

Homeostasis in leaf water potentials on leeward and windward sides of desert shrub crowns: water loss control vs. high hydraulic efficiency

Patricia A. Iogna · Sandra J. Bucci ·
Fabián G. Scholz · Guillermo Goldstein

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Abstract Phenotypic plasticity in morphophysiological leaf traits in response to wind was studied in two dominant shrub species of the Patagonian steppe, used as model systems for understanding effects of high wind speed on leaf water relations and hydraulic properties of small woody plants. Morpho-anatomical traits, hydraulic conductance and conductivity and water relations in leaves of wind-exposed and protected crown sides were examined during the summer with nearly continuous high winds. Although exposed sides of the crowns were subjected to higher wind speeds and air saturation deficits than the protected sides, leaves throughout the crown had similar minimum leaf water potential (Ψ_L). The two species were able to maintain homeostasis in minimum Ψ_L using different physiological mechanisms. *Berberis microphylla* avoided a decrease in the minimum Ψ_L in the exposed side of the crown by reducing water loss by stomatal control, loss of cell turgor and low epidermal conductance.

Colliguaja integerrima increased leaf water transport efficiency to maintain transpiration rates without increasing the driving force for water loss in the wind-exposed crown side. Leaf physiological changes within the crown help to prevent the decrease of minimum Ψ_L and thus contribute to the maintenance of homeostasis, assuring the hydraulic integrity of the plant under unfavorable conditions. The responses of leaf traits that contribute to mechanical resistance (leaf mass per area and thickness) differed from those of large physiological traits by exhibiting low phenotypic plasticity. The results of this study help us to understand the unique properties of shrubs which have different hydraulic architecture compared to trees.

Keywords Hydraulic conductance · Osmotic potential · Patagonia · Stomatal conductance · Wind

Introduction

Leaves are the plant organs most exposed to atmospheric conditions and consequently are one of the plant parts most sensitive and plastic to environmental changes. Leaf traits within the plant crown may change if the microenvironment within the crown varies depending on several factors such as light level and wind speed. Plants respond to physical factors through different phenotypes suitable for each particular habitat. Phenotypic plasticity is the ability of a genotype to express different morphological, anatomical and physiological traits in response to varying environmental conditions (Pigliucci 2001). Plasticity pertains not only to the whole plant but also to different parts of the plant. The plastic nature of the modular construction of plants (De Kroon et al. 2005) may provide a certain degree of independence between parts of the same

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P. A. Iogna · S. J. Bucci · F. G. Scholz (✉) · G. Goldstein
Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET), Buenos Aires, Argentina
e-mail: fgscholz@yahoo.com

P. A. Iogna · S. J. Bucci · F. G. Scholz
Grupo de Estudios Biofísicos y Eco-fisiológicos (GEBEF),
Universidad Nacional de la Patagonia San Juan Bosco,
Comodoro Rivadavia, Argentina

G. Goldstein
Laboratorio de Ecología Funcional (LEF), FCEyN, Universidad
de Buenos Aires, Buenos Aires, Argentina

G. Goldstein
Department of Biology, University of Miami, P.O. Box 249118,
Coral Gables, Florida, USA

individual resulting in intra-canopy plasticity (Sack et al. 2006) by which each leaf may respond differently to a particular microclimatic condition. Most of the examples of intra-canopy plasticity are related to different light environments within the crown of a single plant (e.g., Ellsworth and Reich 1993; Richardson et al. 2001; Givnish et al. 2004; Sack et al. 2006; Wyka et al. 2012). However, wind is also an environmental trigger for changes in leaf structure, morphology and/or anatomy within a single individual (García-Verdugo et al. 2009). The degree of leaf plasticity in response to wind depends on its frequency and/or intensity, plant size, crown shape and leaf traits. Plants of diverse ecosystems exposed to persistent mechanical stresses such as wind loading and other mechanical perturbation generate a slow developmental response termed “thigmomorphogenesis” (Jaffe 1973) which has been well studied, particularly in stems (Telewski and Jaffe 1986; Niklas et al. 2000; Prunyn et al. 2000). However, wind has other effects on leaves as a result of forced convection and the increases in water deficits (Smith and Ennos 2003), which has received less attention. Wind can alter the transpiration rate by removing or reducing the boundary layer (Campbell and Norman 1998). The thicker the boundary layer, the greater boundary layer resistance and the slower the water vapor diffusion from leaves to the atmosphere (Jarvis and McNaughton 1986; Meinzer et al. 1995). The thickness of the boundary layer is controlled primarily by wind and leaf shape.

Water loss depends not only on leaf form and geometry, but also on cuticle properties and stomatal conductance (g_s). Plants exposed to frequent and strong winds can cope with high evaporative losses through architectural, morphological, and physiological changes (Niklas 1992). At a short-term scale, plants can respond to a rapid increase in evaporative demand from the atmosphere by controlling stomatal aperture (Tyree and Sperry 1988). Medium-scale changes can involve cellular osmotic and elastic adjustments to maintain leaf turgor (White et al. 2000). When the stress remains constant for a long period of time, it may result in changes in growth rates, in leaf and stem xylem structure (Nardini and Salleo 2005), and in total leaf area and leaf structure (Van Gardingen et al. 1991; Niklas 1996; De la Barrera and Walter 2006).

Long distance water transport in large trees requires pronounced gradients in water potentials from roots to leaves to occur during periods of transpiration. The difference in water potentials along the water transport systems is a prerequisite for water movement and it is consistent with variations in environmental conditions from the upper portion of the crown exposed to high radiation, evaporative demands and relatively high wind speed to the lower section of the crown with lower

irradiances and relatively low evaporative demands and wind speed (e.g., Bauerle et al. 1999; Woodruff et al. 2004). However, shrubs, particularly small shrubs such as those growing in cold deserts, would have smaller water potential gradients between leaves and branches of the crown due to the short distance between plant parts and to the absence of a gravitational component of water potential, compared to trees. Any disequilibrium, for example, different leaf water potentials across the crown, will trigger the internal redistribution of tissue water content that may effect adjustment of the whole plant water economy. Furthermore, the lack of homeostasis, that is the maintenance of similar plant water status despite changes in soil water availability or environmental conditions (Tardieu and Simonneau 1998), may result in catastrophic dysfunction in the branches and leaves of shrubs due to relatively low leaf water potentials. Some studies done in trees have found differences in leaf or stem hydraulic conductance and vulnerability to embolism depending on the position of the leaves and shoots in the crown as the result of different micro-environmental conditions (e.g., light and vapor pressure deficit; Cochard et al. 1999; Lemoide et al. 2002; Sellin and Kupper 2004; Gebauer et al. 2012). To our knowledge there are no studies evaluating the effects of micro-environmental gradients within the crown of small shrubs on hydraulic traits.

