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Wood density and anatomy of three *Eucalyptus* species: implications for hydraulic conductivity

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Abstract

Aim of the study: To characterize wood anatomical traits of three *Eucalyptus* species that differ in wood density and ecological requirements, and to examine the relationships between some anatomical features, wood density, and theoretical xylem hydraulic conductivity (K_s).

Area of study: We analyzed 86 trees from three sites of Argentina (Entre Ríos and Buenos Aires Provinces).

Methods: The sampled trees were *Eucalyptus globulus*, *E. grandis* and *E. viminalis* ranging from 11 to 15 years old. One stem disc was cut from each tree to determine wood density and identify quantitative anatomical features of vessels and fibers. Vessel composition (S, size - to-number ratio, a measure of vessel size distribution) and lumen fraction (F, the total sapwood area available for water transport) were estimated.

Results: *E. grandis*, the species with the highest growth rates, presented the highest theoretical K_s . This was associated with anatomical features such as a high density of wide vessels resulting in high F. On the other hand, *E. viminalis*, the species with the lowest growth rates and highest resistance to environmental stress, showed lower K_s as a result of a low density of wide vessels. These two species differed not only greatly in wood density but also in fiber characteristics. In the case of *E. globulus*, vessels were relatively narrow, which resulted in the lowest theoretical K_s , fibers were small, and wood density intermediate.

Research highlights: F had greater influence on K_s than S. The anatomical characteristics and wood density could only partly explain the differential growth or resistance to stress of the studied species.

Keywords: functional wood anatomy; lumen fraction; theoretical hydraulic conductivity; vessel composition; wood density.

Abbreviations used: Vd: vessel diameter; A: mean vessel lumen area; N: vessel density; F: lumen fraction (A \times N); S: vessel composition (A/N); K_s : theoretical xylem-specific hydraulic conductivity; D: basic density; 1F: non vessel lumen fraction; Fwt: fiber wall thickness; Fd: fiber diameter; Fld: fiber lumen diameter; MAI: stem mean annual increment.

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Introduction

Xylem structure varies along the tree at the same time that changes with age (Gartner, 1995), and has important implications for the tree functions (Zanne *et al.*, 2010). Regarding angiosperm xylem, in addition to their main support function, fibers might participate indirectly in water transport, by increasing vessel

implosion resistance under negative pressure (Jacobsen *et al.*, 2005), or by providing water to reduce tension in xylem (Pratt *et al.*, 2007). Despite the lack of consensus (e.g. Charrier *et al.*, 2016), several authors argue that parenchyma might also play a key role in embolism repair, by providing the solutes necessary to generate an osmotic gradient. Such gradient might allow water entry into cavitated vessels from nearby cells that would act

as capacitors (Tyree *et al.*, 1999; Hacke & Sperry, 2003; Salleo *et al.*, 2006; Brodersen *et al.*, 2010). It follows from this that the anatomical complexity of angiosperm wood contributes to an efficient water transport process, which depends directly on vessel characteristics and indirectly on the set of accompanying cells.

Wood density may be considered a measure of internal wood structure, reflecting the combination of features of the various cellular elements that compose wood (Naidoo *et al.*, 2006). This trait is associated with not only mechanical wood strength (Gartner, 1995) and fiber walls, but also with the structure of vessel and parenchyma cells, which have thinner walls and larger lumens (Naidoo *et al.*, 2006). Wood density therefore correlates with cavitation resistance, and the thickening of vessel and fiber walls could be related to the stress that xylem vessels must withstand during high tension periods (Hacke *et al.*, 2001). To adjust water supply, plants may alter wood properties in several ways, varying lumen fraction, that is, the fraction of sapwood occupied by vessel lumens (F), vessel composition (S, calculated as the quotient of mean vessel lumen area to vessel density, a measure of vessel size distribution), and the total area of sapwood supplying a given unit of leaf area (Zanne *et al.*, 2010). An analysis of 3005 angiosperm species showed that F and S do not correlate but instead change independently in response to different selection factors (Zanne *et al.*, 2010). Given that xylem-specific hydraulic conductivity (K_s) is a measure of xylem porosity (Tyree & Ewers, 1991) it is therefore influenced by vessel size and density. Functionally, it is associated with photosynthetic carbon assimilation rates (Hubbard *et al.*, 2001; Santiago *et al.*, 2004), plant growth (e.g. Kondoh *et al.*, 2006; Brodrigg *et al.*, 2005) and survival. Also, several studies have shown a strong adaptive value of resistance to hydraulic conductivity losses (vulnerability to cavitation) in different woody species, suggesting an association between increasing cavitation resistance and increasing aridity (Pockman & Sperry, 2000; Maherali *et al.*, 2004). From the global analysis relating F and S to theoretical (estimated) K_s , Zanne *et al.* (2010) have found that hydraulic efficiency is more influenced by the total amount of space available to move water (F) than by the particular distribution of that space within the vessels indicated by S. However, it seems that F is a more conservative character across species, whereas S has shown a wide range of variation across 3000 angiosperm species and therefore has proved to be the variable that has most influenced the variability in K_s .

