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The total path length hydraulic resistance according to known anatomical patterns: what is the shape of the root-to-leaf tension gradient along the plant longitudinal axis?

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Running title: Path length hydraulic resistance in theory vs. practice

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Abstract

Xylem conduit diameter widens from leaf tip to stem base and how this widening affects the total hydraulic resistance (R_{TOT}) and the gradient of water potential (Ψxyl) has never been thoroughly investigated.

Data of conduit diameter of *Acer pseudoplatanus*, *Fagus sylvatica* and *Picea abies* were used to model the axial variation of R_{TOT} and Ψxyl.

The majority of R_{TOT} (from 79 to 98%) was predicted to be confined within the leaf/needle. This means that the xylem conduits of stem and roots, accounting for nearly the total length of the hydraulic path, theoretically provide a nearly negligible contribution to R_{TOT}. Consequently, a steep gradient of water potentials was predicted to develop within the leaf/needle base, whereas lower in the stem water potentials approximate those of rootlets.

Our results would suggest that the strong partitioning of R_{TOT} between leaves/needles coupled with basal conduit widening is of key importance for both hydraulic safety against drought-induced embolism formation and efficiency, as it minimizes the exposure of stem xylem to high tensions and makes the total plant's conductance substantially independent of body size.

Keywords: hydraulic resistance, leaf, xylem, stem, water potential, tension gradient, conduit widening.

Introduction

According to the cohesion-tension theory, water within the xylem flows against gravity from the soil to the canopy due to the lower water potential developed in the transpiring leaves (Dixon and Joly, 1895; Angeles *et al*., 2004). The evaporation of water in the mesophyll and the consequent vapor diffusion into the atmosphere (i.e. leaf transpiration) is the main mechanism driving the water uplift. The surface tension at the liquid/vapor interface does not overcome the adhesion forces in the nanopores of the mesophyll cell walls inside the substomatal cavities (estimated in the range of 5-10 nm, Tyree and Zimmermann, 2002), determining the formation of water menisci inside these cavities and the transmission of subatmospheric pressures along the whole hydraulic path until the rootlets. The water lost with transpiration is replenished by the bulk water flowing from roots to leaves in a metastable state, through a network of dead and hollow conduits (i.e. the xylem) (Nobel, 2012; Brown, 2013).

Plant water relations are widely studied based on the analogy to electric circuits, where water flow (F) is analogous to the electric current generated by a difference in potential energy of water (water potential, Ψ, MPa) between the two extremities of the circuit (soil-to-root and leaf-to-atmosphere interfaces), the media through which water flows are resistances connected in series (e.g., xylem conduits), and other plant structures may serve as capacitors (e.g., parenchyma cells) (Tyree and Zimmermann, 2002). According to the Darcy's law, the water flow is determined by the ratio between the difference in water potential between two points along the hydraulic path ($\Delta\Psi$) and the total hydraulic resistance (R) of the medium passed through (Reid *et al*., 2005):

$$F = \Delta\Psi / R \qquad \text{Eq. 1}$$

The $\Delta\Psi$ between leaves and soil (Ψ_{leaf} - Ψ_{soil}) is strongly influenced by the surrounding environmental conditions. When the atmosphere is dry and hot, the transpiring leaves lose more water from the mesophyll cell walls, with even lower water potential (i.e. higher tension) being propagated down along the xylem path. Instead, when soil gets drier, the xylem water potential decreases accordingly, determining a reduction in $\Delta\Psi$ between leaves and soil.

The total hydraulic resistance of the plant hydraulic architecture can be simplified as the sum of resistances connected in series along the hydraulic path (Fig. 1). For the nearly entire path length, water flows through a complex network of conductive elements, similar to capillary tubes (i.e., the dead, hollow and thick-walled xylem conduits). Instead, at the extremities of the hydraulic path, water moves for very short distances along different structures. In both the leaf mesophyll and rootlets, water does not flow through "tubes", but must cross cell membranes and walls of a series of living cells (Tyree and Zimmermann, 2002).

Between the two extremities where water must flow through the living cells of rootlets and leaves, the total hydraulic resistance of the whole xylem architecture connecting roots to leaves is essentially determined by conduit number and conduit anatomical traits, such as their size (lumen diameter and length) and their pit properties (density and pore size) (Hacke and Sperry, 2001; Choat *et al*., 2008). According to the Hagen-Poiseuille law, the hydraulic resistance (r) of a single xylem conduit lumen can be assimilated to that of a capillary tube (Tyree and Ewers, 1991):

$$r = \frac{128 \cdot \eta \cdot l}{\pi \cdot d^4} \qquad \text{Eq. 2}$$

where η is the dynamic viscosity of water (10^{-9} MPa·s at 20°C), l is the length and d the diameter of the conduit. It follows that changes in conduit size, especially d, along the hydraulic path can have a great effect on its total hydraulic resistance (Comstock and Sperry 2000).

