



## Xylem efficiency vs. safety: Acclimation to drought of seedling root anatomy for six Patagonian shrub species

M. Durante\*, P.H. Maseda, R.J. Fernández

Facultad de Agronomía, Universidad de Buenos Aires, IFEVA-CONICET, Av. San Martín 4453, Buenos Aires, Argentina

### ARTICLE INFO

#### Article history:

Received 22 September 2009

Received in revised form

11 May 2010

Accepted 1 December 2010

Available online 31 December 2010

#### Keywords:

*Anarthrophyllum rigidum*

*Chuquiraga aurea*

*Chuquiraga kingii*

Hydraulic efficiency

Hydraulic safety

*Lycium chilense*

*Mulinum spinosum*

### ABSTRACT

With the aim to seek evidences on the possible trade off between hydraulic efficiency and hydraulic safety, from both an evolutionary and an acclimation point of view, we compared root xylem anatomy and derived functional properties for seedlings of six Patagonian shrub species grown in a greenhouse under two levels of water availability (control and drought). Root central cylinder area, vessel diameter ( $b$ ) and double-wall thickness ( $t$ ) were measured; from these data, the sum of vessel radii to the fourth power and wall strength  $[(t/b)^2]$  were calculated as indicators of hydraulic efficiency and safety respectively. Across species, we observed only a weak negative correlation between hydraulic efficiency and hydraulic safety. Within species, *Lycium chilense*, the species with the most mesic leaves of the group, showed significant acclimation to drought for both functional attributes, lowering efficiency and increasing safety by developing a higher proportion of small and more resistant xylem vessels.

© 2010 Elsevier Ltd. All rights reserved.

### 1. Introduction

Plant water-conduction systems are an important component of plant tolerance to drought (Maherali et al., 2004), with hydraulic efficiency and hydraulic safety being the most important functional properties (Tyree and Zimmermann, 2002). Hydraulic efficiency is defined by xylem hydraulic conductivity, the capacity to transport water in the liquid phase, and it depends on vessel number and size (especially diameter). Hydraulic safety is the property that allows the xylem to maintain its function under biological and mechanical stress. Sequences of freezing–melting, or tensions generated by water deficits can lead to the formation of embolism, lowering hydraulic conductivity (Hacke and Sperry, 2001). Other biotic and abiotic agents such as browsing or wind can affect the xylem. Then, xylem conductivity, in a particular situation, depends on hydraulic efficiency and how it is affected by the presence of embolisms, determined by its hydraulic safety.

There is evidence that water-stress adapted species resist higher tensions but at a cost of a reduced water conductivity compared with more mesophytic species (Pockman and Sperry, 2000; Hacke and Sperry, 2001; McElrone et al., 2004). There is also evidence of an

evolutionary interspecific trade off between efficiency and freezing resistance (Davis et al., 1999). However, less data are available on plastic responses of a species when exposed to drought stress (see Maseda and Fernández, 2006). If xylem vessels produced under drought conditions have a reduced diameter (Lovisolo and Schubert, 1998; Corcuera et al., 2004; Holste et al., 2006) and proportionally thicker walls, then they would be capable of tolerating higher tensions without collapsing (Hacke et al., 2001), but at the cost of a reduced water-conduction efficiency. Thus, what has been established as an inter-species trade off would be mirrored at the intra-species level.

As functional properties are related to anatomy, structural traits can be used to assess hydraulic efficiency and hydraulic safety. Hydraulic efficiency depends on vessel diameter, length, density and degree of vessel connections (Tyree and Zimmermann, 2002). According to the Hagen–Poiseuille law the flow rate of a capillary is proportional to the fourth power of its radius; then, the sum of vessel radii to the fourth power ( $\sum r^4$ ) is commonly used when studying conductivity (Sperry et al., 1994; McCulloh et al., 2003). Hydraulic safety is proximately related to microscopic traits, but is reflected in macroscopic traits, especially wood density (Hacke et al., 2001). According to the air-seeding hypothesis, drought-induced cavitation (the trigger for embolism) occurs when a small air bubble is drawn in by suction through the largest pore of the pit membrane into a functional vessel (Sperry and Tyree, 1988). Then, the size of the largest pore determines the xylem resistance to

\* Corresponding author. Tel.: +54 11 4524 8070x8106.