Eucalyptus is one of the most important genera in forestry—eucalypts plantations occupy over 20 million hectares worldwide (Iglesias-Trabado & Wilstermann, 2009). It is a very diverse genus with approximately

700 species in a wide variety of ecological niches. The wood of this genus exhibits particular characteristics: diffuse porosity; mostly solitary vessels; diagonal vessel arrangement; simple perforation plates; alternate and vested intervessel pits with elliptical included aperture; vasicentric tracheids; fibers with circular or angular contour differentiated into two types, one with simple pits and another with bordered pits (fiber-tracheids); vasicentric-paratracheal and/or diffuse-apotracheal axial parenchyma; and ray parenchyma with uni-, bi-, tri- and rarely tetraseriate rays (Villegas & Rivera, 2002). This complex anatomy results in a wide range of wood densities within the genus, ranging from less than 400 kg m⁻³ to over 1000 kg m⁻³ (Zanne *et al.*, 2009). This density variability may partly reflect the high adaptive radiation of this genus, although comparatively few studies have related wood structure and function to environmental stress resistance. However, studies using different approaches have suggested that *Eucalyptus* wood structure (anatomy and density of branches) is associated with functional traits involved in stress tolerance or avoidance (e.g. Monteoliva *et al.*, 2015; Barotto *et al.*, 2016), and that it determines the distribution patterns of different species in an aridity stress gradient (Pfautsch *et al.*, 2016). Analysis relating branch anatomy and function in three *Eucalyptus* species showed that vessel diameter distribution is a key trait correlated not only with measured maximum K_s , but also with the species vulnerability to cavitation (Barotto *et al.*, 2016). That analysis did not include the traits F and S used by Zanne *et al.* (2010), so it is not clear whether S accounts for the variation in K_s among *Eucalyptus* species. In order to contribute to the knowledge of potential ecological implications of different wood anatomies within a single genus (i.e. within an expected quite narrow variation in F and S), we propose to analyze some wood anatomical traits of stems of three *Eucalyptus* species that differ in wood density and ecological requirements. These species are cultivated in different regions of Argentina, South America, and reflect in part the requirements of their provenance.

E. grandis Hill ex Maiden develops at sites with more than 1000 mm of annual rainfall. It is a light-weight wood species (at least, the genotypes used for commercial purposes in South America), with a mean wood density of about 420 kg m⁻³. It is frost-sensitive, but tolerates high temperatures and certain levels of drought stress. In the regions where it is cultivated (Mesopotamia region, Argentina) it shows a stem mean annual increment (MAI) of between 26 and 50 m³ ha⁻¹ year⁻¹ (genetically highly improved material).

E. globulus Labill develops at sites with more than 600 mm of annual rainfall. It is one of the most

cultivated *Eucalyptus* species worldwide because of its excellent wood characteristics for paper production. It is a mid-weight wood tree, with a mean wood density of about 600 kg m⁻³. It does not tolerate frost or high temperatures, but it can withstand drought stress (up to certain levels). In Argentina (southeast region of Buenos Aires province), it exhibits a MAI of around 30 m³ ha⁻¹ year⁻¹ (MAA, 2010).

E. viminalis Labill develops in positive relief sites, with deep well-drained soils and scarce hydric retention. It has mid-weight wood (mean wood density of 650 kg m⁻³). It has great tolerance to drought and frost stress. In Argentina (west of Buenos Aires province), it shows a MAI of around 25 m³ ha⁻¹ year⁻¹ (Ferrere *et al.*, 2008).

The aim of conducting this study was to characterize stem wood anatomy (morphometric characteristics of vessels and fibers) of the three *Eucalyptus* species mentioned above, and to examine the relationship between anatomical features, as described by Zanne *et al.* (2010), and wood density with hydraulic efficiency as estimated by theoretical hydraulic conductivity (K_s). The following hypotheses were tested: (i) Considering the weight exerted by F and S on K_s in the whole range of angiosperms, we expected that the theoretical K_s in the eucalypt species under study would be more influenced by vessel lumen fraction (F) than by vessel composition (S) (exponential coefficient of F (β) > exponential coefficient of S (δ) in the K_s prediction formula. See next section for equation). However, taking into account the importance of diameter distribution on measured K_s in *Eucalyptus* spp, we also expected that S would have a higher relative impact on K_s than in the survey by Zanne *et al.* (2010) (exponential coefficient of S (δ) > 0.5, which is the value found by Zanne and collaborators); and (ii) Based on the average growth rates, the order of the species regarding theoretical K_s was expected to be as follows: *E. grandis* > *E. globulus* = *E. viminalis*.

Materials and methods

Plant material

We sampled 86 individuals developing on three locations. In the case of *E. grandis*, we analyzed 4 clones growing in Concordia (Entre Ríos province, Argentina (31°22' S; 58° 07' W; 43 m asl) that belong to the network of genetic improvement field trials of INTA (National Institute for Agricultural Technology). More details about these clones can be found in Tesón *et al.* (2011) and Tesón *et al.* (2012). Data from *E. viminalis* also belong to individuals from INTA's network of genetic improvement field trials, growing in Guaminí

(35° 52'S; 60° 43'W; 265 m.a.s.l.) and Del Valle (37° 6'S; 62° 26'W; 297 m.a.s.l.), Buenos Aires province, Argentina. The *E. globulus* trees are individuals with two seed origins, local and Valdivian, grown in a plantation in Necochea (Buenos Aires province, 38° 57'S; 58° 57'W; 17 m.a.s.l.), which were sampled as part of another research project whose results have only been partially published (Igartúa & Monteoliva, 2010).