At the stem level, it has been widely demonstrated that the diameter of xylem conduits continuously varies along the stem, being rather narrow at the tree top and becoming progressively wider towards the stem base (see Anfodillo *et al*., 2013 for a review). The scaling of conduit diameter with the distance from the stem apex commonly follows a trend well approximated to a power function ($Y=a\cdot X^b$). However, the rate of conduit enlargement has been also reported to be steeper close to the tree apex and gradually approaching a plateau towards the stem base, especially in tall trees (Petit et al. 2010; Williams *et al.* 2019), where the benefits gained with conduit widening at the tree base may trade off with factors such as carbon costs associated for the construction of the xylem vasculature (Mencuccini et al. 2007; Prendin et al. 2018). The scaling exponent (*b*) of the basipetal widening of stem xylem conduits has been commonly reported to converge to the value of 0.2 irrespective of species, tree size or environmental conditions (Anfodillo *et al.* 2006, 2013, Olson *et al.* 2014, 2018). Belowground, the axial variation in conduit diameter is more variable than aboveground. However, xylem conduits of roots were always found to be wider than those of stem in both broadleaved (McElrone *et al*., 2004; Petit *et al*., 2010; Jacobsen *et al*., 2018) and conifers (Petit *et al*., 2009; Lintunen and Kalliokoski, 2010; Prendin *et al*., 2018). The hydraulic consequence of such an axial configuration is that the overall increase in conduit diameter basally would theoretically compensate for the path length effect to the total hydraulic resistance. As a consequence, the increase in the hydraulic resistance cumulated from the distal unit of the hydraulic path downwards (R_{TOT}) would be much less than linear, so that most of R_{TOT} remains confined within a very short distance from the stem apex (West *et al*., 1999; Petit and Anfodillo, 2009; Petit *et al*., 2010). A few studies provided empirical hydraulic measurements supporting that the axial distribution of resistances along the longitudinal stem/branch axis were very consistent with modeled distribution of resistances due to the axial widening of xylem conduit diameters (Yang and Tyree, 1993; Petit *et al*., 2008). Although other ultrastructures of xylem conduits (e.g., type of wall thickening, end-wall anatomy, pit structures, pit size and number) effectively contribute to the total xylem resistance, yet they were often reported to contribute to the total conduit resistance in proportion to the lumen resistance (Martre *et al*., 2000; Sperry *et al*., 2005; Pittermann *et al*., 2006; Christman and Sperry, 2010). Consistently, pit number and size have been reported to be strongly correlated to conduit diameter (Becker *et al.* 2003; Sperry *et al.* 2006; Lazzarin *et al.* 2016; Losso *et al.* 2018; Jacobsen *et al.* 2018).

The elements of the stem xylem are directly connected to the elements composing the leaf xylem network, where conduits are on average narrower than those in the stem (Lintunen and Kalliokoski 2010; Petit and Anfodillo 2013), and decrease in diameter from the petiole to the narrow minor veins (Coomes *et al.* 2008; Sack *et al.* 2012; Petit and Anfodillo 2013; Lechthaler *et al.* 2019).

Nonetheless, the contribution of leaves to the total plant resistance remains still controversial. Recent studies have highlighted that the total leaf hydraulic resistance is substantially determined in equal proportion by the resistances in series of the leaf xylem and of the outside-xylem paths in the mesophyll (Cochard *et al.* 2004; Trifiló *et al.* 2016; Scoffoni *et al.* 2017). Moreover, it has been argued that the dissipation of very low water potential within the short distances inside the leaf would require very high hydraulic resistances (Buckley and Sack 2019). On the contrary, other studies based on empirical hydraulic measurements reported instead a lower contribution of leaves to the total hydraulic resistance compared to stem and branches (e.g., Tsuda & Tyree, 1997; Sobrado, 2007).

Despite the existence of the abovementioned axial variations in xylem anatomy, yet their effects on the axial gradient of xylem water potential (Ψxyl) along the soil-plant-atmosphere continuum (SPAC) have never been deeply investigated. Most commonly, the total xylem resistance has been considered as a sort of “unit resistance”, intrinsically assuming no axial variations in resistivity (i.e., resistance per unit length), thus implicitly assuming Ψxyl to vary linearly between the two extremities of the hydraulic path (e.g., Venturas *et al.*, 2017).

This work aims to evaluate the effect of the axial variation in conduit diameter along the whole leaf-to-stem hydraulic path in shaping the stem-to-leaf gradient of water potential (Ψx). We combined our own measurements on axial variation in conduit diameter along the main leaf/needle axial vein with published and unpublished data on the axial variation from the stem apex to base for two angiosperms (*Acer pseudoplatanus* L., Petit *et al.*, 2008, and *Fagus sylvatica* L., Petit *et al.* unpublished data) and one gymnosperm species (*Picea abies* (L.) Karst., Petit *et al.* unpublished data). The published data include anatomical information on the conduit diameter variation with tree height, obtained from several anatomical sections performed at different heights along the stem. We implemented these data into a simple hydraulic model to assess the effect of the distribution of resistances along a single root-to-leaf chain of conduits in shaping the gradient of water potential along the entire vascular path from the ultimate (i.e., distal) element of the leaf/needle venation network to the rootlets (Fig. 1).