The Patagonian steppe, one of the largest cold deserts of the world, is subjected to long periods of water deficit, and nearly continuous and strong westerly winds. In this study we investigated how wind affects leaf hydraulics and water relations characteristics of two dominant shrubs of the Patagonian cold desert in Argentina (*Colliguaja integerrima* Gillies et Hooker ex Hooker and *Berberis microphylla* Jussieu) under field conditions. These two species were used as model systems for understanding effects of wind on water relations of leaves of the crowns of small woody plants exposed to substantial differences in wind speed. The main hypothesis of the study is that adjustments in leaf morphological and physiological traits help to maintain minimum leaf water potential homeostasis between wind-exposed and protected crown sides, by: (a) improved hydraulic efficiency (more water supply per unit leaf surface area), and/or by (2) physiological and morphological changes resulting in reduced water losses. Even though we have not made measurements under controlled conditions (e.g., in a wind tunnel) we have addressed simultaneously several environmental conditions (wind, humidity and temperature), water relation traits, hydraulic architecture characteristics and biomass allocation patterns in relation to wind effects (windward and leeward crown sides) in a comprehensive and multi-factorial study with two dominant shrub species of the Patagonian desert.

Materials and methods

Site, species and sampling

The research was carried out near Comodoro Rivadavia city (150 slm; 45°57'S and 67°31'W), Argentina. The precipitation falls mainly in the autumn and the mean annual value is 287 mm. The mean annual temperature is 12.9 °C and daily mean temperature ranges from 20 °C in January (summer) to 7 °C in July (winter). Mean annual wind speed is 7.5 m s⁻¹ with mean maximum values during spring and summer, mostly from the west, of about 11.9 m s⁻¹ (Beeskow et al. 1987). The vegetation is a typical Patagonian herbaceous-shrubby steppe. Two dominant evergreen woody species were selected for this study: *C. integerrima* (Euphorbiaceae) and *B. microphylla* (Berberidaceae).

Ten individuals of similar size per species (mean height and crown leaf area index for *C. integerrima* 86.1 ± 4.57 cm and 0.97 ± 0.08 m² m⁻², respectively, and for *B. microphylla* 123.1 ± 3.66 cm and 1.39 ± 0.11 m² m⁻², respectively) were selected to avoid differences in wind velocity between crown sides due to variation in crown size. All measures were made during summer (December 2010 and January 2011). Leaves were sampled in the crown area spanning the southwest-west exposure of the canopy ("wind-exposed" side or "windward" hereafter) and on the opposite side of the canopy experiencing the lowest wind exposure (northeast-east or "leeward" hereafter) for each shrub. To investigate the degree of wind reduction caused by the crown during typical summer days, wind speed was characterized at leeward and windward for three individuals of each species every 5 min during 3 days using two meteorological stations. A CR10X Campbell datalogger equipped with a Vaisala HMP45C (Helsinki, Finland), used to measure air temperature and relative humidity, and a MetOne (Grants Pass, OR) 034A anemometer/vane units (accuracy ± 1.1 %), used to determine the wind speed and direction, were placed on each side of the crown. Air saturation deficit (*D*) was calculated from air temperature and relative humidity (RH). In *C. integerrima* the sensors were placed at 50 cm height, whereas for *B. microphylla* they were installed at 100 cm height.

Leaf structure and anatomy

Leaf mass per unit area (LMA; dry mass/fresh area) was determined in 50–100 fully expanded sun leaves per species and the two wind exposures, depending on the total number of leaves per plant. After the fresh surface area (*A_L*) was determined with a scanner, the leaves were oven-dried at 70 °C for 72 h and weighed to obtain their dry mass.

Leaf thickness was calculated for ten leaves per species and wind exposures according to Sack et al. (2003) as lamina volume divided by *A_L*. Leaves were submerged in a recipient with deionized water, placed over a digital balance (±0.001 g) to determine the fresh volume using the Archimedes principle.

Stomatal density and guard cell length were measured from nail polish impressions in the central region of the blade on ten leaves per species and exposure. Stomatal density was estimated by counting the number of stomata per field viewed at 40× magnification. Area measurements were carried out using ImageJ software. These values were converted to stomata per squared millimeter. Stomatal pore index (SPI) was calculated as in Sack et al. (2003):

$$\text{SPI} = \text{stomatal density} \times (\text{guard cell length})^2.$$

Leaf vessel diameter was measured in ten anatomical transverse sections of the mid-vein per species and wind exposure. Vessel diameter was measured from photographs taken with an optical microscope at 40× magnification using ImageJ. The total area of the photos was determined to calculate the vessel area per squared micrometer of leaf xylem.

Leaf hydraulic conductance

The vacuum pump method was used to estimate leaf lamina hydraulic conductance (*K_{Lleaf}*) (Kolb et al. 1996; Sack et al. 2002) of ten leaves per species and wind exposure collected at predawn. This method involves pulling deionized and degassed water through the leaf by applying a series of partial vacuums with a vacuum bomb. Leaves were sealed inside a vacuum flask with moist paper towel and their petioles (both species have very short petioles) emerged through the center of a rubber stopper to the balance. We assumed that the small portion of stem did not affect the results of the measurements because the resistance in the leaves is significantly higher than the resistance in the stem (Sack and Holbrook 2006). Pressure levels used ranged between 17 and 77 kPa and these were applied in three steps of 20 kPa. The maximum vacuum level was applied first, followed by decreasing vacuum levels; however, applying vacuum levels in a sequence of different magnitude does not affect the measurement estimates (Kolb et al. 1996; Nardini et al. 2001). Readings were made when the water flow was stable for 10–15 min after applying each vacuum level. Leaf conductance was obtained from the slope of the least-squares regression of flow rate versus the applied vacuum. The resulting leaf hydraulic conductance was scaled by the leaf surface area to obtain *K_{Lleaf}* (kg m⁻² s⁻¹ MPa⁻¹).

Theoretical petiole xylem hydraulic conductance normalized by leaf area (*K_i*; kg m⁻¹ s⁻¹ MPa⁻¹) for each

species and wind exposure was determined according to Tyree and Ewers (1991):

$$K_t = ((3.14159 * \rho * d^4) / (128 * \eta)) / LA$$

where ρ is water density (kg m^{-3}), d^4 is vessel diameter to the fourth power (m^4), η is water viscosity (MPa s) and LA is the leaf area.