We collected one sample per tree from 30 *E. viminalis*, 36 *E. grandis* and 20 *E. globulus*. Sapwood from *E. grandis* and *E. viminalis* was sampled with an increment borer at 1.3 m above ground level along the north-south radius, while samples from *E. globulus* were obtained by destructive sampling (slices) at the same height. *E. grandis* and *E. viminalis* individuals were 14 years old, whereas *E. globulus* were 11 years old (n=14) and 15 years old (n=6).

Sample processing

Cross sections (20 μ m) were cut from the last two growing periods using a sliding microtome. Sections were then stained for 5 minutes in safranin (1%), dehydrated in a graded alcohol series, and mounted in Entellan® for microscopic analysis. Images of the stained cross-sections were captured with a digital camera (Olympus DP71, Japan) mounted on a research microscope (Olympus BX50, Japan), using either 4x or 40x objectives.

Anatomical variables

Image analysis software (ImagePro Plus, v. 6.3, Media Cybernetics, USA) was used to analyze captured images for the following parameters : fiber wall thickness (Fwt, μ m, $n = 100$ per sample), fiber diameter (Fd, μ m, $n = 100$ per sample), fiber lumen diameter (Fld, μ m, $n = 100$ per sample), vessel lumen diameter (Vd, μ m, $n = 100$ per sample), mean vessel lumen area (A, mm², $n = 100$ per sample), and vessel density (N, mm⁻², $n = 5$ captured images per sample). Double fiber wall thickness was measured from radial and tangential walls and their average divided by 2. Fiber lumen diameter was measured in radial and tangential directions. Vessel lumen diameter and area was measured in the transverse section and the number of vessels in each 1 mm² field was counted to determine vessel density. Basic density (D, kg·m⁻³) was obtained as the ratio of dry weight to saturated volume.

From the initial variables mentioned above, the following were calculated:

- Lumen fraction (F, unitless), which is a measure of the relative amount of sapwood area available for water transport. It was calculated from mean vessel lumen

area (A) and vessel density (N) for each individual sample.

- Vessel composition (also known as “vulnerability index”, S, mm⁴), proposed as a measure of vessel size distribution within the full space occupied by vessels, and calculated as the ratio between mean vessel lumen area (A) and vessel density (N).

- Theoretical xylem-specific hydraulic conductivity (K_s , kg·m⁻¹·MPa⁻¹·s⁻¹), which was estimated by the HagenPoiseuille law, as follows:

$$K_s = \frac{\left[\frac{(\pi \rho d^4)}{\eta} \right]}{128 \cdot N}$$

where ρ is water density (kg·m⁻³), d is vessel lumen diameter (m), η is water viscosity (MPa·s) and N is vessel density (m⁻²). K_s was estimated considering the proportional contribution of the different-sized vessels in each tissue (i.e. not from the mean vessel diameter).

Non- vessel lumen fraction (1-F, unitless), a measure of the sapwood area occupied by non-vessel cells (fibers, vascentric tracheids and parenchyma) as well as vessel walls, which resulted from subtracting the lumen vessel fraction (F) from the stem cross-sectional area.

Statistical analyses

One-way ANOVA was used to test for differences in selected variables between species. Differences were further analyzed with Tukey multiple pairwise comparison test ($\alpha=0.05$). Contingency tables were used to establish differences among species in vessel size distribution. Shifts in vessel characteristics were analyzed through variance of the four selected vessel variables (A, N, F and S), at a general inter-specific level as well as for each species. Based on the results, we examined the level of variation that S and F presented with respect to the variation showed by the other two variables, as follows:

$$\text{var}(A) + \text{var}(N) = 0,5 \cdot [\text{var}(S) + \text{var}(F)]$$

The maximum-minimum ratio for S and F was calculated for each species as well as at general level. This is a measure of the relative dispersion of each variable:

$$M_m = \frac{\max(x_i)}{\min(x_i)}$$

F and S influence over K_s was estimated according to Zanne *et al.* (2010), as follows:

$$K_s = \alpha \cdot F^b \cdot S^6$$

Estimation of equation parameters was carried out using nonlinear regression techniques. Goodness of fit and significance of parameter estimates were also analyzed. Influence over K_s was examined and quantified through the level of variation experienced as a result of changes in magnitude in the variables of the model.

All statistical analyses were carried out with InfoStat statistical software (v. 2012, InfoStat group, FCA, Universidad Nacional de Córdoba, Argentina).

Results

There were significant differences for every analyzed variable, especially between two of the studied species (Table 1).