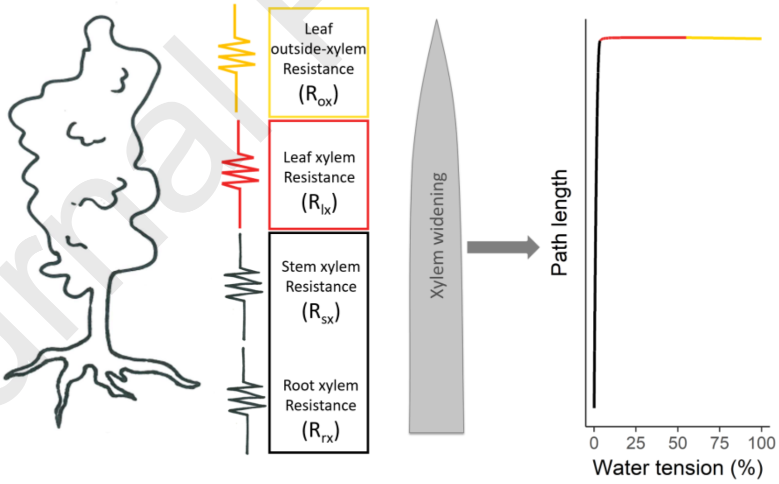

Figure 1. Graphic representation of the distribution of the different hydraulic resistances connected in series along the aboveground hydraulic path from the rootlets to the leaf mesophyll. Due to the axial variation of conduit diameter along the axes of leaves (yellow) and stems (black) and to the magnitude of the outside-xylem resistance in the leaves (red), almost the total water tension gradient is confined within the leaf (red, yellow lines) whereas the variation in the stem is almost negligible (black line). - Online color only

Material and methods

Anatomical analyses

We focused our analyses on two angiosperms (*Acer pseudoplatanus* L. and *Fagus sylvatica* L.) and one gymnosperm (*Picea abies* (L.) Karst).

At the end of the growing season 2017 (to ensure that the leaves were fully developed), we collected three to seven leaves/needles per species from trees growing at the botanical garden of the University of Padova (http://www.ortobotanicopd.it/en). For each leaf/needle, we cut 3-10 segments at different distances from the leaf/needle tip, and embedded them in paraffin (Anderson and Bancroft 2002). Stem cross-sections for *F. sylvatica* and *P. abies* individuals were cut at different distances from the tree apex (Petit *et al.* unpublished). Micro-sections of both stem and leaves were then cut with a rotary microtome Leica RM 2245 (Leica Biosystems, Nussloch, Germany) at 14-15 µm. For the leaves, we cut sections every 2 cm along the midrib and petiole whereas the needles of *P. abies* were cut along the longitudinal axis at every ~1mm (for more information on cut frequencies for the stem sections see Table S1). All sections were stained with a solution of safranin and Astra blue (1% and 0.5% in distilled water, respectively) and permanently fixed on glass slides with Eukitt (BiOptica, Milan, Italy). Slides of leaf sections were scanned with a D-Sight 2.0 scanner (A. Menarini diagnostic, Firenze, Italy) at 100x magnification. Images of stem cross-sections were captured at 100× magnification using a light microscope connected to a digital camera (Nikon Eclipse 80i, Nikon, Tokyo, Japan) and considering the outermost ring. Relative images of leaf and stem sections were analyzed with ROXAS v. 3.0.139 (von Arx and Dietz 2005; von Arx and Carrer 2014) for the automated measurement of the xylem vessel diameters. Slides of the needle sections were observed under a light microscope (Nikon Eclipse80i; Nikon, Tokyo, Japan) connected to a digital camera at 400x magnification, and the diameter of the six biggest tracheids was manually measured using a linear measurement tool. For each leaf/needle section, the hydraulic diamter (Dh) was calculated as:

$$Dh = \Sigma d^5 / \Sigma d^4 \qquad \text{Eq. 3}$$

where d is the diameter of a given vessel.

For *A. pseudoplatanus*, we obtained from published data the estimated Dh at different distances from the stem apex (Table S1 and references therein).

Statistical analyses

Data of Dh and distance from the leaf/needle tip (L) were Log10-transformed in order to meet the normality and homoscedasticity assumptions, and fitted with a linear regression to estimate the y-intercept and the widening coefficient (i.e., the slope b) for each species and organ (leaf/needle or stem) (Table 1).

Hydraulic model

For each species, the estimated basal widening pattern of conduit diameter along the leaf midrib/needle axis and further below along the stem were implemented into a hydraulic model for the assessment of the axial variation in the cumulative path resistance and in the xylem water potential along the longitudinal hydraulic path of a theoretical tree, simplified as a single pipe of concatenated conduits of 30 m of total length.

We calculated the theoretical hydraulic resistance (r) of each conductive element at each 1 mm of the hydraulic path according to Hagen-Poiseuille (Eq. 2). The cumulative path resistance (R_{TOT}) was calculated as the sum of all conduits starting from the apical (i.e., distal) element and moving down until the stem base.

R_{TOT} was calculated according to three different patterns of axial variation in conduit diameter: (i) according to our empirical observations of axial conduit widening along the stem

(R_{sx}); (ii) according to our empirical observations of axial conduit widening along the stem and leaf midrib/needle (R_{sx} + R_{lx}); (iii) according to our empirical observations of axial conduit widening along the stem and leaf midrib/needle, plus the resistance of the outside-xylem path (R_{ox}) added as the most distal element of the hydraulic path (R_{sx} + R_{lx} + R_{ox}), with a value of 50% of the total leaf/needle resistance (i.e., R_{ox}=R_{sx} + R_{lx}) (Cochard *et al.* 2004; Trifiló *et al.* 2016; Scoffoni *et al.* 2017).