Stomatal and minimum conductance

Stomatal conductance (g_s) was measured in three leaves per plant ($n = 10$) and wind exposure of *C. integerrima* using a steady-state porometer (LI-COR 1600). Measurements were made between 0900 and 1100 hours during 3 days in the summer. Leaves of *B. microphylla* were too small to get reliable g_s measurement with our instrument despite using a small chamber aperture (0.5 cm^2).

The minimum stomatal conductance (g_{\min}) was calculated using minimum transpiration (E_c). Leafy twigs were cut in the field and the cut end was immediately placed in distilled water for 2 h and covered with black plastic bags to allow for hydration. Single hydrated leaves ($n = 10$ per species and wind exposure) with the cut end sealed with nail polish were allowed to freely transpire on a laboratory bench, at photosynthetically active radiation of $<10 \text{ mmol photons m}^{-2} \text{ s}^{-1}$, and at air temperature and RH approximately constant for 16 h. The leaves were weighed at different time intervals (30–120 min) depending on hydration level. Plots of loss of weight vs. time were used to calculate transpiration of the leaf surfaces. The loss of leaf weight increases at first very rapidly (large transpiration rate), and then at a slower rate (after stomatal closure) to reach a nearly asymptotic value at the end of the measurement period (phase of E_c after complete stomatal closure). Minimum conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$) was calculated according to Hoard et al. (1996):

$$g_{\min} = E_c / (D / P)$$

where D is the air saturation deficit (kPa) and P is the atmospheric pressure (kPa) under laboratory conditions.

Leaf water potential and pressure–volume relations

Midday leaf water potential (Ψ_L) was measured in ten leafy shoots per species and wind exposure during 3 days in the summer using a pressure chamber (PMS1000; Corvallis, OR).

Pressure–volume curves (Tyree and Hammel 1972) were obtained for four shoots per species and wind exposure using the bench-drying technique. The leaves have very short petioles and consequently we had to use shoots with several leaves for the measurements. We took care in maintaining similar ratios of leaf to stem tissue of the shoots when comparing wind exposures to reduce the

effects of stem water content on the interpretation of the pressure volume curves. The shoots were cut at predawn in the field, immediately covered with black plastic bags to avoid dehydration, and transported to the laboratory. Measurements begun not later than 1 h after collection. Samples were non-hydrated to avoid alteration in water relations characteristics, which have been observed in species of arid ecosystems (e.g., Meinzer et al. 1986). After each determination of balance pressure with the pressure chamber, shoots were immediately weighed to the nearest 0.001 g and left to transpire freely on the laboratory bench until the next measurement. After all balance pressure–weight measurements were completed, the twigs were oven-dried at $70 \text{ }^\circ\text{C}$ until constant mass and weighed. Saturated weights of the samples (which were non-hydrated) to determine the relative water content were estimated from adjacent samples obtained from the same individual by determining hydrated/dry weight ratios. The tissue water relation parameters estimated were the osmotic potential at full turgor (π^{100}) and at the turgor loss point (π^0), the elastic modulus and the 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 the symplastic water fraction (SWF) to estimate the symplastic water volume. Saturated osmotic potential was converted to osmolality by multiplying saturated osmotic potential by $410 \text{ mOsmol MPa}^{-1}$. 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. ε was used to better represent tissue elastic properties across the full range of turgor values including the wilting point.

Plasticity index and statistical analysis

To assess phenotypic variation induced by wind we used the plasticity index (PI) (Valladares et al. 2002). This index was calculated for a particular morpho-anatomical or physiological trait within a species as the difference between the maximum (X_{\max}) and the minimum mean value (X_{\min}) divided by the maximum mean value (per trait and species):

$$PI = (X_{\max} - X_{\min}) / X_{\max}$$

PI was determined for each shrub, and an average value per trait was obtained for each species. The PI scales from 0 to

1. This standardized form of the index allows comparisons across variables expressed in different units and with different variation ranges.

All morphophysiological characteristics within a species and environmental variables were analyzed for normal distribution using the Kolmogorov–Smirnov, and Student’s test was used for assessing for differences at leeward and at windward within species. Mean PI between trait groups (morpho-anatomical and physiological traits) were also tested using Student’s test. The SPSS statistical package (SPSS, Chicago, IL) was used and the level of significance used was $P < 0.05$.

Results

Wind speed at leeward and at windward of each plant were substantially different (Fig. 1a, b; t -value -39.6 for *B. microphylla* and -35.2 for *C. integerrima*; $P < 0.001$ for both species). The differences between both crown exposures were higher for *B. microphylla*. Mean daily wind speeds for *B. microphylla* were 4.93 and 1.46 m s^{-1} at windward and leeward, respectively, whereas for *C. integerrima* they were 3.18 and 1.11 m s^{-1} at windward and leeward, respectively. Maximum wind speed measured during the study period was 15.55 m s^{-1} . The differences in wind gust speed were up to 4.38 m s^{-1} higher at windward than at leeward for *C. integerrima* and 8.8 m s^{-1} for *B. microphylla* (t -value -39 for *B. microphylla* and -32 for *C. integerrima*; $P < 0.01$ for both species). Consistent with the higher wind speed at windward, D was also

higher at windward in both species (t -value -3.48 for *B. microphylla* and -35.17 for *C. integerrima*; $P < 0.05$) due to an increase in air temperature and a decrease in relative humidity at this side of the crown (Fig. 1c, d). Mean D was 0.6 kPa at windward and 0.5 kPa at leeward for *B. microphylla* and 0.9 kPa at windward and 0.7 kPa at leeward for *C. integerrima*. Despite the higher D at windward, leaves of both species were able to maintain similar minimum Ψ_L on both sides of the crown (Fig. 2). The minimum Ψ_L was about -3 MPa in *B. microphylla* and -2 MPa in *C. integerrima* regardless of wind exposure.