E-mail addresses: [durante@agro.uba.ar](mailto:durante@agro.uba.ar) (M. Durante), [maseda@agro.uba.ar](mailto:maseda@agro.uba.ar) (P.H. Maseda), [fernandez@agro.uba.ar](mailto:fernandez@agro.uba.ar) (R.J. Fernández).

cavitation, which seems to be related with pit membrane area per vessel (Wheeler et al., 2005). Besides having less porous walls, resistant conduits should be strong enough to withstand the bending stress occurring between water and gas-filled vessels. Hacke et al. (2001) found, across 48 species, that the higher the conduit wall thickness ( $t$ ) relative to its maximum span ( $b$ ), the higher the resistance against implosion by negative pressure, and the lower the water potential inducing 50% loss of hydraulic conductivity ( $P_{50}$ ). This interspecific relationship, however, did not hold between poplar clones (Cochard et al., 2007).

To seek evidences on the possible trade off between efficiency vs. safety in an acclimation context, we performed a greenhouse experiment comparing six shrub species and assessing their plasticity when exposed to drought. The main objectives were: (1) To evaluate possible differences in the root anatomy and derived xylem functional properties of six shrub species from semiarid Patagonia; and (2) To assess their plasticity when exposed to water stress. Our predictions were also two: that growing without hydraulic restrictions, species with higher safety will have lower hydraulic efficiency; and that when exposed to water stress, species will increase safety and decrease efficiency.

## 2. Materials and methods

### 2.1. Species

Seeds of *Anarthrophyllum rigidum* (Gillies ex Hook. et Arn.) Hieronymus, *Chuquiraga aurea* Skottsberg, *Chuquiraga kingii* Ball, *Lycium chilense* Miers ex Bertero and *Mulinum spinosum* (Cav.) Persoon were collected as a pool sample from at least 10 plants per species, in places of high abundance of each species, near Río Mayo (approx. Lat. 45°25'S, Long. 70°20'W). Seeds of two *M. spinosum* populations were collected: "West" and "East"; they differ in chromosome number (Maseda, data not published), and thus can be considered as two different species.

### 2.2. Plant culture

Several seeds from each species (except *A. rigidum*) were placed on moistened filter papers in plastic boxes (10 × 25 cm) within a growth chamber in darkness at 6 °C (Soriano, 1960). Seeds of *A. rigidum* were mechanically scarified before being placed in similar boxes within a dark growth chamber but at 20 °C. Once seeds germinated, after 10–45 days, 200 seedlings of similar size of each species were selected and transplanted to forestry trays (Dass-plastic-40) in a controlled-temperature glasshouse. Commercial substrate (Klasmann) was used and all the seedlings were kept at field capacity during the entire acclimation process. After 60–90 days (end of winter) 90 seedlings of homogeneous size for each species were selected and transplanted to 1-l plastic pots, containing fine sand (<250 μm). The base of each pot was fitted with a fine nylon cloth to allow air and nutrient solution exchange, yet preventing root passage. All plants had been kept well watered during two weeks until treatments of drought began. Drought was imposed using a modification of the method proposed by Snow and Tingey (1985), as described by Fernández and Reynolds (2000). This procedure ensures that drought intensity becomes independent of plant size. An hydrosoluble commercial fertilizer (KSC phitactyl II, Roullier s.a.) which contained N:P:K (23:5:5) and micronutrients was used for mineral nutrition of plants at 3 g l<sup>-1</sup>.

### 2.3. Experimental conditions

The experiment was performed in a controlled-condition glasshouse, under natural irradiance, in an experimental field at the

School of Agriculture, Facultad de Agronomía, Universidad de Buenos Aires (Lat. 34°35'S, Long. 58°28'W). The aim was not to mimic field conditions or to be able to extrapolate our results to field situations; rather, this setting was devised for the objective of singling out the effect of drought on root anatomy. During the whole 18-week experiment glasshouse temperature was recorded every 30 min with a thermocouple connected to a micrologger (Model 21X, Campbell Scientific, Inc., Logan, UT). The experiment had a split-plot 2-way factorial design, with six species and two levels of drought (control and drought). Twenty groups of 18 pots each (three pots per species, one plant per pot) were placed in a 140-l plastic container housing a 28-cm-tall column of commercial Styrofoam (no. 0140; Smithers-Oasis; Kent, Ohio, USA) with a water table at different depths. The foam had been repeatedly rinsed with water, as recommended by the manufacturer, before the installation of plants. The 20 containers were randomly assigned to two water levels, with 10 replications each. Based on a previous pilot experiment, we chose water levels to obtain two drought intensities: 100% (control, C) and 51% (drought, D) of field capacity. These stress levels were attained by partially filling the containers until the nutrient solution was 5 cm (control) and 17.5 cm (drought) below the base of the pots. Keeping a constant nutrient solution height ensures uniform and repeatable water availability in the pots (Saulescu et al., 1995). The soil is kept constantly half-wet; hence, equilibrium moisture levels are reached in a few minutes, ensuring that drought intensity is independent of plant size.