At each mm along the hydraulic path, the water potential was estimated according to Eq. 1, and the estimation of water flow (*F*) under the assumption of the conservation of mass. The total path resistance was calculated as the sum of each single element resistance and an arbitrary $\Delta\Psi$ was applied between the most distal and the proximal element of the hydraulic path. The water potential at the base of a given xylem element (Ψ_{X1}, considered cylindrical) was calculated as:

$$\Psi_{X1} = F * r + \Psi_{X0} \qquad \text{Eq. 4}$$

where *r* is the conduit resistance and Ψ_{X0} is the water potential at the base of the next xylem element.

We then expressed Ψ_X as relative variation in tension between the most apical (distal) element of the hydraulic path (i.e., 100% of tension) and the stem base (0% of tension).

The model also simulated the theoretical effects of different soil ($\Psi soil$ from 0 to -2 MPa) and air dryness (Ψair from -40 to -80 MPa) (Brown and van Haveren 1972) on the variation in the water potential (Ψ_X) along the whole hydraulic path from the substomatal cavities until the soil/root interface, assuming full functionality of all xylem conduits (i.e., no losses of conductance due to embolism formation). The water potential calculated for the apical element of the hydraulic path, (i.e., at the outside xylem level) accounted also for the leaf osmotic adjustment to compensate for the gravitational pressure drop of 0.01 MPa/m.

Results

The hydraulic diameter of xylem conduits (*Dh*) in the angiosperm leaves increased along the midrib from the leaf tip to the petiole (Fig. 2, 3) following a power trajectory characterized by a scaling exponent of *b*=0.41 (CI 95%: 0.37; 0.44, Table 1) in *A. pseudoplatanus* and *b*=0.33 in *F. sylvatica* (CI 95%: 0.18; 0.47, Table 1). On the contrary, the tracheid *Dh* of the analyzed *P. abies*' needles remained around 3.5 µm without showing significant axial trends (Fig. 2, 3, Table 1).

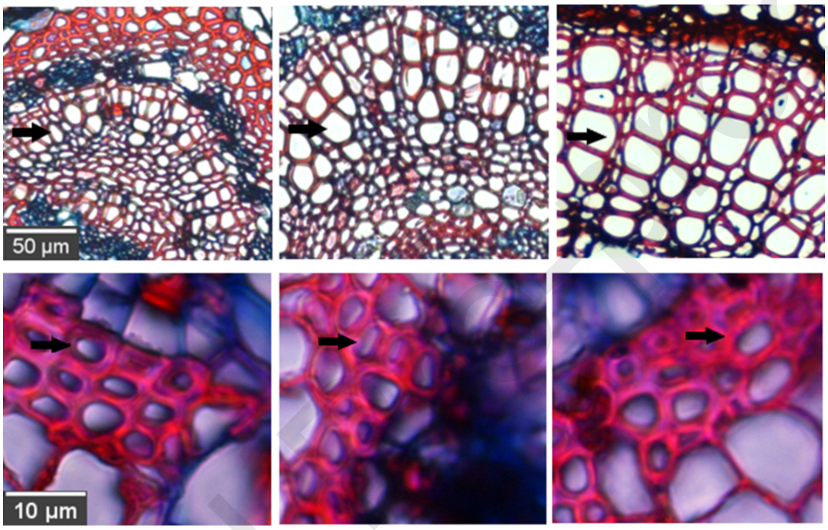

Figure 2. Anatomical sections taken at 20, 60, 180 mm from the leaf tip for *Acer pseudoplatanus* (upper images) and at the tip, middle and base of a *Picea abies* needle of 15 mm in length. The black arrows indicate the xylem conduits. - Online color only

The pattern of xylem *Dh* variation along the axis of the whole hydraulic path with leaves/needles and stem connected in series, revealed for *P. abies* a steep increase in *Dh* at the needle junction into the stem apex, whereas in both angiosperms the *Dh* variation from the leaf petiole to the stem apex was rather smooth (Fig. 3, Table 1).