Wind exposure did not modify leaf size (Fig. 3a), whereas there was a tendency to reduce the number of leaves per branch in both species (Fig. 3b). Leaf shape tended to change between crown sides, but the differences

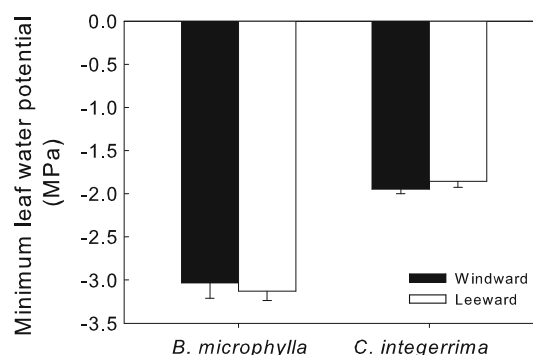


Fig. 2 Minimum leaf water potential at windward (solid bars) and leeward (open bars) for *B. microphylla* and *C. integerrima*. Bars are mean values ($-SE$) ($n = 10$)

Fig. 1 Typical pattern of mean wind speed (m s^{-1}) (a, b) and air saturation deficits (D ; kPa) (c and d) at windward (solid line) and leeward (dotted line) sides of the crown of *Berberis microphylla* and *Colliguaja integerrima*, respectively, during 3 days of January 2011

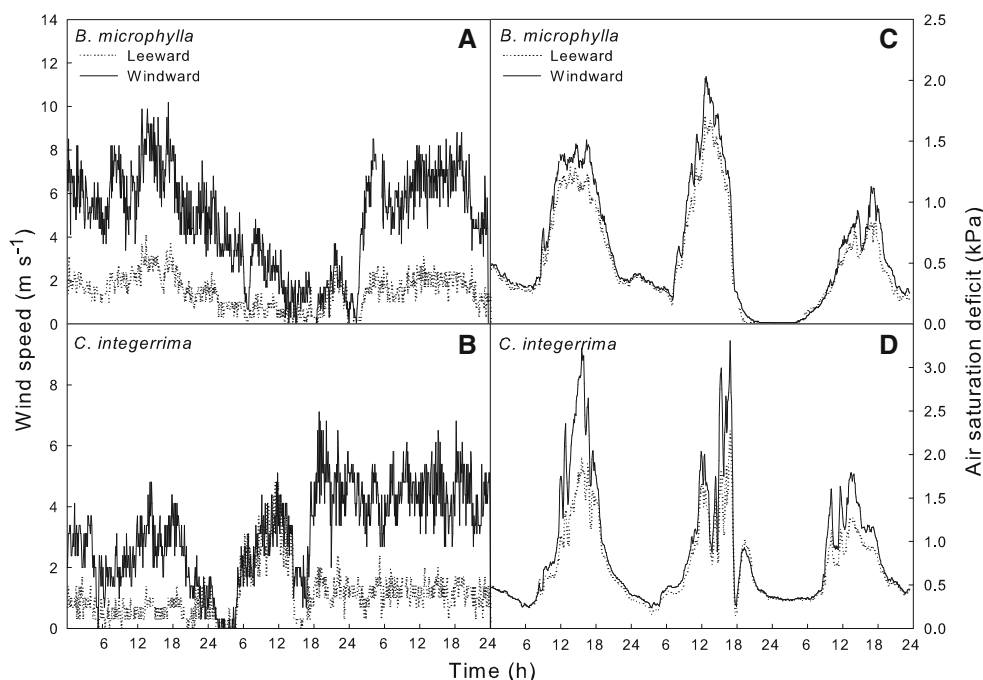
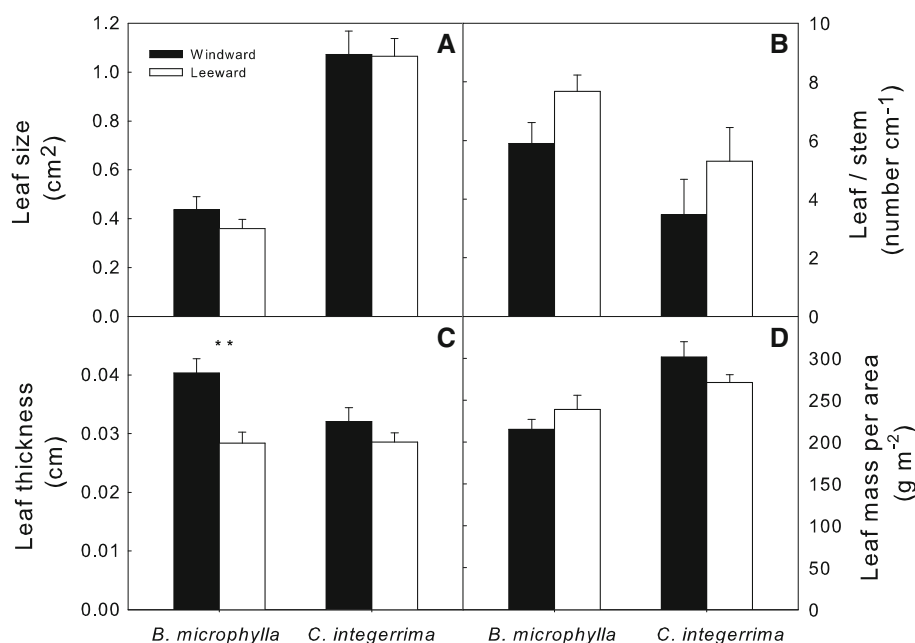


Fig. 3 **a** Leaf size (cm^2), **b** number of leaves per stem length, **c** leaf thickness, and **d** leaf mass per area (LMA) at windward (*solid bars*) and leeward (*open bars*) for *B. microphylla* and *C. integerrima*. Bars are means values + SE ($n = 10$). Significant differences between windward and leeward are indicated as $**P < 0.01$



were not significant. Leaf length was 33.8 ± 1.19 mm and 32.9 ± 1.55 mm at windward and leeward, respectively, for *C. integerrima*, and 17.26 ± 0.99 mm and 18.47 ± 1.01 mm at windward and leeward, respectively, for *B. microphylla*. Crown leaf area index tended to be lower at windward than at leeward in both species (for *C. integerrima* 0.87 and $1.07 \text{ m}^2 \text{ m}^{-2}$ at windward and leeward, respectively; and for *B. microphylla* 1.29 and $1.49 \text{ m}^2 \text{ m}^{-2}$ at windward and leeward, respectively). Leaves of *B. microphylla* were significantly thicker at windward than at leeward (t -value 3.73 ; $P < 0.01$) but there was no difference in leaf thickness for *C. integerrima* (Fig. 3c). LMA was not significantly different between crown sides in both species (Fig. 3d).

Traits influencing gas exchange were affected by the position of the leaves in the crown. Stomata length (μm) tended to be smaller (18.8 ± 0.92 at windward and 20.09 ± 0.66 at leeward for *C. integerrima* and 18.79 ± 0.48 at windward and 19.52 ± 0.68 at leeward for *B. microphylla*). Stomatal density was significantly higher in leaves from the windward than from the leeward side of the crown in both species (Fig. 4a; t -value -5.24 for *B. microphylla* and -2.99 for *C. integerrima*; $P < 0.01$); nevertheless, stomatal pore index was higher at windward only in *B. microphylla* (Fig. 4b; t -value -3.36 ; $P < 0.05$). g_s was lower at windward in *C. integerrima*: windward morning g_s was $57.9 \text{ mmol m}^{-2} \text{ s}^{-1}$ and at leeward it reached values of $61.4 \text{ mmol m}^{-2} \text{ s}^{-1}$.