Eucalyptus grandis presented fibers with a larger cross section than those of the other two species (thicker fiber wall and wider fiber diameter and fiber lumen diameter), and large mean vessel area, with intermediate values between the other two species (Fig. 1, Table 1). Vessel density was the highest in this species (twice as high as *E. viminalis*, but statistically similar to *E. globulus*), resulting in the highest F value. *E. grandis* was characterized by high lumen fraction (F), distributed in a similar number of vessels in each diameter class, but including in the largest diameter classes (> 160 μ m) more vessels than the other species (Fig. 2). Theoretical K_s reflected this trend, presenting the highest value. In *E. grandis*, S showed a low value, resulting from dividing a quite large mean diameter by a large number of vessels. S was similar to that observed in *E. globulus*, in spite of the high difference in F and K_s between species (Table 1), as well as in the diameter size distribution (Fig. 2). *E. viminalis* had the highest mean vessel area but the lowest vessel density, which resulted in high S. Its vessel size distribution (Fig. 2) resulted in K_s at intermediate values between the other two species, but not differing statistically from *E. globulus* (Table 1). Likewise, it showed the smallest fiber dimensions, with the thinnest fiber wall (Fwt) and values of fiber diameter and fiber lumen diameter (Fd, Fld) similar to those of *E. globulus*. The latter species was characterized by the smallest vessel area (A) and vessel diameter (Vd), with quite high vessel density (N), not differing statistically from *E. grandis* (Table 1). The vessel size distribution (Fig. 2) was significantly different from that of the other two species ($\chi^2 < 0.001$). *E. globulus* showed the lowest theoretical K_s (but not statistically different from that of *E. viminalis*) and, as stated before, a low value of S similar to that of *E. grandis* (Table 1, Fig.1).

Wood density ranged from 0.421 g/cm³ in *E. grandis* to 0.665 g/cm³ in *E. viminalis*, with an intermediate

Table 1. Vessel and fiber morphometry, basic density, and theoretical xylem-specific hydraulic conductivity (K_s) for three *Eucalyptus* species grown in Argentina. Mean \pm standard deviation

Variable		<i>E. grandis</i>	<i>E. viminalis</i>	<i>E. globulus</i>
Fibers	Fwt μm	3.24 c* ± 0.38	2.22 a ± 0.28	2.73 b ± 0.49
	Fd μm	20.49 b ± 2.38	12.58 a ± 0.68	13.61 a ± 0.80
	Fld μm	14.01 b ± 2.36	8.14 a ± 0.53	8.13 a ± 1.03
Vessels	Vd μm	96.57 ab ± 19.72	110.99 b ± 13.73	89.63 a ± 17.21
	A μm^2	9673.38 ab ± 3624	11268.17 b ± 2258	7518.02 a ± 2240
	N $\text{n}^\circ.\text{mm}^{-1}$	14.93 b ± 8.45	7.78 a ± 2.08	12.34 b ± 3.80
	F	0.144 b ± 0.005	0.087 a ± 0.004	0.092 a ± 0.004
	S mm^4	0.0006 a ± 0.0001	0.0015 b ± 0.0002	0.0006 a ± 0.0001
	1-F	0.856 a ± 0.0035	0.913 b ± 0.0041	0.908 b ± 0.0038
	Conductivity	$K_s \text{ Kg.m}^{-1}.\text{MPa}^{-1}.\text{s}^{-1}$	88.28 b ± 4.25	53.76 a ± 9.84
Density	D g.cm^{-3}	0.421 a ± 0.02	0.665 c ± 0.05	0.545 b ± 0.04

*Different letters indicate significant differences between species (Tukey test, $\alpha=0.05$). Fwt: fiber wall thickness, Fd: fiber diameter, Fld: fiber lumen diameter, Vd: vessel diameter, A: mean vessel lumen area, N: vessel density, F: lumen fraction ($A \times N$), S: vessel composition (A/N), K_s : theoretical xylem-specific hydraulic conductivity, D: basic density, 1F: non-vessel lumen fraction (fibers and parenchyma).

value obtained for *E. globulus* (Table 1). As expected from wood density data, 1-F (non-vessels lumen fraction) was higher in *E. viminalis* and *E. globulus* than in *E. grandis*, but did not differ between the former two species in spite of having significantly different wood density (Table 1).

Variation in vessel characteristics

From a functional standpoint, not only does the average size of wood characters matter, but also its variation. For example, a few large vessels (with small relative weight in the arithmetic mean) may be responsible for a significant increase in xylem water transport capacity, as was observed in *E. grandis* (Table

1, Fig. 2). At the genus level, S was the feature that showed the greatest variation (coefficient of variation (CV): 69.6%), followed by N, A, and F (CV: 55.6%, 48.1% and 33.8%, respectively). The same trend was observed for *E. grandis*, while in *E. globulus* and *E. viminalis* the order changed, with S remaining as the most variable feature, followed by F, A and N (data not shown).

In general (in all three species), vessel composition (S) explained 87.3% of total variation described by A and N, compared to only 12.7% accounted for by changes in lumen fraction (F). In turn, percentages also varied between species although similar trends were observed. At one extreme, in *E. grandis*, S explained 95.5% of total variation, while in *E. globulus* it only accounted for 65.6% of total variation.