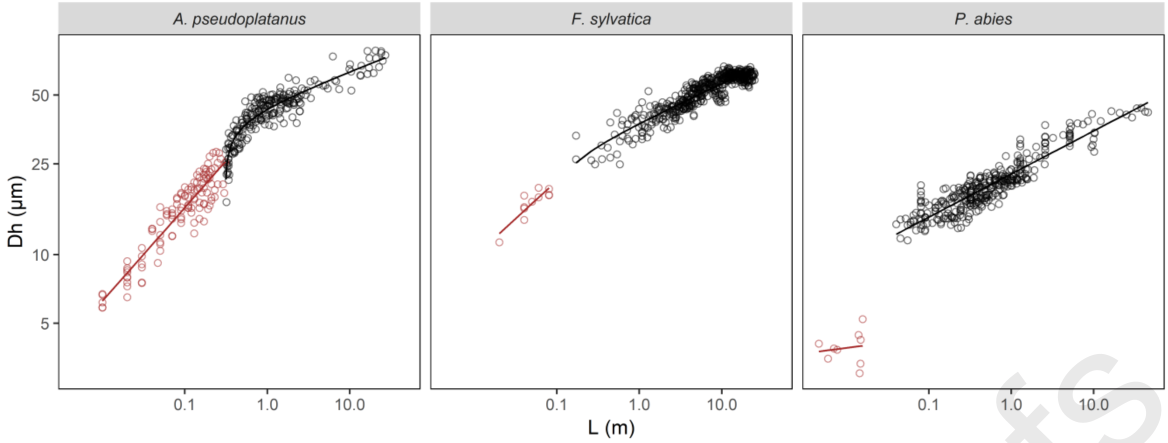

Figure 3. Variation of the hydraulic diameter (*Dh*) with the distance from the apex of leaf/needle (*L*) along the leaf/needle (red symbols) and stem (black symbols, data from Anfodillo *et al.*, 2006; Petit *et al.*, 2008) axes. Power scaling parameters of the relationship between *Dh* and axial length (*L*) assessed separately for leaves and stems are reported in table 1. - Online color only

Table 1: Parameters (*y*-intercept, a; slope, b; their 95% confidence intervals; R^2 and p-value) of the linear regressions of Log10*Dh* vs. Log10*L* describing the conduit widening along the leaf/needle or stem longitudinal axis, where *L* (in mm) is the distance from the apical element of the leaf/needle or stem, respectively. Data of *Dh* along the stem were taken from literature (*Acer pseudoplatanus*, Petit *et al.*, 2008) and from unpublished works (*Fagus sylvatica*, *Picea abies)*.

Species		*a*	*b*	*R^2*	*p-value*	*a 95% CI*	*b 95% CI*	*Citation*
Acer pseudoplatanus	*Leaf*	0.39	0.41	0.85	**9.32e-49**	0.32; 0.45	0.37;0.44	
	Stem	1.22	0.14	0.85	**4.68e-94**	1.20; 1.25	0.13; 0.15	Petit *et al.*, 2008
Fagus sylvatica	*Leaf*	0.66	0.33	0.75	**2.4e-6**	0.41; 0.90	0.18;0.47	
	Stem	1.34	0.13	0.79	**3.3e-164**	1.32; 1.35	0.13;0.14	Unpublished
Picea abies	*Leaf*	0.54	0.04	0.02	0.36			
	Stem	0.85	0.16	0.82	**7.1e-140**	0.83;0.87	0.16;0.17	Unpublished

The estimated variation in the cumulative resistance (R_{TOT}) with the increasing path length (L) showed that the hydraulic resistances are not homogeneously distributed along the hydraulic path (Fig. 4), but elements of highest resistance are located in the apical part of the hydraulic path.

Along the longitudinal axis of the stem, R_{TOT} increased from the apex downwards at rates progressively decreasing towards the stem base (Fig. 4a). When the contribution of leaf/needle conduits (*Rlx*) to the total path resistance was added to that of stem conduits (*Rsx*), the model predicted that 65% and 95% of the total hydraulic resistance was confined within the needle in the conifer and within the leaf midrib in angiosperms, respectively (Fig. 4b). When also the contribution of the outside xylem resistance (*Rox*) was considered for the build-up of the total path resistance (i.e. 50% of the total leaf/needle resistance), almost the entire R_{TOT} (98% for *A. pseudoplatanus*, 92% for *F. sylvatica* and 85% for *P. abies*) was confined at the leaf level (Fig. 4c).

Accordingly, the same trends were predicted for the variation in tension (expressed as the percentage of Ψxyl from the leaf tip to the stem base, with most of the gradient confined within the leaf/needle (Fig. 4d).