### 2.4. Morphological variables

At the time pots were placed in the containers, an initial harvest of 30 seedlings of each species was made. These plants were never placed in the containers but in every other respect were treated in the same way as those used for the rest of the experiment. The final harvest was done 4 months after drought treatments began, using all 10 plants from each treatment (one repetition per species in each container). Each plant was separated into leaves, stems, and roots; all plant material was dried for 48 h at 80 °C to determine dry weight. Before drying, a subsample of the leaves was measured with a leaf-area meter (Li-Cor 3100; Li-Cor Inc., Lincoln, Nebraska). Besides, subsamples of the root system were immediately scanned for length determination with an image-device system, and the first 5 cm of the main root of each plant was conserved in FAA (5% formaldehyde, 5% acetic acid, 90% ethanol) until anatomical measurements. Finally, total biomass for each treatment was calculated. Specific leaf area (SLA) was calculated as the ratio between leaf area and leaf dry mass. SLA is related with plant functional attributes like photosynthetic capacity, leaf life span, and adaptations to water stress (Reich et al., 1995; Niinemets, 2001). Since drought-adapted plant species (i.e. xerophytic) show generally lower values of SLA than species from places without water restrictions (i.e. mesophytic) (Mitchell et al., 2008), we used SLA as an indicator of species mesophytism.

### 2.5. Anatomical variables

From the root subsamples conserved in FAA, freehand root transverse sections were cut at 2 cm from the main root apex and stained with safranin 0.5% to increase contrast. Digital images were taken with a 640 × 480 pixel monochrome video camera attached to a compound microscope. The magnification used was ×20 for *L. chilense* and ×40 for the rest of the species. The UTHSCSA Image Tool 3.0 free software (<http://ddsdx.uthscsa.edu/dig/itdesc.html>) was used for image processing. To obtain vessel diameter

(b); vessel area was measured first; then, diameter was calculated assuming vessel shape as circular. To avoid confusion with other elements, only vessels larger than 10  $\mu\text{m}$  were measured. Vessel-wall thickness was calculated as the mean double wall between one vessel and its neighboring vessels. The central cylinder area was also measured, and vessel density was calculated as the ratio between vessel number and central cylinder area. Following (Sperry et al., 1994), we calculated the hydraulically weighted diameter as  $2(\sum r_i^5 / \sum r_i^4)$ .

Then, xylem functional properties were derived from anatomical measures. First, we estimated, based on the Hagen–Poiseuille law, the relative hydraulic efficiency (RHE) as the ratio between the sum of vessel radii to the fourth power and total conduction area (Eq. (1)).

$$\text{RHE} = \frac{\sum_{i=1}^n r_i^4}{\text{Central cylinder area}} \quad (1)$$

where  $r$  express the vessel radius; thus, RHE express the conduction capacity of each  $\mu\text{m}^2$  of the conduction system.

Second, to estimate hydraulic safety of each individual, we used the index proposed by Hacke et al. (2001): the wall strength  $[(t/b)_h]$ . The terms  $t$  and  $b$  are wall thickness and vessel diameter for conduits of mean hydraulically weighted diameter ( $h$ ), respectively. For each individual, the measured vessel closest to the mean hydraulically weighted diameter was used to estimate  $(t/b)_h$ .

It is important to consider that both functional properties derived from anatomy are not completely independent from each other, since vessel diameter is used for estimating both RHE and  $(t/b)_h$ , and differences in the frequency distribution of vessel diameter classes will affect both. However, for efficiency estimation all measured vessels are included, whereas for safety estimation only the closest one to the mean hydraulic diameter ( $b_h$ ) is. Thus, RHE and  $(t/b)_h$  are not affected by the same anatomical variables: differences in the central cylinder area or in vessel number will affect RHE but not  $(t/b)_h$ , and differences in the relation between wall thickness and vessel diameter will affect only  $(t/b)_h$ . Therefore, unlike what may have been expected from a superficial analysis, a negative correlation between RHE and  $(t/b)_h$  is not inevitable (“spurious”), but informative (Peters, 1991).

To evaluate the acclimation to drought of each species, we calculated a plasticity index (PI, Eq. (2)), where  $C$  and  $D$  represent any of the xylem functional attribute values [RHE or  $(t/b)_h$ ] for control and drought treatments respectively.

$$\text{PI} = \frac{C - D}{C + D} \quad (2)$$

PI ranges between  $-1$  and  $1$  (similar to Valladares et al., 2000); values close to  $0$  indicate lack of plasticity and extreme values (either positive or negative ones) maximum plasticity.