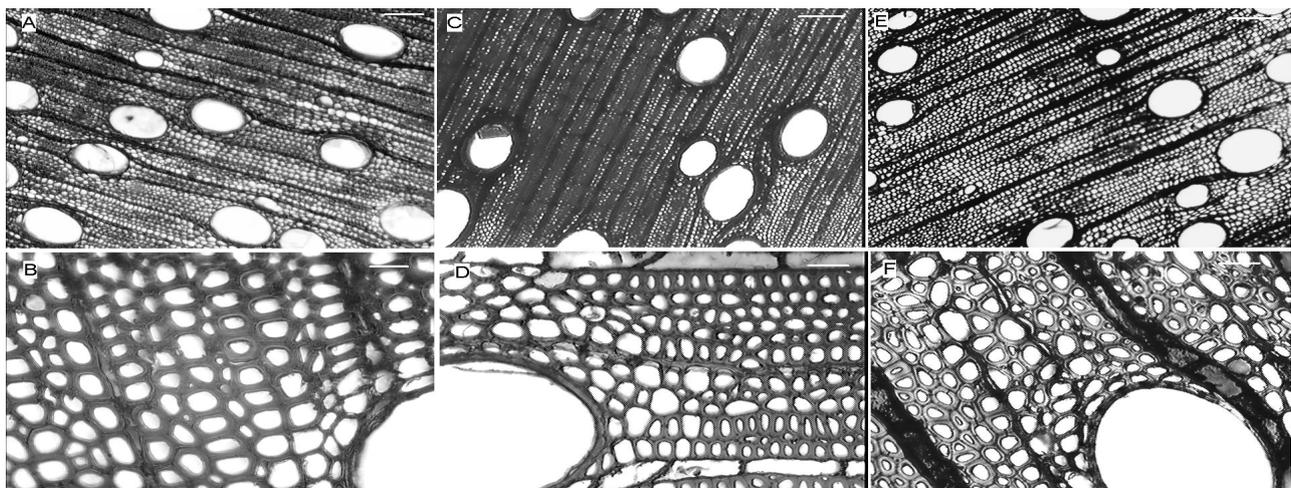


Figure 1. CT images (top panel: 4x; lower panel: 20x) of *E. grandis* (A and B), *E. viminalis* (C and D), *E. globulus* (E and F). Images A, C and E show the most numerous solitary pores in *E. grandis* (A) compared to the other species. Images B, D and F show details of fibers, radii and vasicentric tracheids. In *E. grandis* (B) the fibers are large (wider and larger lumen), whereas in *E. viminalis* (D) and *E. globulus* (F) they are small. Scale bar A, C and E=100 μm , scale bar B, D and F=20 μm .

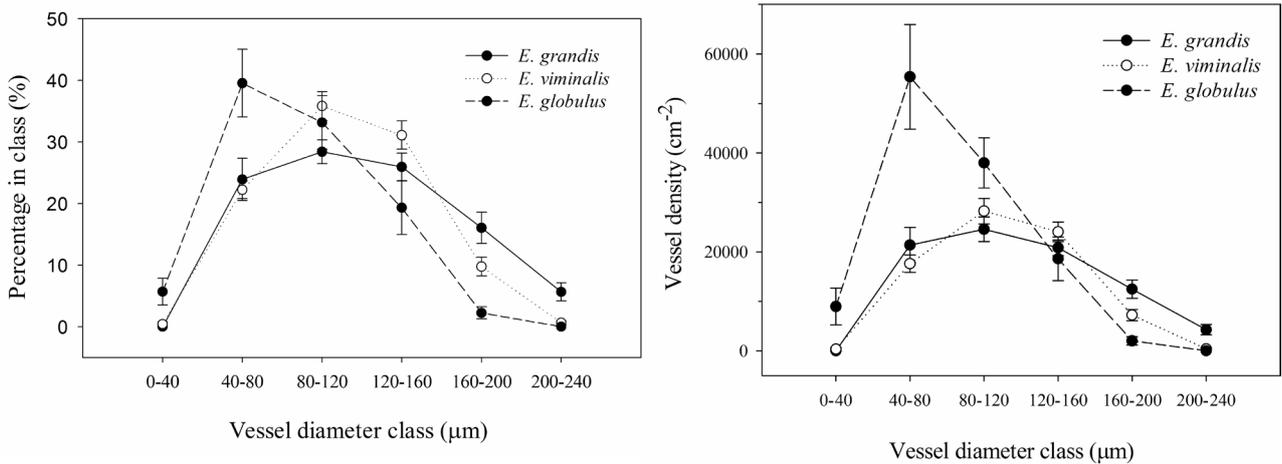


Figure 2. Vessel distribution in diameter class (µm). Mean and standard error.

Another indicator expressing the great variation of S compared to F was the maximum-minimum ratio. At general level, S varied 50-fold while F showed a 6-fold variation. While this trend was also recorded within species, it is noteworthy that *E. grandis* differed from the other two species, with a 50-fold and 3-fold variation in S and F, respectively. On the other hand, both *E. globulus* and *E. viminalis* exhibited an 8-fold variation in S, while F varied 5-fold and 3-fold, respectively.