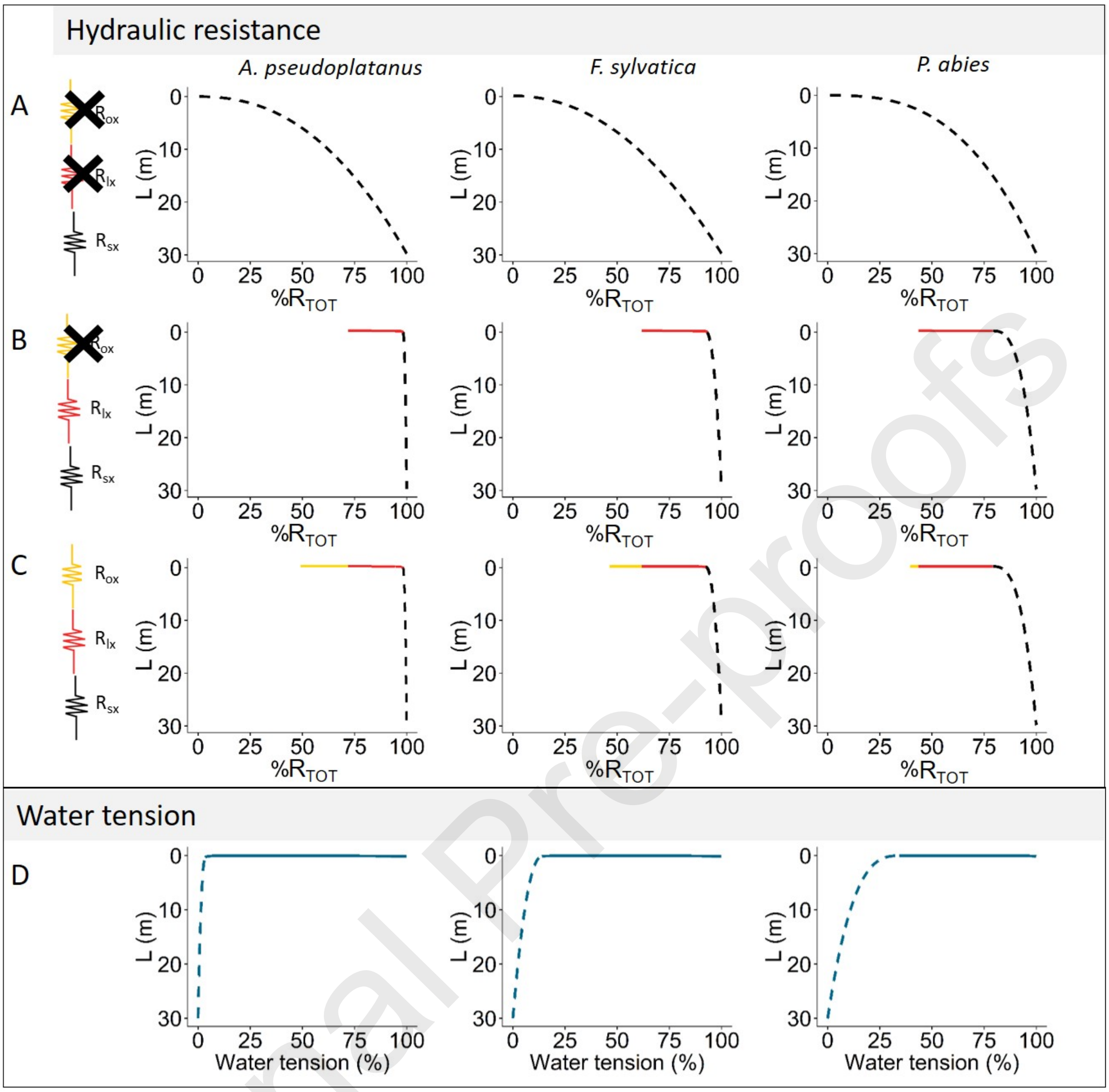

Figure 4. Relative axial variation of the cumulated resistance (R_{TOT}) with the path length (L) considering (a) only the stem vasculature (R_{sx}, black dotted line), (b) the integrated vasculatures of stem and leaves/needles ($R_{sx} + R_{lx}$, black + red line), and (c) the total pathway, i.e. accounting also for the outside-xylem resistance in the leaves/needles ($R_{sx} + R_{lx} + R_{ox}$, black + red + yellow line). (d) Relative variation in tension between the most apical (distal) element of the hydraulic path (i.e., the air/liquid interface on the mesophyll cell walls, 100% of tension) and the stem base (0% of tension). The dotted and solid lines represent the hydraulic path along the branch/stem/root and leaf/needle axes, respectively. - Online color only

By running a series of simulations for considering all the resistances along the hydraulic path (*Rsx*+*Rlx*+*Rox*), and applying virtually possible conditions of soil water potentials (*Ψsoil*=0 to -2 MPa) and air water potentials in the substomatal cavities (*Ψair*=-40 to -80 MPa), the model predicted that the gradient of xylem water potential rapidly increases along the leaf midrib from the tip to the petiole (Fig 5, dotted line, simulation made for *A. pseudoplatanus*). Notably, at the petiole base, the *Ψx* was predicted not to reach very low values, and was mostly dependent on *Ψsoil* and much less influenced by air dryness (*Ψair*).

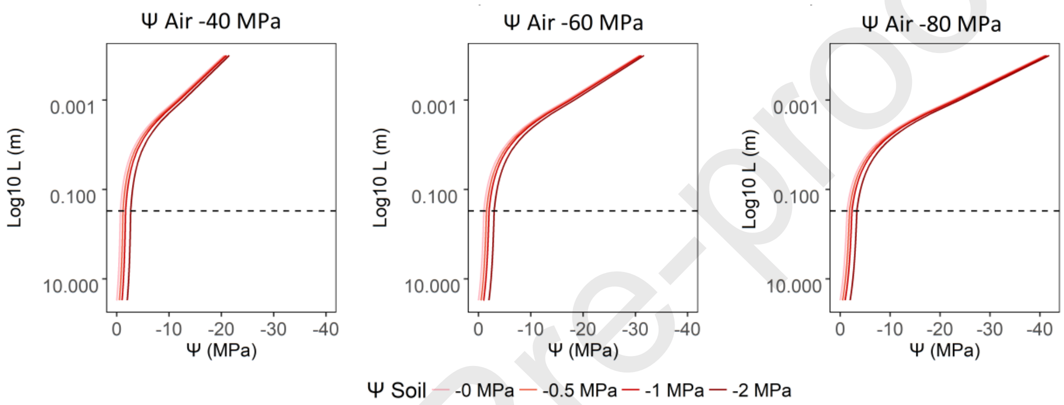

Figure 5. Theoretical effects of different soil (*Ψsoil* from 0 to -2 MPa) and air dryness (*Ψair* from -40 to -80 MPa) on the variation in the water potential (*Ψ*) along the whole hydraulic path from the most apical (distal) element of the hydraulic path (i.e., the air/liquid interface on the mesophyll cell walls) until the soil/root interface (*L* = 30 meters). The dotted line indicates the separation between the hydraulic paths along leaf and stem. - Online color only

Discussion

Our results provided novel insights into the key role played by the axial scaling of xylem conduits along the whole hydraulic path on the efficiency and safety of the hydraulic transport system.