Leaf mass loss increased asymptotically with time (Fig. 5). In both species the mass loss was slower in leaves fully exposed to wind than in more protected leaves (lower slope in exposed leaves; t -value -2.7 and $P < 0.05$ for

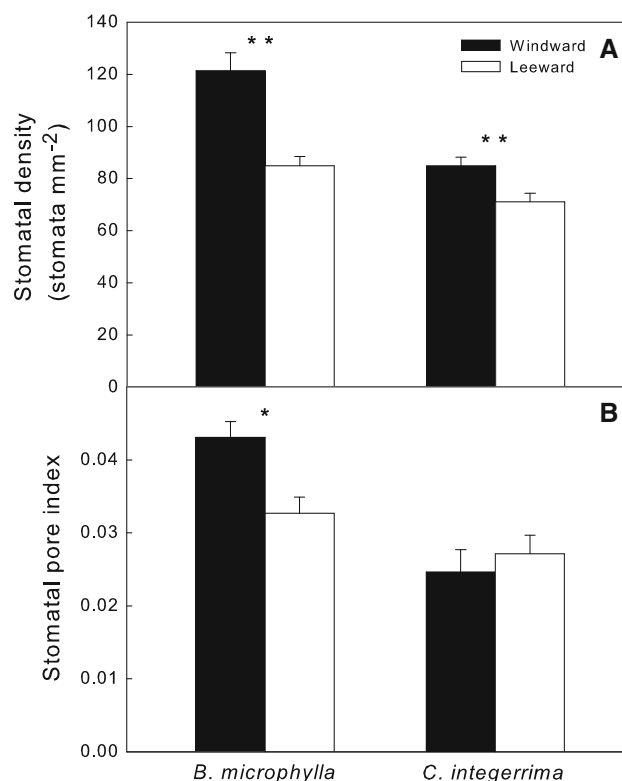


Fig. 4 **a** Stomatal density (stomata mm^{-2}) and **b** stomatal pore index at windward (*solid bars*) and leeward (*white bars*) for *B. microphylla* and *C. integerrima* leaves. Bars are means values + SE ($n = 10$). Significant differences are indicated as $*P < 0.5$ and $**P < 0.01$

B. microphylla and -4.34 , $P < 0.01$ for *C. integerrima*). g_{min} , determined after the inflexion point, tended to be lower at windward, in particular in *C. integerrima*, where there was a reduction of 20 % in exposed leaves

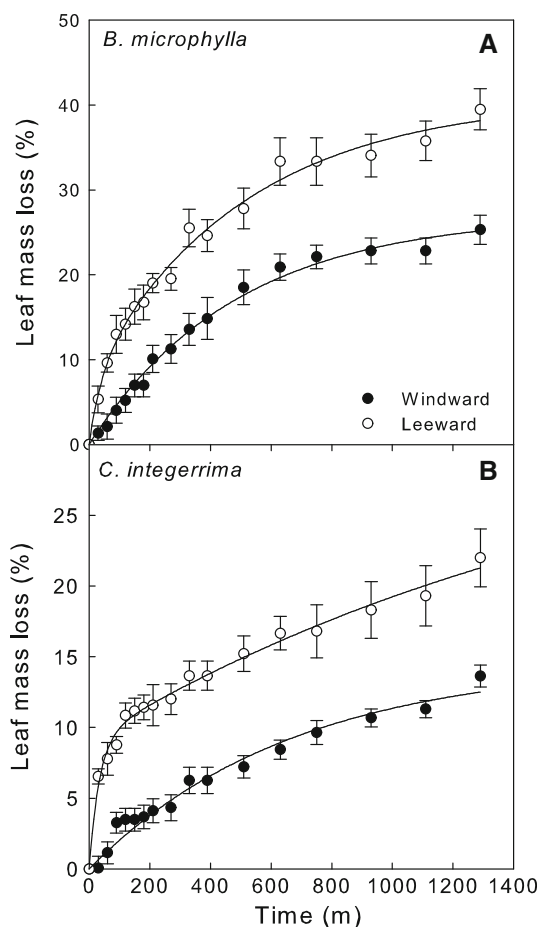


Fig. 5 Leaf-drying curves (leaf mass loss as a function of time) for detached leaves of *B. microphylla* (a) and *C. integerrima* (b) exposed to windward (closed circles) and to leeward (open circles) environmental conditions. Water loss is expressed as a percentage of the initial leaf mass. Each value represents the mean value \pm SE for ten leaves. Lines are the exponential increase to a maximum function fitted to the data ($y = 7.7 \times [1 - \exp(-0.02x)] + 33 \times [1 - \exp(-0.002x)]$ and $y = 13 \times [1 - \exp(-0.002x)] + 14 \times [1 - \exp(-0.002x)]$ for *B. microphylla* at leeward and windward, respectively, and $y = 9 \times [1 - \exp(-0.03x)] + 24 \times [1 - \exp(-0.0005x)]$ and $y = 14 \times [1 - \exp(-0.0015x)]$ for *C. integerrima* at leeward and windward, respectively)

($3.1 \text{ mmol m}^{-2} \text{ s}^{-1}$ at leeward and $2.2 \text{ mmol m}^{-2} \text{ s}^{-1}$ at windward; $P < 0.05$), while in *B. microphylla* the reduction was 16.7 % ($1.8 \text{ mmol m}^{-2} \text{ s}^{-1}$ at leeward and $1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ at windward).

The pressure–volume relationships of shoots indicated a significant increment (less negative values) in osmotic water potential at π^{100} , at π^0 (Fig. 6a, b) and a reduction in the amount of osmotically active solutes (Fig. 6c) at windward in both species. Shoots of *C. integerrima* at leeward had up to nine times more solutes than at windward. The ε tended to increase at windward in *B. microphylla* (Fig. 6d).

Differences in anatomical traits were only significant for *C. integerrima*. For example, leaf xylem vessels in the

transversal section of the midveins had a significantly higher diameter and substantially lower density at windward than at leeward (Fig. 7a, b). Leaf physiological traits also associated with the hydraulic function were consistently significantly different between the different sides of the crown for *C. integerrima*, but were similar for *B. microphylla* (Fig. 8). Theoretical petiole xylem hydraulic conductivity (K_t) was higher in windward leaves of *C. integerrima* (t -value 4.47, $P < 0.01$), while leaf hydraulic conductance (K_{Leaf}) was substantially higher for the same crown exposure, but differences were not significantly different. Whereas K_t in this species was twofold higher at windward, K_{Leaf} was three times higher for the same crown exposure.