Influence of F and S over hydraulic conductivity

Differences between species were obtained in parameters β and α of the model relating theoretical K_s to F and S (Table 2). This model showed an adequate fit for all analyzed species, and the parameters used were highly significant. The model applied to the pooled data of the three species resulted in the following parameters values: β= 1.5 and α=0.5.

Differences found in β for *E. viminalis*, *E. globulus* and *E. grandis* represent approximately a 21- and 41-fold variation in K_s for each 10-fold increase in F, respectively (Fig. 3a). The δ values found are similar for *E. globulus* and *E. grandis*, and a little lower for

E. viminalis. They produce a 2.7 and 2.6-fold variation in K_s for a 10-fold increase in S, respectively (Fig. 3b). This indicates that for the three species under consideration, the hydraulic conductivity is more sensitive to changes in lumen fraction (F) than to changes in vessel composition (S).

This can also be seen in Fig. 4, where F and S were plotted against K_s showing that the variation in F cuts the conductivity isolines more perpendicularly, while S does so more obliquely. The greatest general variance of S compared to F results in a greater contribution of the former to the variation between A (x axis, Fig. 4) and N (y axis, Fig. 4).

Discussion

F and S influence on theoretical K_s of three *Eucalyptus* species

By adjusting the model that relates theoretical K_s (estimated through Hagen-Poiseuille Law) to F and S, using the three species dataset, we obtained values for β and δ similar to those estimated by Zanne *et*

Table 2. Estimated value of the model parameters ($K_s = \alpha \cdot F^\beta \cdot S^\delta$)

Species	Parameter	Estimate	p-value	R ² adj	MSE
<i>E. globulus</i>	α	39201.72	0.0002	98.89	2.15
	β	1.53	< 0.0001		
	δ	0.44	< 0.0001		
<i>E. grandis</i>	α	50797.04	0.0001	97.94	35.50
	β	1.61	< 0.0001		
	δ	0.44	< 0.0001		
<i>E. viminalis</i>	α	21207.75	0.0002	97.27	7.95
	β	1.32	< 0.0001		
	δ	0.42	< 0.0001		

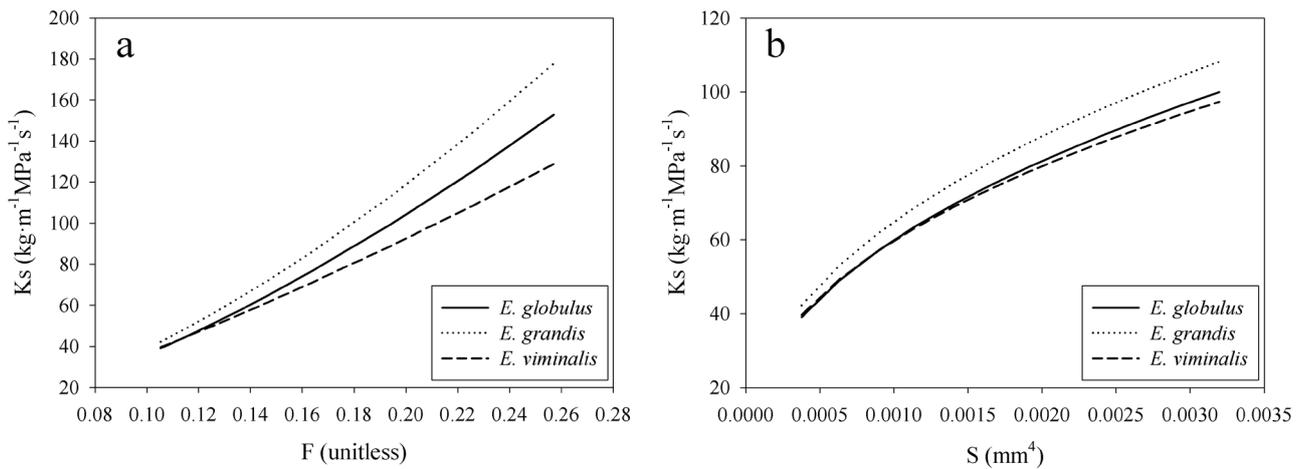


Figure 3. Changes in theoretical xylem-specific hydraulic conductivity (K_s) as a function of a) lumen fraction (F) and b) vessel composition (S) for three *Eucalyptus* species grown in Argentina.

al. (2010) for all angiosperm species, that is, 1.5 and 0.5, respectively. As shown in Zanne *et al.* (2010), we also observed a lack of orthogonality between S and F, and a greater influence of F on K_s than that of S. However, in contrast to their results, the variation in K_s was more influenced by F than by S. In addition, contrary to what we expected, the relative weight of S on K_s (δ coefficient) was slightly lower in each of the studied species (or similar in the pooled analysis of the three species) than in the general survey by Zanne *et al.* (2010). This means that vessel composition, estimated by S, has a low influence on K_s , in contrast to previous findings showing a high correlation between measures of vessel diameter distribution and measured maximum K_s (Barotto *et al.*, 2016). The

relative influence of S on K_s , which was low compared to F, is also a counterintuitive result considering the high relative weight of large vessels on K_s , since this is directly proportional to the 4th power of vessel diameter (Hagen Poiseuille Law). However, despite its lower relative weight, S has a significant influence on K_s , as its greater variability indicates that the plant can alter its conductive system to achieve greater conductive efficiency without modifying substantially the lumen fraction involved, which was a feature of great stability in all studied species. At the same time, and because conductivity increases approximately with the square of A, but only with the first power of N, altering vessel composition could have major influence on potential K_s . Nonetheless, in quantitative terms, the model applied to the three *Eucalyptus* species studied suggests that the great variation of S only produced a change in theoretical conductivity of 17 times, whereas F produced a change of 32 times.