Our anatomical analyses revealed that xylem conduits of leaves/ needles are the narrowest of the whole hydraulic path. In angiosperm leaves, the axial scaling of conduit diameter (*b*~0.4) was much higher than that of stem of our trees (*b*~0.14), consistent with previous studies

(Coomes *et al.* 2008; Petit and Anfodillo 2013; Lechthaler *et al.* 2019). On the contrary, needles showed no axial trends in conduit diameter.

At the stem level, the total hydraulic resistance (R_{TOT}) progressively cumulated from the stem apex downwards, but much less than linearly (Petit and Anfodillo 2009). Although our model simplified the xylem architecture, this result is fully consistent with some detailed physiological data. Indeed, measurements of axial hydraulic resistances carried out on stems of *Acer saccharum* Marsh. (6-9 m in length) of progressively reducing length by removing segments from the apex (Yang and Tyree 1993) are highly consistent with our anatomical based data (Fig. 6). Seemingly, but with the opposite approach (i.e., reducing stem/branch length starting from the base), the axial components of the total hydraulic resistance of stem and branches in *Acer pseudoplatanus* L. assessed with hydraulic measurements well matched the predictions based on the axial variation in vessel diameter (Petit *et al.* 2008).

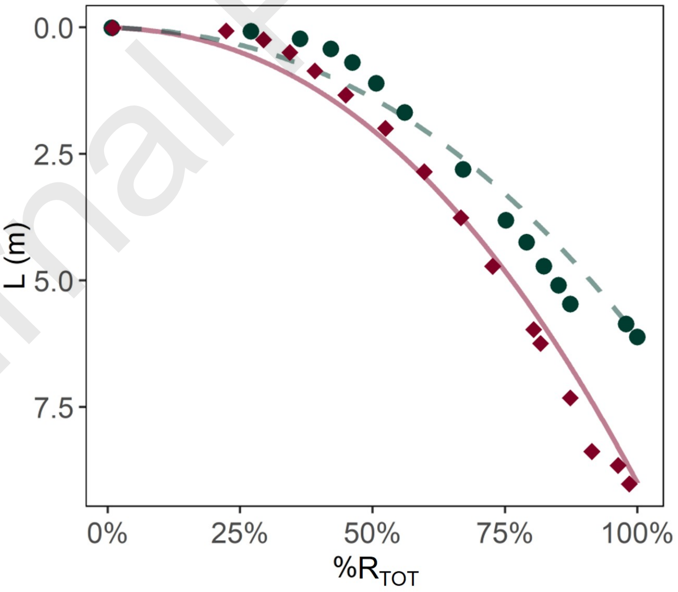

Figure 6. Relative axial variation of the cumulated resistance (*%R_{TOT}*) with path length (*L* (m)) in two branches of *Acer saccharum*. Dark green circles and purple diamonds are real data obtained from Yang & Tyree 1993 for two branches of 6 and 9 meter in length. We considered vessel widening with an exponent 0.14 as for our *Acer* trees (see table 1). Dark green dashed line and solid purple line are model expectation. - Online color only

These good matching of physiological data and intra-individual variation in conduit diameter implies that the contribution of other conduit ultrastructures to the total conduit resistance is a constant fraction of lumen resistance. Indeed, it had been often reported that different cell anatomical features, such as vessel length, wall-thickening type, end wall angle and morphology, size and density of pits, effectively contribute to the total conduit resistance in proportion to the lumen resistance (Comstock and Sperry 2000; Martre *et al.* 2000; John S. Sperry *et al.* 2005; Pittermann *et al.* 2006; Christman and Sperry 2010) with a proportion estimated as 56-64% in both angiosperms and gymnosperms (Sperry et al. 2005; Pittermann et al. 2006). However, detailed analyses on how this proportion varies along the hydraulic path have still to be carried out.

The overall axial trend in conduit diameter along the whole hydraulic path suggested that leaves/needles account for most of the total R_{TOT} (~80%), with a minor contribution of the stem xylem (Fig. 4a). The combined effects of leaf/needle length, its conduit widening, and the variation in conduit size at the junction between leaf/needle and stem seemed to determine such a partitioning of R_{TOT}. Given the steep rate of widening in leaves, it follows that leaf R_{TOT} would be nearly independent of leaf size, and so its contribution to R_{TOT}. On the contrary, leaf/needle length would become an important factor in partitioning R_{TOT} more in leaves/needle than the remaining xylem path (i.e., branch, stem and root axes) in the absence of conduit widening along the leaf/needle axis, as for our conifer species.

