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Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and cavitation patterns of *Acer pseudoplatanus* leaves

Silvia Lechthaler^{1*}, Pierluigi Colangeli², Moira Gazzabin¹ and Tommaso Anfodillo¹

¹Department Territorio e Sistemi Agro-Forestali, University of Padova, Padova, Italy.

²Department of Ecology and Ecosystem Modelling, University of Potsdam, Potsdam, Germany.

*Corresponding author: silvia.hth@gmail.com; tel: 00393481820404

pierluigi.colangeli@gmail.com

moira.gazzabin@studenti.unipd.it

tommaso.anfodillo@unipd.it

Highlights:

This study shows that vessels anatomy varies along the midrib and that wider vessels embolize first.

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Abstract

The structure of leaf veins is typically described with a hierarchical scheme (e.g. midrib, 1st order, 2nd order), that is used to predict variation in conduit diameter from one order to another overlooking possible variation within the same order. We tested whether xylem conduit diameter changes within the same vein order, with consequences on resistance to embolism.

We measured the hydraulic diameter (Dh), and number of vessels (VNo) along the midrib and petioles of *Acer pseudoplatanus* leaves. We estimated the leaf area supplied (LA_{sup}) at different points of the midrib and how variation in anatomical traits affected embolism resistance.

Our results showed that Dh scales with distance from the midrib tip (L) with a power of 0.42, and that VNo scales with LA_{sup} with a power of 0.66. Total conductive area scales isometrically with the LA_{sup} . Embolism events along the midrib occurred first in the basipetal part and afterwards at the leaf tip where vessels are narrower. The distance from the midrib tip well predicts the variations in vessels diameter along the 1st order vein in sycamore maple leaves and this anatomical pattern seems to have an effect on hydraulic safety since wider vessels at the leaf base embolize first.

Keywords: leaf hydraulic architecture, leaf cavitation, leaf midrib, vessel number, vessel diameter, total conductive area.

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Introduction

The leaf is the terminal part of the water transport system in plants and is where a substantial amount of hydraulic resistance is located (Sack and Holbrook, 2006). The physiological mechanisms and morphological traits of leaves largely determine how water deficit affects plants. The control of stomatal conductance induced by leaf dehydration is the primary mechanism involved in limitation of embolism events (Scoffoni *et al.*, 2017a). However, in prolonged conditions of soil water shortage, the leaf veins are prone to embolism. Major veins are reported to be more vulnerable to embolism than minor veins (Brodribb *et al.*, 2016b,a; Scoffoni *et al.*, 2017b) and, for angiosperms, leaf vessel diameter is narrower in the minor veins compared to the major veins (Coomes *et al.*, 2008; Scoffoni *et al.*, 2017b; Carvalho *et al.*, 2017). Conduit diameter is an important trait to evaluate hydraulic conductivity and a correlation between conduit diameter and vulnerability to embolism has been shown in both stem (Cai and Tyree, 2010; Olson *et al.*, 2018; Liu *et al.*, 2019) and leaf (Brodribb *et al.*, 2016b,a; Scoffoni *et al.*, 2017b). Therefore, understanding how and why vessel diameter changes within the leaf is crucial for predicting the efficiency of water transport and how embolism events might spread throughout the hydraulic network.

To study the hydraulic architecture in leaves the vein network is usually divided following a hierarchical grouping of the leaf veins: the 1st order veins that run from the base of the lamina to the leaf apex, the 2nd order veins that branch from it, the 3rd and higher orders that decrease in diameter and form the reticulated network that transport the water close to the substomatal cavities (Fig. 1A) (Hickey, 1973; Ellis *et al.*, 2009; Sack *et al.*, 2012; Carvalho *et al.*, 2017; Gleason *et al.*, 2018). This hierarchical vein grouping has been used to test whether the leaf vascular system follows the structure proposed by Murray's law. This law predicts that variation in conduit diameter occurs only among different vein orders, but not within the same order, and that the sum of all inner radii cubed at one order (n), is equal to the sum of all inner radii cubed at a more distal order ($n+1$) (McCulloh *et al.*, 2003, 2009; Gleason *et al.*, 2018). Murray's law predicts that the total conductive area across all vein orders increases from the 1st order vein (midrib) to the 2nd (and higher), but remains invariant within the same vein order (McCulloh *et al.*, 2003, 2009; Gleason *et al.*, 2018). From our knowledge the number of studies that evaluate possible variations within the same vein order are scarce. A study on leaves of ash species showed variation in conduit diameter along leaf rachis, where vessel diameter widened from apical leaflet base to petiole, suggesting that vessel diameter scales with leaf length (Petit and Anfodillo, 2013). Variation of conduit diameter with path length was theoretically predicted by West, Brown and Enquist model (WBE model) for tree stems (West *et al.*, 1999) and this is supported by several studies conducted on different species