### 3. Results

Differences between species for root anatomical and functional properties were first assessed in the absence of drought (Fig. 1, open bars). *L. chilense* was clearly different from the rest of the species, showing the highest vessel number and central cylinder area and the lowest vessel density. Besides, *L. chilense*, *A. rigidum* and *C. aurea* showed the highest hydraulically weighted mean vessel diameter (Fig. 1A–D). The higher vessel number and diameter (Fig. 1A, D) of *L. chilense*, compared to the other species, caused that it had the largest sum of radii to the fourth power (between 2.5 and 15.0 times higher). However, as a result of its lower vessel density (Fig. 1C), its conduction system was not the most efficient per xylem area unit, but the third one, after *A. rigidum* and *C. aurea* (Fig. 1E).

These three species, besides having the highest specific hydraulic conductivity, were also the ones with the lowest wall strength (Fig. 1F).

Experimental drought caused a general reduction in total biomass (Table 1), which was statistically significant only for *M. spinosum* W. ( $p < 0.0001$ ) and marginally so for *L. chilense* ( $p = 0.06$ ). However, drought affected root anatomy mainly in *L. chilense* and *A. rigidum*. Both species decreased their vessel number (Fig. 1A), *A. rigidum* decreased the central cylinder area (Fig. 1B), and *L. chilense* decreased vessel density (Fig. 1C) and hydraulically weighted mean vessel diameter (Fig. 1D). Besides, these two species showed changes in the frequency distribution of vessel diameter, leading to a higher proportion of narrower (less efficient) vessels under drought (Fig. 2A.); this was also observed for *C. kingii*, but not for *M. spinosum* genotypes (E and W), which both had an inverse response, with fewer smaller vessels and more medium-size vessels under drought (data not shown). For *L. chilense* and *A. rigidum*, wall thickness increased less proportionally than diameter (i.e. slope much lower than the 1:1 line in Fig. 2B), implying that smaller vessels tended to have higher wall strength. For each species, regressions between these two traits were not different between water treatments. Drought changed significantly root functional properties in *L. chilense* and *A. rigidum*, while the other four species were unaffected. Both species reduced, under drought conditions, their relative hydraulic efficiency (Fig. 1E), and only *L. chilense* increased wall strength (Fig. 1F).

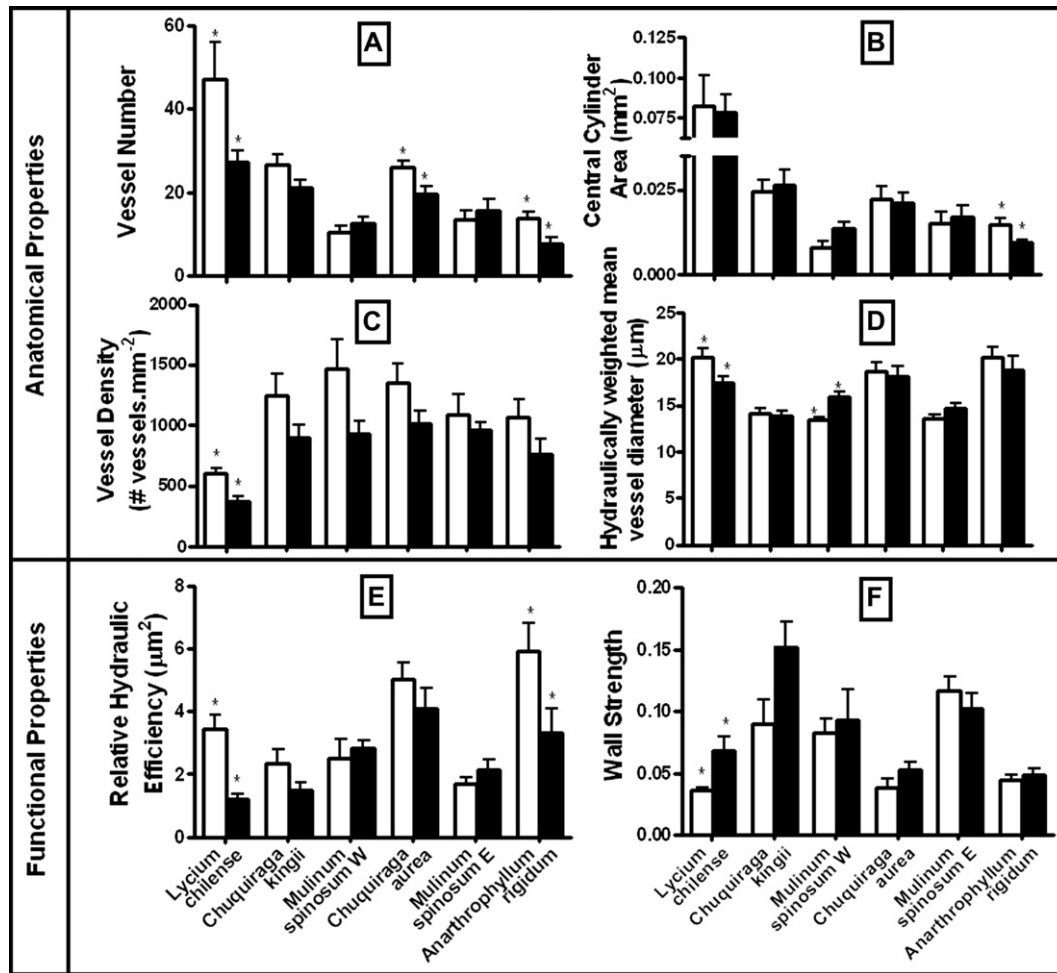
We observed only a trend towards a negative relationship between wall strength and relative hydraulic efficiency (Fig. 3). A permutation test performed for each treatment individually (Spearman non-parametric rank correlation coefficient), hinted a negative relationship between the two variables for controls ( $p = 0.06$ ), not significant for the drought treatment ( $p = 0.17$ ). Acclimation to drought for each species on relative hydraulic efficiency and wall strength can also be observed in Fig. 3: arrows show the changes caused by drought for *L. chilense* and *A. rigidum*, the species with the highest plasticity in the studied variables (Fig. 1E, F).