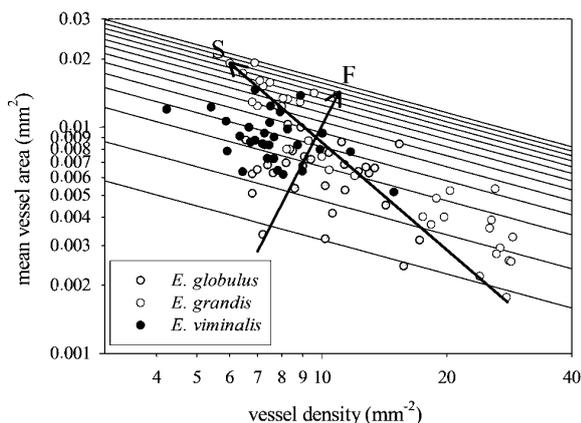


Figure 4. Mean vessel lumen area (A) vs. vessel density (N) for three *Eucalyptus* species grown in Argentina, plotted on log-scaled axes. Solid lines denote isolines representing constant values of theoretical xylem-specific hydraulic conductivity (K_s). Bold lines with arrows show the direction of increasing lumen fraction (F) and vessel composition (S).

Possible ecological implications of the stem wood anatomy of three *Eucalyptus* species

The results of this study were in agreement with the second proposed hypothesis. Based on the average growth observed in the areas where the studied species are planted in Argentina, and because of the functional relationship between water transport capacity and growth (Tyree & Ewers, 1991; Vander Willigen & Pammenter, 1998; Kondoh *et al.*, 2006), it was expected that *E. viminalis* would present a similar K_s than *E. globulus*, and both species would present a lower K_s than *E. grandis*. As expected, K_s of *E. viminalis* did not significantly differ from K_s of *E. globulus*. However, the mean K_s was relatively high, with an intermediate value between that of *E. grandis* and *E. globulus*.

This relatively high K_s suggests that, compared to *E. globulus*, *E. viminalis* is a species with a high capacity for water transport, at least at stem level, which could imply that the growth levels it typically achieves are below its potential. This is probably due to the fact that, being a species with high resistance to adverse environmental conditions, *E. viminalis* is usually grown in areas with some kind of growth-limiting factor (drought, low temperatures). This is in agreement with the results obtained by Barotto *et al.* (2016) in a study of these two species planted in a common place in Argentina (analysis without environmental constraints) that compare hydraulic conductivity and vulnerability to cavitation (at branch level). Their results showed that, compared with *E. globulus*, *E. viminalis* had higher specific hydraulic conductivity (both measured and theoretically estimated from anatomy) and higher cavitation resistance.

In accord with the hypothesis, *E. grandis* had the highest theoretical K_s , which could explain, at least in part, the high growth rates of the clones analyzed in this study (between 45 and 55 m³ ha⁻¹ yr⁻¹ at the age of 14 years, in years with high water availability, Tesón and Licata, EEA INTA Concordia, unpublished data). The high theoretical K_s for *E. grandis* was the consequence of high A and high N, which together caused F to be also the highest of the three species. These anatomical features are consistent with a high water transport capacity (high hydraulic efficiency), but probably with high vulnerability to drought and frost-induced cavitation. Vulnerability to cavitation curves in *E. grandis* (same clones as those analyzed here) indicate that this species has high vulnerability to drought-induced xylem cavitation, with a xylem water potential corresponding to 50% loss of hydraulic conductivity (Ψ_{50}) of -1.5 MPa (Tesón *et al.*, 2012). These P50 values are also consistent with values reported for *E. grandis* planted in South Africa (Vander Willigen & Pammenter, 1998). These water potential values, and even lower ones, are achieved at leaf level under normal conditions during the growing season in the sites where *E. grandis* is cultivated in Argentina. First, it is important to point out that this high vulnerability to cavitation is not related to large vessels in *E. grandis* branches. On the contrary, this species has the smallest vessels at branch level (Monteoliva *et al.*, 2017), compared to *E. viminalis* and *E. globulus*. In the three species, it seems that, at least at branch level, there is no trade-off between xylem safety and efficiency, but the opposite. Species with large vessels and high k_s (such as *E. viminalis*) are the safest (Barotto *et al.*, 2016). There is no information about vulnerability to cavitation at stem level, and we cannot extrapolate results from branches to stems. So, in the case of *E. grandis* branches, it seems that the functional