(Anfodillo *et al.*, 2006; Olson *et al.*, 2014, 2018; Petit *et al.*, 2014). WBE model predicts that conduit widening from tree top to base is involved in compensating the potential increase of hydraulic resistance imposed by increasing plant height (i.e. increase of path length) and that conduit diameter (Dh) should scale with tree height (L) with an exponent of about 0.20 approximately following a power law (West *et al.*, 1999; Anfodillo *et al.*, 2006):

$$Dh = aL^b \quad \text{Eqn 1}$$

Where a is conduit diameter measured at the most distal position and b , the exponent, is the degree of widening. WBE model assumes leaves as invariant terminal units that do not affect the scaling relationship of anatomical traits, such as conduit diameter and number, in the stem. Despite WBE model aroused some controversy on the assumption of invariant terminal units (Kozłowski and Konarzewski, 2004, 2005; Mäkelä and Valentine, 2006), the prediction of pathlength as the main driver for stem conduit diameter was confirmed by several studies on different species and environments (Anfodillo *et al.*, 2006, 2013; Lintunen *et al.*, 2010; Olson *et al.*, 2014, 2018). To evaluate if vessel diameter varies with path length also in the leaf, we considered the midrib as “stem” and distal part of the midrib close to the substomatal cavities at the leaf tip as terminal unit. The aim of this study is to test whether leaf vessel diameter and vessel number scale with path length, in this case represented by midrib length, as observed in the stem, or if vein order is the only predictor in leaf hydraulic architecture. Finding variations in vessel diameter and number along the midrib would suggest that the prediction of WBE model that assumes vessels diameter to scale with pathlength is valid also for the leaf. Finding instead no variation in vessel diameter along the midrib would support the prediction of Murray’s law that assumes that vessel diameter changes only between vein orders. Moreover, given that vessel diameter in veins is associated with hydraulic conductivity and susceptibility to embolism (Scoffoni *et al.*, 2017b), the spread of embolism along the midrib would follow the pattern of vessel diameter. We tested two possible patterns: in the case of a vessel diameter widening pattern along the midrib we would expect wider vessels at the base of the vein to embolize first and the narrow vessels at the midrib tip to embolize later. In the case of constant vessel diameter pattern in the midrib we would expect embolism to initiate randomly along the midrib.

Finally, we tested how total conductive area scales with leaf area along the midrib. Total conductive area is affected by the vessel lumen area (VA) and vessel number (VNo). From WBE model prediction we would expect that total conductive area (TCA) scales with leaf area (LA) with an exponent > 1 . The WBE model predicts that there is a constant vessel number per unit of leaf area ($VNo \propto LA^1$) and that vessel diameter widens with increase in path length with an exponent of 0.20, without approximations for tree height (see Anfodillo *et al.*, 2006; Olson *et al.*, 2018) and that leaf

area scales with path length with an exponent of 3 (West *et al.*, 1999). Using these exponents we calculate that vessel area ($VA \propto Dh^2$) scales with leaf area with an exponent of 0.13 ($VA \propto LA^{(0.20 \times 2/3)}$). Summing the exponents of $VNo \propto LA^1$ and $VA \propto LA^{0.13}$, total conductive area is expected to scale with leaf area with an exponent of 1.13.

Murray's law predicts that the total conductive area across all vein orders increases from the 1st order vein (midrib) to the 2nd (and higher), but remains invariant within the same vein order (McCulloh *et al.*, 2003, 2009; Gleason *et al.*, 2018). Following the predictions of Murray's law, we would expect no variation of total conductive area along the midrib.