### 4. Discussion

We assessed root xylem anatomy and derived functional attributes for seedlings of six Patagonian shrub species. Mean conduit diameter of the six species was under  $30 \mu\text{m}$ , considered the threshold below which resistance to freezing-induced embolism is ensured (Davis et al., 1999). *L. chilense* was clearly different from the rest of the species in xylem anatomy and functional properties (Fig. 1). It also differed in external attributes, having higher SLA and total biomass (Table 1). Based on its SLA, we considered it the most mesophytic (less xerophytic) of the studied species (see Mitchell et al., 2008).

We found a weak negative correlation between hydraulic efficiency and hydraulic safety among species for control treatment (Fig. 3). Pockman and Sperry (2000) found a similar trade off between resistance to drought-induced cavitation (causing embolism) and conduction efficiency for 15 Sonoran desert species along a moisture gradient in the field. In that study, they showed that resistance to cavitation of the species was positively related with the minimum water potential observed in the field; then, species from humid places were not able to survive in drier sites. Hydraulically safe species, on the other hand, may be excluded from humid places because of the limited growth, and thus lower competitive ability, probably caused by their transport inefficiency (Sperry, 2000; Maseda and Fernández, 2006).

Within species, we expected that, when exposed to water stress, plants would increase hydraulic safety and would decrease



**Fig. 1.** Drought effects on root anatomical and functional properties (mean  $\pm$  s.e.) for the six Patagonian shrub species, ordered by decreasing SLA. Open and close bars represent control and drought treatment respectively. Asterisks indicate statistically significant differences between treatments ( $p < 0.05$ ).

hydraulic efficiency. *L. chilense*, with the highest SLA, showed a response in both functional attributes and *A. rigidum* presented only a response in hydraulic efficiency. This is in line with previous work showing drought-induced plasticity in the root anatomy of species from more humid environments, like *Vitis vinifera* (Lovisololo and Schubert, 1998), *Quercus ilex* (Corcuera et al., 2004) and *Phaseolus vulgaris* (Holste et al., 2006), and with the lack of plasticity of species from arid environments like *Hymenoclea salsola* and *Ambrosia dumosa* (Mencuccini and Comstock, 1997). Nevertheless,

**Table 1**

Patagonian shrub species, ordered by decreasing specific leaf area (SLA). Data is expressed as mean (s.e.).

