al. 2002). Although we found a significant negative correlation between cell turgor loss and stomatal sensitivity (Fig. 6a), similar to the findings of Galmes et al. (2007) for Mediterranean plants, this does not necessarily imply a causal relationship. The possibility that other physiological traits such as the loss of leaf hydraulic conductance could also be involved in stomatal closure cannot be excluded. The non-effective stomatal closure in some of the studied species in response to leaf desiccation could have a positive effect in term of carbon gain and thus may help to optimize returns on xylem investment (Brodribb and Holbrook 2004; Hao et al. 2010). On the other hand, this behavior could be disadvantageous in terms of increasing the risk of hydraulic failure under drought stress.

We suggest that the relatively high tissue elasticity in shallow-rooted species, compared to that of the deep-rooted species, contributes to their rapid recovery when water stress is alleviated, by allowing greater carbon utilization in cell repair processes and more rapid growth after rain pulses, common in the Patagonian Steppe during the summer, increases soil water availability in the upper soil layers. Studies of shallow-rooted Patagonian shrub species has indicated that they have a high degree of growth responsiveness to summer water pulses (Golluscio et al. 2009; Kowaljowa and Fernández 2011). This behavior, in addition to the high xylem-specific and leaf hydraulic conductivity, support the idea that shallow-rooted Patagonian plants behave opportunistically by growing fast only when water is available for short periods of time in the upper soil layers during the growing season. With the results from this study, we provide a mechanistic explanation for the fast growth responses of shallow-rooted shrubs. On the other hand, the high leaf tissue rigidity of species with deep roots is consistent with the high $\Delta\Psi$ (large driving force for water uptake and transport to the leaves) observed, which could contribute to compensate for the low xylem-specific hydraulic conductivity (Bucci et al. 2009, 2011). Both species groups might be able to sustain growth to a similar extent, due to different compensatory mechanisms for sustaining gas exchange (low k_L vs. high $\Delta\Psi$, and high k_L vs. low $\Delta\Psi$).

Decrease in tissue elasticity during winter and freezing injury

Seasonal variations in tissue elasticity can be a consequence of changes in environmental conditions and leaf phenology (Bowman and Roberts 1985; Abrams and Menges 1992). In the present study, we observed seasonal changes in ε, i.e., higher elastic tissues during summer (hot and dry season) than in winter and spring. Although some works have shown an increase in ε with leaf aging (Abrams 1990; Abrams and Menges 1992; Patakas and Noitsakis 1997), we partially discard that possibility for the spring changes in ε because all study species produce new leaves during early spring. We used the same cohort of leaves to measure water relationship traits during spring (recently expanded leaves), summer (mature leaves), and winter (senescent or start of senescence leaves). Although the largest differences in tissue elasticity were observed between summer and winter, there was a tendency in six of the seven study species to have more rigid tissues in leaves recently expanded than in mature leaves. Those changes in tissue elasticity could be induced by environmental conditions (drought in summer and low temperatures in spring and winter), in addition by the leaf development state.

Reversible changes in ε temperature dependence have been attributed to variation in cell wall properties associated with deposition and posterior degradation of pectins (Stefanowska et al. 1999; Solecka et al. 2008).

Elastic adjustment had a lower contribution to turgor maintenance than osmotic adjustment during the summer because only the species with lower π^{100} were able to avoid turgor loss. Thus, we ruled out elastic adjustment as an important mechanism for maintaining leaf water status for Patagonian shrubs, and we suggest that seasonal changes in cell wall elasticity observed in these species are predominantly a mechanism for coping with extreme subzero temperatures. Morpho-physiological changes have been associated with increased frost tolerance in some evergreen species (Ball et al. 2002; Callister et al. 2008). A strong correlation was found between the temperature at which 50 % of membrane damage occurs in leaves (LT^{50}) and ε for Patagonian shrubs in an area with milder temperatures than in the present study (Zhang, unpublished information). In that research, species with more rigid tissue walls such as *B. heterophylla* and *C. integerrima* could tolerate lower temperatures (close to −20 °C) than species with elastic walls such as *S. filaginoides* (−13 °C). Here, we also found a negative correlation between leaf damage by freezing and ε. Contrary to Zhang, who observed substantial tissue damage at −20 °C, we found only slight to moderate membrane damage at −20 °C (a maximum of 25 % in *S. filaginoides* to a minimum of the 1 % in *B. heterophylla*). These differences in temperature thresholds for leaf injury may be associated with lower mean air temperatures in our more continental study site (near the Andean mountains) compared with the site studied by Zhang near the Atlantic coast.