Mean PI differed between morpho-anatomical and physiological traits within each species (Table 1). Physiological traits exhibited higher plasticity than morpho-anatomical traits in both species but only in *C. integerrima* were the differences significant (t -value -3.5 , $P < 0.01$). There were no significant PI differences between species.

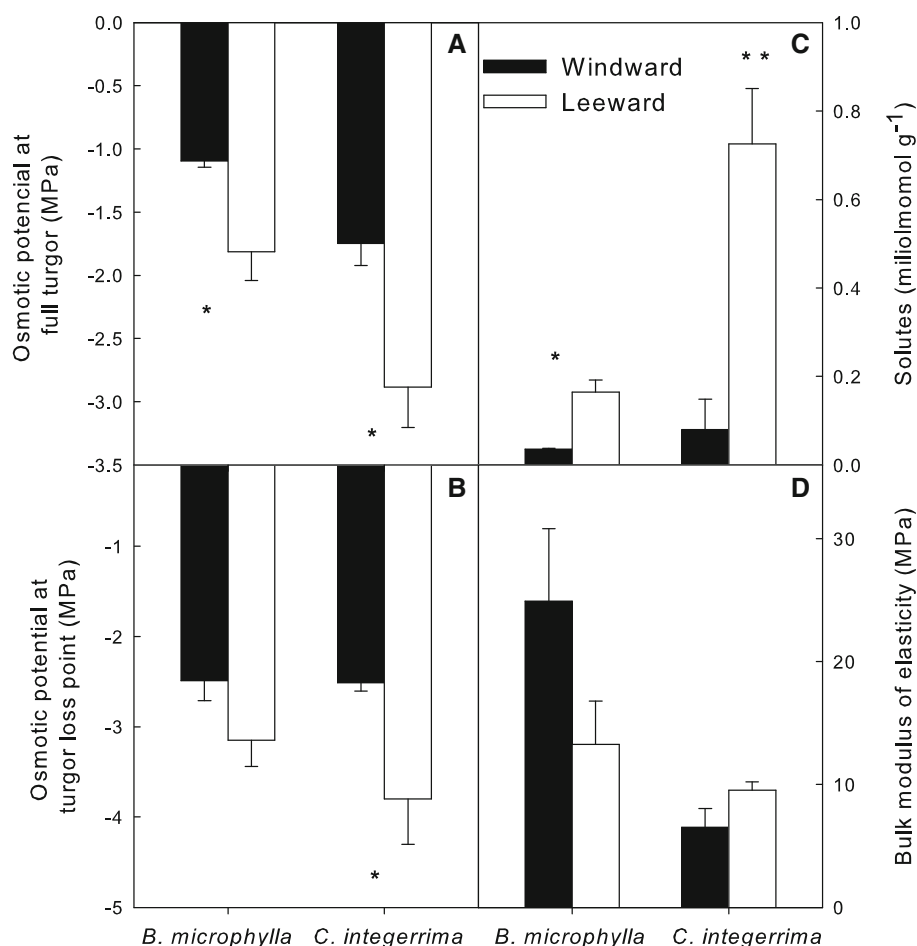
Discussion

Plasticity in morpho-anatomical vs. physiological leaf traits

The sheltering effect of crowns resulted in differences in morphophysiological traits in Patagonian shrubs. The speed of the westerly wind was reduced 59.7 and 32.4 % between exposed and protected sides of the plants in *B. microphylla* and *C. integerrima*, respectively. These differences in forced convection and D surrounding the leaves between sides of the crown resulted in leaf anatomical, morphological and physiological changes that reveal a certain degree of intra-canopy leaf phenotypic plasticity. Although we cannot rule out that other factors can influence intra-canopy plasticity as this behavioral response integrates the effect of multi-environmental factors (Sack et al. 2006), we think that differences in leaf properties found between crown sides may be partially explained by wind because leaf samples were collected from the outer layer of the canopy where the effects of self-shading are minimal (crowns are low and open and there is no overlap among crowns).

To assess plant phenotypic plasticity in response to wind is challenging because both mechanical (morpho-anatomical) and physiological traits are simultaneously affected by this factor (Smith and Ennos 2003; Anten et al. 2010). However, the species examined in our study did not show plastic changes in mechanical traits as the PI was relatively low for traits that have been associated with responses to mechanical stimulation (e.g., LMA, vein density). The only

Fig. 6 **a** Osmotic potential at full turgor (MPa), **b** osmotic potential at the turgor lost point (MPa), **c** solute content (mOsmomol g⁻¹) and **d** bulk leaf modulus of elasticity (MPa) of leaves at windward (*black bars*) and leeward (*open bars*) of *B. microphylla* and *C. integerrima*. Bars are mean values + SE of ten leaves per species and wind exposure. Significant differences are indicated as ** $P < 0.01$ and * $P < 0.05$



leaf trait related to mechanical properties that exhibited significant changes with wind exposure was leaf thickness in *B. microphylla* (the higher the wind speed, the higher the thickness). Thicker leaves in general tend to have higher structural resistance (e.g., work to shear, force to punch and to tear; Onoda et al. 2011), probably conferring greater resistance against damage from the wind.

Low plasticity of LMA (or its inverse: specific leaf area) is surprising since several studies have shown that this leaf trait is highly plastic (Bucci et al. 2006; Gratani et al. 2006; Wyka et al. 2012). However, higher plasticity of LMA has been generally found in response to different levels of irradiance (Poorter et al. 2009), as this trait reflects the investment in biomass in relation to leaf surface for light capture. Although LMA correlates positively with leaf structural toughness, strength, and stiffness of plants in other ecosystems (Read and Sanson 2003; Read et al. 2005), the result found in this study suggests that LMA does not have a substantial role in determining the resistance of leaves to mechanical stress (Niklas 1992). The lack of response of LMA under mechanical stress induced by wind observed in this study is consistent with findings of Anten et al. (2006) and Garcia-Verdugo (2011).

The main effect of wind in this study appears to be the consequence of the relatively high air saturation deficit in the wind-exposed side of the crown. Physiological traits related to avoidance of water loss exhibited higher plastic responses than morpho-anatomical traits which could allow a higher resistance to mechanical stress. For plants growing in water-limited environments, it is essential for them to develop mechanisms for the avoidance of water loss and to find an optimal hydraulic architecture that can provide both efficient and safe water transport.