meaning of the high vulnerability to cavitation and resistance to water flow is to cavitate relatively soon during a drought acting as a fuse to protect the stem. Post-drought recovery in *E. grandis* has been observed in variables such as stem sap flux density and growth (Tesón *et al.*, 2011). However, it is yet unknown whether the cavitated branches recover their K_s or if the plant resilience relies on the formation of new tissues. It has been argued that, in angiosperms with high vulnerability to cavitation, high wood capacitance and efficient repair mechanisms would be essential parts of water transport strategy (Meinzer & McCulloh, 2013), which would be associated with low wood densities and small or negative safety margins (difference between the minimum daily water potential and cavitation threshold), leading to daily losses of K_s . This theory does not apply to *E. grandis* branches since they present high vulnerability to cavitation but high wood density, higher than the other studied *Eucalyptus* (Monteoliva *et al.*, 2017). However, the low wood density of *E. grandis* stems, coupled with its high vessel lumen fraction, but also with its high fiber lumen fraction, suggest that it may have high capacitance to buffer internal water deficits and thus contribute to efficient water transport under high evaporative demand conditions (drought avoidance strategy). In contrast to the highly vulnerable branch xylem of *E. grandis*, Ψ_{50} of *E. viminalis* and *E. globulus* growing in Argentina was -3.76 MPa and -2.44 MPa respectively (Barotto *et al.*, 2016), indicating quite high resistance to cavitation in the branches of these species and suggesting a different hydraulic strategy than that of *E. grandis*. In this regard, these species present a lower stem capacity than *E. grandis* for providing water to the leaves, resulting from their lower theoretical K_s and higher wood density (probably related to lower capacitance), but at the same time, presenting branches with higher K_s , lower vulnerability to cavitation and lower wood density. The combination of traits observed in *Eucalyptus* species challenges the well-established hypothesis about hydraulic functioning in woody species, as suggested by Meinzer & McCulloh (2013), and deserves future research combining studies at stem, roots and branch level.

In the present study, when analyzing stem density, anatomy and theoretical K_s , a general trade-off did arise between wood density and K_s , which is in agreement with results reported by Pfautsch *et al.* (2016) for *Eucalyptus* growing in Australia. However, as in the case of *Eucalyptus* branches (Barotto *et al.*, 2016), this should not necessarily imply a trade-off between hydraulic efficiency and safety. The particular and complex wood anatomy of *Eucalyptus*, composed of solitary vessels surrounded by vasicentric tracheids and parenchyma, allows for the possibility of avoiding this

trade-off (Barotto *et al.*, 2016). Moreover, there is no information available about embolism repair capacity in any *Eucalyptus* species, nor has their capacitance been quantified, so many questions about wood functional role in this genus remain unanswered.

Furthermore, *E. grandis* was the species that showed the greatest variation in vessel characteristics, suggesting that it would possess high genetic variability in these traits and/or a relatively high plasticity (greater than in the other studied species) to adjust its conductive system to accommodate to environmental conditions (e.g. climatic differences during the sampling period). Phenotypic plasticity in wood (dendroplasticity) is a fundamental mechanism for the adaptability of tree species to changing environmental conditions (e.g. Martinez-Meier *et al.*, 2008, 2009). Its measurement in relation to the climatic conditions of the study sites will determine whether *E. grandis* is actually more plastic than *E. viminalis* and *E. globulus*, or if the environmental conditions where individuals grew had a greater range of variation, which resulted in a greater variability of wood characters. Beyond this, this study has quantified the effect of changes in F and S on theoretical water transport capacity (Table 2, Figs. 2 and 3), which could help explain the functional role of the anatomical changes observed both between and within individuals, for the three *Eucalyptus* species studied.

Conclusions

The present study analyzes fiber and vessel anatomy of three *Eucalyptus* species used in commercial plantations. These species differ in both wood density and general site requirements for their development. In agreement with the suggested hypothesis, *E. grandis*, the species with higher growth rates, presented the highest water transport capacity (greatest theoretical K_s) at the stem level, which has been associated with anatomical features such as wide vessels and high vessel density. On the other hand, *E. viminalis*, the species with lowest growth rates (at least in current plantations in Argentina) and highest resistance to environmental stress (drought and frost) showed lower K_s than that of *E. grandis*, but not the lowest of the three studied species. Moreover, it presented wide vessels, although at low density. These two species, *E. grandis* and *E. viminalis*, showed differences in fiber characteristics as well as the highest contrast in wood density. Despite presenting high growth rates, *E. globulus* has an anatomy characterized by relatively narrow vessels, which result in the lowest theoretical K_s , and also small-sized fibers. Even though mean vessel size is important in determining K_s , this study indicates that the total area

occupied by lumens (F) has a greater relative influence on K_s than size distribution within that area. In spite of this, F was more stable within and among species, whereas S showed a greater variation, suggesting that it has a more plastic nature and/or it is more susceptible to undergoing variations as a product of natural selection (genetic variation) within the studied genus.

The anatomical characteristics measured can only partly explain the differential growth or resistance to stress of the three studied species. Further studies need to be conducted to analyze other anatomical wood components such as vasicentric tracheids and parenchyma, as well as capacitance of different wood types. Additionally, to explain the functional-adaptive role of wood in *Eucalyptus*, further research needs to be done to simultaneously measure anatomical features of different plant organs, growth, and physiological variables in response to environmental stress.

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