The differences in tissue wall elasticity reflected alternate freezing resistance mechanisms employed by the different shrub species. A plausible explanation for the negative correlation between the percentage of membrane damage and ε found in this study could be greater mechanical resistance of tissue walls to physical pressure exerted by extracellular ice growth in species with high ε, thus excluding ice from the cell. On the other hand, a higher cell resistance to collapse in species with high ε can lead to reduction in cell dehydration during freezing, which is one of the factors that may cause freezing injury. The causes of differences in the properties of cell walls that affect their ability to act as barrier against propagation of extracellular ice are uncertain (Yamada et al. 2002). However, some studies (Wisniewski et al. 1991; Rajashekar and Burke 1996) have indicated that differences in micro-capillaries of cell wall may be involved in avoiding the ice propagation into the cell and the water movement out. Low porosity in cold-acclimated plants is partially attributable to pectin deposition, which together with extensin deposition are considered compounds imparting rigidity to tissue walls (Weiser et al. 1990; Cavender-Bares 2005; Solecka et al. 2008). We have not determined the cell wall structure, so further investigation of the properties of cell walls is necessary for clarification of the mechanisms of adaptation to freezing of cold desert species.

In conclusion, the species studied varied in the degree of osmotic adjustment during summer depending on rooting depth and access to different types of water sources. Only species with deeper roots were capable of maintaining positive turgor throughout year. On the other hand, species that lost turgor during the summer drought (species with shallow roots and without osmotic adjustment) did not completely close their stomata, perhaps allowing them to maintain CO_2 assimilation during the driest period. In addition, the shallow-rooted species had more elastic cell walls and high k_L that may allow them to respond rapidly to upper soil layers recharge during rain pulses that occur during the summer. Although all species had more elastic cell walls during the summer, osmotic adjustment was the main water relationship adaptation to cope with drought. Shallow-rooted species generating lower driving forces for water uptake exhibited greater water transport efficiency. Increasing cell wall rigidity during winter appeared to protect cell membranes from mechanical injury caused by ice crystals forming in apoplastic spaces, consistent with our predictions. The results of this study suggest that all the species studied are adapted to withstand low subzero temperatures, as substantial damage in leaves was not observed at least down to −20 °C.

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Conflict of interest The authors have no conflict of interest.

References

Abrams MD (1988) Sources of variation in osmotic potentials with special reference to North American tree species. For Sci 34:1030–1046

Abrams MD (1990) Adaptations and responses to drought in *Quercus* species of North America. Tree Physiol 7:227–238

Abrams MD, Menges ES (1992) Leaf ageing and plateau effect on seasonal pressure–volume relationships in three sclerophyllous *Quercus* species in south–eastern USA. Funct Ecol 6:353–360

Ball MC, Wolfe J, Canny M, Hofmann M, Nicotra AB, Hughes D (2002) Space and time dependence of temperature and freezing in evergreen leaves. Funct Plant Biol 29:1259–1272

Barradas VL, Ramos-Vázquez A, Orozco-Segovia A (2004) Stomatal conductance in a tropical xerophilous shrub land at a lava substratum. Int J Biomet 48:119–127

Bowman WD, Roberts SW (1985) Seasonal and diurnal water relations adjustments in three chaparral shrubs. Ecology 66:738–742

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Brodribb TJ, Holbrook NM (2004) Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms. New Phytol 162:663–670

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Hinojosa JA, Hoffmann WA, Franco AC (2004) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. Tree Physiol 24:1119–1127

Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Campanello P, Scholz FG (2005) Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plants in Neotropical savanna trees. Trees 19:296–304

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Franco AC, Zhang Y, Hao G-Y (2008) Water relations and hydraulic architecture in Cerrado trees: adjustments to seasonal changes in water availability and evaporative demand. Braz J Plant Physiol 20:233–245