The regulation of Ψ_L which resulted in similar minimum Ψ_L in the leaves of both sides of the crown, as observed in these study species, probably contributes to maintain adequate carbon gain while restricting the rate of water loss under conditions of higher D . These results suggest that different mechanisms help to regulate water loss and Ψ_L changes in response to increasing D in the most exposed side of the crown of shrubs in an ecosystem such as the Patagonian steppe where soil water availability is limited during most of the year (Bucci et al. 2009; Scholz et al. 2012). The main mechanism used to explain the homeostatic behavior in leaves has been the sensitivity of stomata to soil water potential and D (Franks et al. 2007).

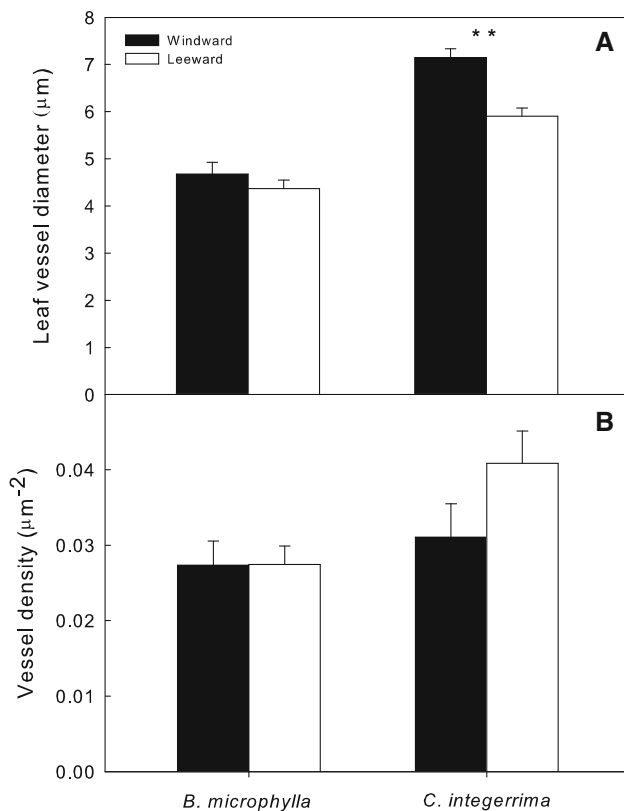


Fig. 7 **a** Leaf xylem vessel diameter (μm) and **b** number of vessels per leaf area (μm^{-2}) for *B. microphylla* and *C. integerrima* at windward (black bars) and leeward (white bars) sides of the crown. Bars are means values + SE ($n = 10$). Significant differences are indicated as ** $P < 0.01$

However, other mechanisms such as the reduction in total leaf area, the increase in leaf specific hydraulic conductivity and water use from internal reservoirs (Bucci et al. 2005, 2008; Zhang et al. 2012), can all contribute to the homeostasis in minimum Ψ_L . In this study we observed two physiological responses that can explain the homeostasis in leaf water status across the crown despite of differences in D between sides of the crown: reduced g_{min} and osmotic adjustment in *B. microphylla* and leaf hydraulic adjustments in *C. integerrima*.

Changes associated with minimizing water loss: turgor loss and reduced leaf epidermal conductance

Osmotic adjustment is the decline of osmotic potential in the cytosolic compartment of the cells by accumulation of solutes which maintain positive cell turgor allowing the maintenance of active metabolic and physiological processes. In a previous study with Patagonian shrubs, Scholz et al. (2012) found in the same species that cell turgor loss occurs with high D , despite g_s being only reduced 40–60 %. In our study the two species showed a decrease in solute concentration in leaf tissues and higher π^0 and

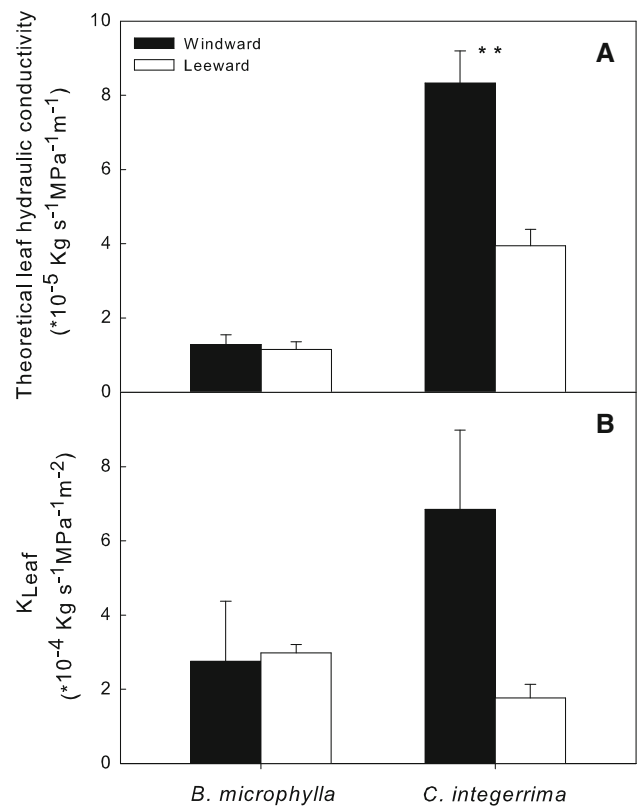


Fig. 8 **a** Theoretical leaf hydraulic conductivity ($\text{kg MPa}^{-1} \text{m}^{-1} \text{s}^{-1}$) and **b** leaf hydraulic conductance ($\text{kg MPa}^{-1} \text{m}^{-2} \text{s}^{-1}$) for *B. microphylla* and *C. integerrima* at windward (black bars) and leeward (open bars) sides of the crown. Bars are means values + SE of ten leaves per species and wind exposure. Significant differences are indicated as ** $P < 0.01$

π^{100} in leaves exposed to higher D , resulting in turgor loss in *B. microphylla* and stomatal closure in both species. We have not measured g_s in *B. microphylla*; however, taking into account the results of Scholz et al. (2012), which show a relatively high sensibility of g_s to D for this species, we can assume that g_s was reduced on the windward side of the crown in *B. microphylla*. The two study species had higher stomatal density and SPI at the windward side of the crown, probably to maintain CO_2 uptake when the wind speed is low. On the other hand, high stomatal sensitivity prevents excessive water loss when D is high.