Notably, when we accounted also for the living cells of the outside-xylem hydraulic pathway to $R_{TOT,}$ the contribution of leaves increases (~90%). (Fig. 4a). In our simulations we implemented a conservative approximation of the outside-xylem resistance (R_{OX}), being equal to 50% of the total leaf/needle hydraulic resistance in both angiosperms and conifer. However, R_{OX} had been reported to vary between 40 to 88 % of the total leaf resistance across angiosperm species (Cochard *et al.* 2004; Trifiló *et al.* 2016; Scoffoni *et al.* 2017), but no data are available for conifer needles. Nevertheless, these results would suggest in general that the higher is the contribution of R_{OX} to the total leaf resistance then the lower is the contribution of the stem xylem to R_{TOT}. This theoretical scenario is consistent with the classical view that the major component of the whole plant hydraulic resistance is concentrated in the leaves (Tyree *et al.* 1993; Yang and Tyree 1994; Nardini and Salleo 2000; Sack and Holbrook 2006), although other empirical hydraulic measurements reported that leaf resistance is significantly lower than the resistance of both stem and roots (Tsuda and Tyree 1997; Sobrado 2007).

Our model predicted that the drop in water potential between leaves (Ψ_{LEAF}) and soil (Ψ_{SOIL}) is not linear, as commonly assumed (e.g.,Venturas *et al.*, 2017). Instead, according to the reported axial anatomical patterns, leaves/needles theoretically accounted for nearly the total hydraulic resistance (Nardini and Salleo 2000; Buckley and Sack 2019), thus resulting to be nearly hydraulically “decoupled” from the stem. Consequently, and most of the water potential gradient ($\Delta\Psi$) is predicted to be dissipated within the leaves, consistent with other theoretical analyses (Buckley and Sack 2019) and with empirical water potential measurements reporting a steep gradient of water potential developing during the day along the midrib of compound leaves (Petit and Anfodillo 2013). The remaining $\Delta\Psi$ between the stem apex and rootlets was predicted to be very small, so that the water potential gradient would be rather flat for nearly the entire path length until the last vascular elements of roots (Fig. 4d and Fig. 5). Consequently, such a precise distribution of resistances along the hydraulic path is theoretically of key importance in limiting the risks of embolism formation due to high transpiration rates (Martin-StPaul *et al.* 2017), as the highest tensions would remain confined at the leaf level where narrower conduits are less vulnerable to embolism formation. Furthermore, it can be argued that the hydraulic resistance of the root symplastic pathway (i.e., the resistance of crossing through the root living cells) is a key determinant of the xylem water potential along the stem (i.e., stem water potential decreasing with increasing root symplasm resistance). Hydraulic measurements on root segments suggested a minor contribution of roots to R_{TOT} (Martínez-Vilalta *et al.* 2002; Andrew J. McElrone *et al.* 2004), consistent with anatomical observations of larger conduits below- than aboveground (Andrew J. McElrone *et al.* 2004; Petit *et al.* 2009, 2010; Lintunen and Kalliokoski 2010; Prendin *et al.* 2018; Jacobsen *et al.* 2018). According to these results, our model would predict that in case of water sub-saturation of soil (Ψ_{SOIL} ~0 MPa), the water potential developing along the whole stem axis would not be very low, even with very dry air. On the contrary, other analyses reported a relevant contribution of roots to R_{TOT} (Pratt *et al.* 2010). Therefore, negative water potentials of similar magnitude would develop all along the hydraulic path of stem and roots. Since conduit diameter is commonly reported to be related to vulnerability to embolism formation (Cai and Tyree 2010; Olson *et al.* 2018; Liu *et al.* 2019), our model provided further theoretical support the hypothesis that height growth is limited in dry environments because taller statures would imply the production of larger conduits basally to compensate for the negative effect of path length on R_{TOT}, but their functionality would be hampered by their higher vulnerability to embolism (Olson *et al.* 2018).

Further investigations are needed to more precisely understand the water relations of whole roots, as they imply the bypassing of impermeable barriers (i.e., the Casparian bands in the endodermis cell walls) through symplastic pathways, where aquaporins actively play a key role in strongly modulating root resistance upon physiological needs (Steudle and Peterson 1998).

In conclusion, we provided evidence for typical anatomical axial patterns along the entire hydraulic path. That leaf/needle resistance is much higher than that of stem and roots is likely the result of natural selection to confine the lowest water potentials within the leaves/needles, thus minimizing the risk of xylem dysfunction in stem and roots, where conduits are more vulnerable. However, the water relations at the extremities of the hydraulic path (leaves and rootlets) remain controversial and require further investigations to clearly understand their relative contribution to the total plant hydraulic resistance.

Contributions

SL and NK collected and prepared samples, carried out measurements and analyses and prepared the first manuscript draft. GP designed the study and coordinated sample collection and contributed to data analyses. All authors contributed to discussion and critically contributed to the final manuscript version.

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Highlights:

- Xylem conduit diameter widens from leaf tip to stem base
- Conduit diameter has a major role in determining the hydraulic resistance
- Considering conduit widening, hydraulic resistance is mostly confined in the leaves
- Hydraulic resistance and water potential gradient in stem are almost negligible.