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Arce ME (2009) Soil water availability and rooting depth as determinants of hydraulic architecture of Patagonian woody species. Oecologia 160:631–641

Bucci SJ, Scholz FG, Iogna PA, Goldstein G (2011) Economía de agua de especies arbustivas de la Estepa Patagónica. Ecol Austral 21:43–60

Callister A, Arndt S, Ades P, Merchant A, Rowell D, Adams M (2008) Leaf osmotic potential of Eucalyptus hybrids respond differently to freezing and drought, with little clonal variation. Tree Physiol 28:1297–1304

Campanella MV, Bertiller MB (2008) Plant phenology, leaf traits, and leaf litter fall of contrasting life forms in the arid Patagonian Monte, Argentina. J Veg Sci 19:75–78

Cavender-Bares J (2005) Impacts of freezing on long distance transport in woody plants. In: Holbrook NM, Zweiniecki MA (eds) Vascular transport in plants. Elsevier, Boston, pp 401–424

Corcuera L, Camarero JJ, Gil-Pelegrín E (2002) Functional groups in *Quercus* species derived from the analysis of pressure–volume curves. Trees 16:465–472

Damascos MA, Barthelemy D, Ezcurra C, Martinez AP, Brion AC (2008) Plant phenology, shoot growth, and branching pattern in *Mulinum spinosum* (Apiaceae), a cushion shrub of the arid Patagonian steppe of Argentina. J Arid Environ 72:1977–1988

Douglas DC, Bockheim JG (2006) Soil-forming rates and processes in Quaternary moraines near Lago Buenos Aires, Argentina. Quatern Res 65:293–307

Durante M, Maseda PH, Fernández RJ (2011) Xylem efficiency vs. safety: acclimation to drought of seedling root anatomy for six Patagonian shrub species. J Arid Environ 75:397–402

Evans RD, Black RA, Link SO (1990) Rehydration-inducedchanges in pressure–volume relationships of *Artemisia tridentata*Nutt. ssp. *tridentata*. Plant Cell Environ 13:455–461

Fisher RA, Williams M, Do Vale RL, Da Costa AL, Meir P (2006) Evidence from Amazonian forests is consistent with isohydric control of leaf water potential. Plant Cell Environ 29:151–165

Franks PJ, Drake PL, Froend RH (2007) Aniso hydric but isohydrodynamic: seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. Plant Cell Environ 30:19–30

Galmes J, Flexas J, Save R, Medrano H (2007) Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant Soil 290:139–155

Goldstein G, Nobel PS (1991) Changes in osmotic pressure and mucilage during low-temperature acclimation of *Opuntia ficus-indica*. Plant Physiol 97:954–961

Golluscio RA, Oesterheld MR (2007) Water use efficiency of twenty-five co-existing Patagonian species growing under different soil water availability. Oecologia 154:207–217

Golluscio RA, Sigal Escalada V, Perez J (2009) Minimal plant responsiveness to summer water pulses: ecophysiological constraints of three species of semiarid Patagonia rangeland. Ecol Manag 62:171–178

Graefe S, Leuschner Ch, Coners H, Hertel D (2011) Root functioning in tropical high-elevation forests: environmental vs. biological control of root water absorption. Environ Exp Bot 71:329–336

Hao G-Y, Sack L, Wang A-Y, Cao K-F, Goldstein G (2010) Differentiation of leaf water flux and drought tolerance traits in hemiepiphytic and non-hemiepiphytic *Ficus* tree species. Funct Ecol 24:731–740

Iogna PA, Bucci SJ, Scholz FG, Goldstein G (2011) Water relations and hydraulic architecture of two Patagonian steppe shrubs: effect of slope orientation and microclimate. J Arid Environ 75:763–772

Kolb KJ, Sperry JS (1999) Differences in drought adaptation between subspecies of sagebrush (*Artemisia tridentata*). Ecology 80:2373–2384

Kosová K, Vitamvas P, Prasil IT (2007) The role of dehydrins in plant response to cold. Biol Plant 51:601–617