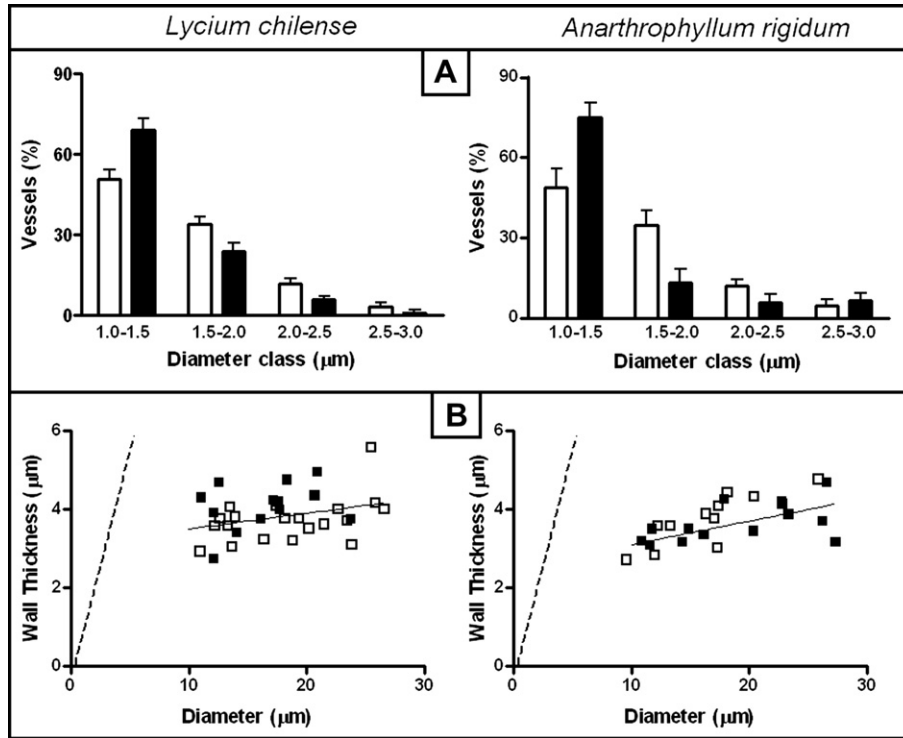
Species	Family	Phenology	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Total biomass (g)	
				Control	Drought
<i>Lycium chilense</i>	Solanaceae	Drought deciduous	77 (4)	2.48 (0.36)	1.67 (0.20)
<i>Chuquiraga kingii</i>	Asteraceae	Evergreen	56 (4)	0.63 (0.12)	0.44 (0.07)
<i>Mulinum spinosum W.</i>	Apiaceae	Drought deciduous	48 (5)	1.41 (0.07)	0.81 (0.03)
<i>Chuquiraga aurea</i>	Asteraceae	Evergreen	47 (3)	1.66 (0.25)	1.26 (0.30)
<i>Mulinum spinosum E.</i>	Apiaceae	Drought deciduous	45 (3)	1.41 (0.07)	1.36 (0.14)
<i>Anarthrophyllum rigidum</i>	Fabaceae	Evergreen	43 (1)	0.80 (0.10)	0.77 (0.10)

this does not necessarily mean that our xerophytic species are not plastic; for example, they could have expressed acclimation to drought by changing variables not measured here, such as root length or hydraulic conductance per unit leaf area (e.g. Trillo and Fernández, 2005).

Differences found here in root functional properties in seedlings grown under different water regimes provide evidence for a plastic trade off between hydraulic efficiency and hydraulic safety. Plasticity in root functional properties was higher for *L. chilense*, the species with the highest specific leaf area; it decreased efficiency and increased safety when exposed to drought (Figs. 1E, F and 3), suggesting that the increase in hydraulic efficiency could come at the cost of a reduced hydraulic safety. However, we observed only a weak negative relationship between relative hydraulic efficiency and wall strength among species, and an interspecific trade off was not evident.

To evaluate if the capacity of species to express changes in their root xylem functional properties under drought (i.e. the degree of plasticity of each species) was associated with its tolerance to drought, regressions between plasticity index and specific leaf area were performed for both root functional attributes (Fig. 4). Rank correlation (permutation) analysis showed no relationship between PI and SLA, and a marginally significant negative correlation for  $(t/b)_i^2$  ( $p = 0.1$ ). In future studies, it would be worthwhile to evaluate these trends for a larger set of species encompassing a wider range of xerophytism.