In addition to turgor loss and partial stomatal closure on the exposed side of the crown, decreased cell wall elasticity observed in leaves of *B. microphylla* would also favor the maintenance of symplastic water content, which may enhance leaf mechanical resistance. Turgor pressure in leaves can contribute significantly to their mechanical properties (Niklas 1988; Hejnowicz and Barthlott 2005). However, leaves of *B. microphylla* did not show signs of wilting, neither did the leaves of *C. integerrima* when Ψ_L dropped below the turgor loss point (P. Iogna, personal observation). These findings suggest that wind does not

Table 1 Plasticity index for leaf structure and anatomy traits, leaf conductance and leaf water relation traits of *Berberis microphylla* and *Colliguaja integerrima*

Trait type	Variables	Plasticity index	
		<i>B. microphylla</i>	<i>C. integerrima</i>
Morpho-anatomical	Leaf size	0.1075	0.0078
	Leaf mass area	0.0773	0.0886
	Thickness	0.2964	0.1091
	Stomatal density	0.3005	0.1629
	Stomatal pore index	0.0737	0.1125
	Vessel diameter	0.0656	0.1737
	Vessel density	0.0019	0.2398
	Vein density	0.0784	0.0913
	Mean	0.1251 ± 0.0390	0.1232 ± 0.024
	Physiological	Leaf hydraulic conductance	0.0770
Minimum leaf hydraulic conductance		0.1511	0.2854
Petiole hydraulic conductance		0.104	0.526
Minimum leaf water potential		0.0308	0.0459
Osmotic potential at full turgor		0.8131	0.3935
Osmotic potential at zero turgor		0.2103	0.3388
Bulk modulus of elasticity		0.8792	0.4625
Solute content		0.7693	0.8896
Mean		0.3994 ± 0.1309	0.4538 ± 0.0090

Values closer to 1 indicate high phenotypic plasticity

produce mechanical damage in the leaves of the studied shrub species.

In addition to stomatal control, cuticular conductance that is only 1 or 2 orders of magnitude lower than minimum stomatal conductance (Kerstiens 2006), can play an important role in determining leaf water status in arid environments. Cuticles in plants non-adapted to high winds can exhibit severe damage if the wind speed is relatively high, and consequently experience increased cuticular conductance and water loss due to disruption of epicuticular waxes (Grace 1974; Hoad et al. 1998). In this study, leaves exposed to drier and windy conditions had cuticles with lower water permeability (lower g_{\min}) than less-exposed leaves. Leaves exposed to higher wind speeds apparently had high deposition of waxes that increased the cuticle thickness and resistance to water loss [lower cuticle conductance (g_c) or g_{\min}]. Although the gravimetric method used here could overestimate g_c because not all stomata completely close in the dark (Howard and Donovan 2007; Scholz et al. 2007), we consider that the technique used accurately represents g_{\min} on a comparative basis.

Hydraulic changes enhance water transport without increases in the driving force

Stem and leaf hydraulic responses to changes in wind speed have been scarcely studied (but see Smith and Ennos 2003; Fluckiger et al. 1978 and Iogna et al. 2011).

Research has been mostly focused on mechanical responses to strong winds. In this study we found large variations in leaf hydraulics as a consequence of different wind speeds, which is not surprising since several studies have shown that leaf hydraulics are highly plastic traits (Aasamaa et al. 2001; Sack and Holbrook 2006). Removal or decreasing boundary layer thickness due to high wind speeds decreases the resistance to water transport from the leaf surface into the atmosphere, and thus we would expect that leaf water use efficiency should be higher in leaves more exposed to wind to prevent a large drop in minimum Ψ_L . In the absence of homeostasis the leaves could approach the threshold for hydraulic dysfunction if those changes are not accompanied by increases in resistance to cavitation (Tyree and Sperry 1989), or reduce xylem cell wall collapse (Blackman et al. 2010). In this study we observed that an increase in hydraulic efficiency in *C. integerrima* contributed to maintaining minimum Ψ_L above the threshold of hydraulic failure.

Whereas in *B. microphylla* homeostasis in Ψ_L was achieved by controlling the water loss by stomata and the cuticle, in *C. integerrima* an improved hydraulic system was responsible for maintaining favorable water status. Although leaf xylem vessel density decreased in the exposed side of the crown in *C. integerrima*, a small increase in vessel diameter was enough to increase the leaf hydraulic conductivity. According to the Hagen-Poiseuille law, the water transport in stems increase by the fourth power of the xylem conduit diameter (Tyree and Ewers

1991). The higher hydraulic efficiency in *C. integerrima* is consistent with the results found by Smith and Ennos (2003) in *Helianthus annuus*, who observed an increase in stem hydraulic conductivity in plants exposed to higher air flow. Similarly, Onoda and Anten (2011) found that the increase in the amount and size of vessels in petioles of *Plantago major* resulted in a larger volume of water transported to the leaves under higher wind speeds.

The hydraulic behavior observed in *C. integerrima* could be disadvantageous in terms of water conservation; however, *C. integerrima* have a deep root system (2 m deep) where soil water availability is relatively high even during the dry season (Bucci et al. 2009). On the other hand, higher hydraulic conductance has been interpreted as adaptive in environments with high *D* (Maherali et al. 2004) facilitating transpiration without increasing the soil to leaf water potential gradient (Maherali and DeLucia 2000). Another possible interpretation of higher K_{Leaf} under higher *D* in *C. integerrima* is that water transport efficiency improves carbon fixation to compensate for the reduced leaf area per branch found on the exposed side of the crown. Other studies have shown that there are no differences in carbon assimilation and transpiration between the exposed and protected side of the crown (Cordero 1999).

The reduction in leaf size is a typical response to increasing wind speeds (Grace 1988; Garcia-Verdugo et al. 2009) because the allocation of assimilates is shifted from the production of leaf material to the production of more resistant stems and roots (Telewski 2006). In this study we have not observed changes in leaf size between crown sides, but there was a reduction in the leaf area supported by the stem (20 % lower leaf area per branch at windward). A high K_{Leaf} is counterbalanced by a lower total leaf area support by stems, resulting in similar total water use per side of the crown.

Conclusion

This is the first study that addresses simultaneously wind effects on several water relation traits, hydraulic architecture and biomass allocation patterns in woody plants from arid ecosystems. This comprehensive and multi-factorial study was performed in two typical dominant shrubby species which exhibited contrasting physiological responses to wind. The lack of strong leaf mechanical responses could be associated with the high energetic costs involved in the in situ production of rigid structures or in the utilization of photoassimilates in other plant organs, such as roots, that may contribute to improve the water balance and anchorage of the plants. Leaf phenotypic changes within the crown helped to prevent the decrease in minimum Ψ_L and thus contributed to the maintenance of homeostasis,

assuring the hydraulic integrity of the studied plants. Whereas *B. microphylla* avoided a decrease in Ψ_L in the exposed side of the crown by reducing water loss through stomatal control, avoiding turgor loss and lowering epidermal conductance, *C. integerrima* improved the leaf water transport efficiency to enhance water transport without increases in the driving force for water loss in the wind-exposed side of the crown.

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