Kowaljowa E, Fernández RJ (2011) Differential utilization of a shallow-water pulse by six shrub species in the Patagonian steppe. J Arid Environ 75(2):211–214

Kubiske ME, Abrams MD (1994) Ecophysiological analysis of temperate woody species on contrasting sites during wet and dry years. Oecologia 98:303–312

Levitt J (1980) Responses of plants to environmental stresses, Vol 1, 2nd edn. Academic, New York

Loik ME, Nobel PS (1991) Water relations and mucopolysaccharide increases for a winter hardy cactus during acclimation to subzero temperatures. Oecologia 88:340–346

Meinzer FC, Rundel PW, Shari MR, Nilsen ET (1986) Turgorand osmotic relations of the desert shrub Larrea tridentata.Plant Cell Environ 9:467–475

Mitchell PJ, Veneklaas EJ, Lambers H, Burgess SO (2008) Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. Plant Cell Environ 31:1791–1802

Myers B, Landsberg J (1989) Water stress and seedling growth of eucalyptus species from contrasting habitats. Tree Physiol 5:207–208

Ngugi M, Doley D, Hunt A, Dart P, Ryan P (2003) Leaf water relations of *Eucalyptus cloeziana* and *Eucalyptus argophloia* in response of water deficit. Tree Physiol 23:335–343

Nilsen ET (1991) The relationships between freezing tolerance and thermotropic leaf movement in five Rhododendron species. Oecologia 87:63–71

Paruelo JM, Aguiar MR, Golluscio RA (1988) Soil water availability in the Patagonian arid steppe: gravel content effect. Arid Soil Res Rehabil 7:67–74

Patakas A, Noitsakis B (1997) Cell wall elasticity as a mechanism to maintain favorable water relations during leaf ontogeny in grapevines. Am J Enol Vitic 48:352–358

Rajashekar CB, Burke MJ (1996) Freezing characteristics of rigid plant tissues. Plant Physiol 111:597–603

Sala OE, Golluscio RA, Lauenroth WK, Soriano A (1989) Resource partitioning between shrubs and grasses in the Patagonian steppe. Oecologia 81:501–505

Solecka D, Zebrowski J, Kacperska A (2008) Are pectins involved in cold acclimation and de-acclimation of winter oil-seed rape plants? Ann Bot (Lond) 101:521–530

Stefanowska M, Kuras M, Kubacka-zebalska M, Kacperska A (1999) Low temperature affects pattern of leaf growth and structure of cell walls in winter oilseed rape (*Brassica napus* L., var. o*leifera* L.). Ann Bot 84:313–319

Thomas H, James AR (1993) Freezing tolerance and solute changes in contrasting genotypes of *Lolium perenne* L. acclimated to cold and drought. Ann Bot 72:249–254

Turner NC, Jones MN (1980) Turgor maintenance by osmotic adjustment: a review and evaluation. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. Wiley, New York, pp 84–104

Tyree MT, Jarvis PG (1982) Water in tissues and cells, vol 12. Springer, Berlin

Tyree MT, Cheung YNS, McGregor ME, Talbot AJB (1978) The characteristic of seasonal and ontogenic changes in the tissue-water relations of Acer, Populus, Tsuga and Picea. Can J Bot 56:635–647

Weiser RL, Wallner SJ, Waddell JW (1990) Cell wall and extensinmRNA changes during cold acclimation of pea seedlings. PlantPhysiol 93:1021–1026

White DA, Beadle CL, Worledge D (1996) Leaf water relations of *Eucalyptus globulus* ssp. *globulus* and *E. nitens*: seasonal, drought and species effects. Tree Physiol 16:469–476

Wilner J (1960) Relative and absolute electrolytic conductance tests for frost hardiness of apple varieties. Can J Plant Sci 40:630–637

Wisniewski M, Davis G, Schaffer K (1991) Mediation of deep supercooling of peach and dogwood by enzymatic modifications in cell-wall structure. Planta 184:254–260

Xin Z, Browse J (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell Environ 23:893–902

Yamada T, Kuroda K, Jitsuyama Y, Takezawa D, Arakawa K, Fujikawa S (2002) Roles of the plasma membrane and the cell wall in the responses of plant cells to freezing. Planta 215:770–778