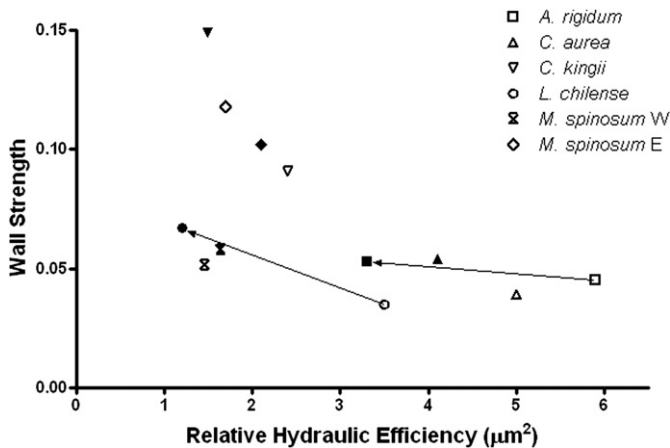


**Fig. 2.** Proportion of xylem vessels (mean ± s.e.) in four diameter classes (A), and regressions between wall thickness and vessel diameter (B) for *L. chilense* (left) and *A. rigidum* (right). Open and closed bars/squares represent control and drought treatment respectively. Solid lines represent the linear regression ( $p$ -values = 0.07 and 0.001 for *L. chilense* and *A. rigidum* respectively). The dashed line shows the 1:1 relationship.

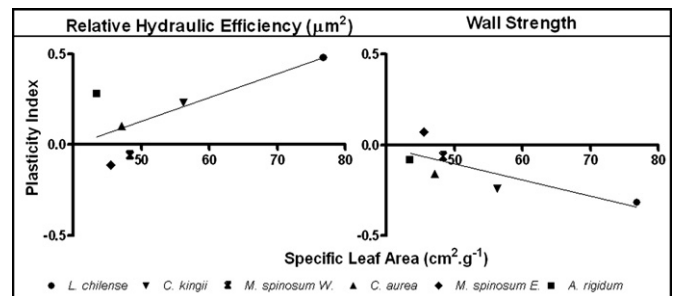
For those species showing more plasticity, wall thickness increased less proportionally than vessel diameter (Fig. 2, lower panels); then, across this drought range, the more efficient the vessel, the easier it would loss functionality (i.e., at a higher water potential; Lo Gullo and Salleo, 1993). Besides, in agreement with previous work (Lovisolo and Schubert, 1998; Corcuera et al., 2004), a higher proportion of small vessels was observed under drought (Fig. 2, upper panels). The combination of both anatomical changes can explain why water stressed plants resulted more resistant to drought and, perhaps more interestingly, can also be used to simulate vulnerability curves and to explain their shape. Plants with a narrow range of vessel sizes (and resistance) would have an

abrupt fall in conductivity once a water-potential threshold is crossed; on the other hand, plants with a broad range of vessel sizes would show a more gradual decrease in conductivity as water potential falls. Thus, we hypothesize that, since cavitation or implosion of individual vessels is not a random process but would begin by the bigger ones, typical size distributions like the ones we found (Fig. 2) would determine a non-linear relation between the percentage of non-functional vessels and percentage loss of conductivity. This could explain why Hietz et al. (2008) found a low (linear) correlation between the embolized area assessed by the xylem staining method and hydraulic methods for estimating percentage loss of conductivity.

In this work, we assessed major functional properties of water-conduction systems of seedlings of six Patagonian shrub species by simple anatomical measurements of roots in a controlled-environment experiment. This approach allowed the comparison of water-conduction systems between species and treatments at once,



**Fig. 3.** Regression between wall strength and relative hydraulic efficiency. Open and closed symbols for control and drought treatments respectively. Arrows indicate drought effect in *L. chilense* and *A. rigidum*, both species that showed plasticity.



**Fig. 4.** Regression between plasticity index and specific leaf area, for the two xylem functional attributes: relative hydraulic efficiency (left) and wall strength (right). Each point represents a species.

fostering our understanding of the trade off between efficiency and safety. It would be worth to include a larger set of species on this type of analysis, taking advantage of the uniform and repeatable soil–water environment it provides. Finally, to be able to extrapolate our findings to natural conditions, further comparisons between anatomically derived estimates and direct hydraulic measurements are needed.

### Acknowledgements

INTA provided for permission to collect seeds at the Río Mayo Experimental Station; Rodolfo Golluscio and Nicolás Trillo enriched this work with useful comments and suggestions; Juan Carlos Villardi helped in the set up of the experiment; Mónica Tourn and Viviana Vasellati gave anatomical advice, and Gabriela Zarlavsky helped with laboratory work.

### References

- Cochard, H., Casella, E., Mencuccini, M., 2007. Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. *Tree Physiology* 27, 1761–1767.
- Corcuera, L., Camarero, J.J., Gil-Pelegrín, E., 2004. Effects of a severe drought on *Quercus ilex* growth and xylem anatomy. *Trees – Structure and Function* 18, 83–92.
- Davis, S.D., Sperry, J.S., Hacke, U.G., 1999. The relationship between xylem conduit diameter and cavitation caused by freezing. *American Journal of Botany* 86, 1367–1372.
- Fernández, R.J., Reynolds, J.F., 2000. Potential growth and drought tolerance of eight desert grasses: lack of trade-off? *Oecologia* 123, 90–98.
- Hacke, U.G., Sperry, J.S., 2001. Functional and ecological xylem anatomy. *Perspectives in Plant Ecology, Evolution and Systematics* 4, 97–115.
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D., McCulloh, K.A., 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126, 457–461.
- Hietz, P., Rosner, S., Sorz, J., Mayr, S., 2008. Comparison of methods to quantify loss of hydraulic conductivity in Norway spruce. *Annals of Forest Science* 65, 502p1–502p7.
- Holste, E.K., Jerke, M.J., Matzner, S.L., 2006. Long term acclimatization of hydraulic properties, xylem conduit size, wall strength and cavitation resistance in *Phaseolus vulgaris* in response to different environmental effects. *Plant, Cell and Environment* 29, 836–843.
- Lo Gullo, M.A., Salleo, S., 1993. Different vulnerabilities of *Quercus ilex* L. to freeze- and summer drought-induced xylem embolism: an ecological interpretation. *Plant, Cell and Environment* 16, 511–519.
- Lovisolo, C., Schubert, A., 1998. Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *Journal of Experimental Botany* 49, 693–700.
- Maherali, H., Pockman, W.T., Jackson, R.B., 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85, 2184–2199.
- Maseda, P., Fernández, R.J., 2006. Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *Journal of Experimental Botany* 57, 3963–3977.
- McCulloh, K.A., Sperry, J.S., Adler, F.R., 2003. Water transport in plants obeys Murray's law. *Nature* 421, 939–942.
- McElrone, A.J., Pockman, W.T., Martínez-Vilalta, J., Jackson, R.B., 2004. Variation in xylem structure and function in stems and roots of trees to 20 m depth. *New Phytologist* 163, 507–517.
- Mencuccini, M., Comstock, J., 1997. Vulnerability to cavitation in populations of two desert species, *Hymenoclea salsola* and *Ambrosia dumosa*, from different climatic regions. *Journal of Experimental Botany* 48, 1323–1334.
- Mitchell, P., Veneklaas, E., Lambers, H., Burgess, S., 2008. Using multiple trait associations to define hydraulic functional types in plant communities of south-western Australia. *Oecologia* 158, 385–397.
- Niinemets, U., 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82, 453–469.
- Peters, R.H., 1991. *A Critique for Ecology*. Cambridge University Press, Cambridge.
- Pockman, W.T., Sperry, J.S., 2000. Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* 87, 1287–1299.
- Reich, P.B., Ellsworth, D.S., Uhl, C., 1995. Leaf carbon assimilation and conservation in species of differing successional status in an oligotrophic Amazonian forest. *Functional Ecology* 9, 65–76.
- Saulescu, N.N., Kronstad, W.E., Moss, D.M., 1995. Detection of genotypic differences in early growth response to water stress in wheat using the Snow and Tingey system. *Crop Science* 35, 928–931.
- Snow, M.D., Tingey, D.T., 1985. Evaluation of a system for the imposition of plant water stress. *Plant Physiology* 77, 602–607.
- Soriano, A., 1960. Germination of twenty dominant plants in Patagonia in relation to regeneration of the vegetation. In: *Proceedings of the Eighth International Grassland Congress*, pp. 154–158.
- Sperry, J.S., 2000. Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* 104, 13–23.
- Sperry, J.S., Nichols, K.L., Sullivan, J.E.M., Eastlack, S.E., 1994. Xylem embolism in ring porous, diffuse porous, and coniferous trees of Northern Utah and Interior Alaska. *Ecology* 75, 1736–1752.
- Sperry, J.S., Tyree, T., 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiology* 88, 581–587.
- Trillo, N., Fernández, R.J., 2005. Wheat plant hydraulic properties under prolonged experimental drought: stronger decline in root-system conductance than in leaf area. *Plant and Soil* 277, 277–284.
- Tyree, M.T., Zimmermann, M.H., 2002. *Xylem Structure and the Ascent of Sap*, second ed. Springer-Verlag, Berlin.
- Valladares, L., Wright, S.J., Lasso, E., Kitajima, K., Pearcy, R.W., 2000. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81, 1925–1936.
- Wheeler, J.K., Sperry, J.S., Hacke, U.G., Hoang, N., 2005. Inter-vessel pitting and cavitation in woody Rosaceae and other vesselless plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell and Environment* 28, 800–812.