Material and Methods

Plant material

We collected five apical shoots of young *Acer pseudoplatanus* L. plants growing in a wooded area of San Vito di Cadore (Northern of Italy). We chose *Acer pseudoplatanus* for the study since it has leaves that at full development can reach more than 15 cm in length and width (petiole excluded). The lamina is thin and the vein network is clearly visible so it is possible to easily individuate the pathway of minor veins. The length and the thickness of the lamina allowed obtaining several anatomical sections along the midrib and tracing the supplied leaf area for each section. Samples were collected at the end of the growing season (August 2016) to ensure that leaves were fully developed and we selected leaves with a similar light exposure. The leaves were preserved in 50% alcohol. Seven healthy leaves were selected for the measurements. For each leaf we measured the leaf area (LA) and prepared anatomical sections along the midrib (ca. 10-16 depending on leaf size) (Fig. 1B, C).

Anatomical sections

Anatomical cross sections were cut at different distances from the midrib tip. As observed for the stem, conduit widening is well fitted by a power law, meaning that the widening is more rapid at the apical part (West *et al.*, 1999; Petit *et al.*, 2008; Anfodillo *et al.*, 2013). To test whether also along the midrib vessel diameter and vessel number changed steeply at the tip of the leaf, we prepared sections starting from the distal tip of the vein at each centimetre for the first 3 cm and every 2 for the rest of the leaf lamina and petiole (Fig.1B). The samples were embedded in paraffin and sections were cut at 14 μ m with a rotary microtome Leica RM 2125 (Leica Biosystems, Nussloch, Germany). We used a solution of safranin (1%) and astra blue (0.5%) to stain the sections for highlighting the lignified cells and better distinguishing the xylem vessels, and then permanently mounted them on slides with Eukitt (BiOptica, Milan, Italy). Slides were scanned with a D-Sight

2.0 scanner (A. Menarini Diagnostics, Florence, Italy) at 100x magnification, and images were analysed with ROXAS software (von Arx, www.wsl.ch/roxas). ROXAS is a software widely used for analysing wood anatomical sections since it automatically measures a large number of “particles” (i.e. vessels or conduits) (Wegner *et al.*, 2013; von Arx *et al.*, 2016). The software counted the number of vessels (VNo), calculated the total conductive area of the midrib section (TCA , calculated as the sum of the vessel areas) and the hydraulic weighted diameter (Dh). The Dh accounts for the contribution of vessels to hydraulic conductance (Dh , Kolb and Sperry, 1999) and was calculated as the sum of vessel diameters to the 5th power (d^5) divided by the sum of vessel diameters to the 4th power (d^4):

$$Dh = \Sigma d^5 / \Sigma d^4 \quad \text{Eqn 2}$$

where d is the diameter measured from the cross sectional area of each vessel.

Leaf area (LA) was obtained by scanning the leaf lamina (1000 dpi) and analysing the image with ImageJ (Rasband, 2018, <https://imagej.nih.gov/ij/>). The high quality scans allowed for the estimation of the portion of leaf area supplied (LA_{sup}) by the vessels at different distances from the leaf tip. On the leaf scan, we traced the points where the anatomical sections were obtained along the midrib. Starting from the leaf tip we traced the sector of leaf area supplied by the vessels at 1 cm from the midrib tip. We accounted for the contribution of the 2nd and 3rd order veins in supplying water to each leaf sector (Fig. 1B, C). We summed the area sectors from leaf tip to petiole to obtain the supplied leaf area (LA_{sup}) for each anatomical section. For this estimation, we assumed that the water flow was unidirectional from base to tip of the lamina and that the distal part of the midrib serves only the distal part of the leaf lamina. We estimated the number of vessels per unit of leaf area (V/cm^2) along the midrib as the number of vessels in the anatomical cross section divided by the supplied leaf area.

Vulnerability to embolism

Three small healthy saplings of *Acer pseudoplatanus* were cut at the base of the stem at root collar level at predawn and, immediately after cutting, placed in black plastic bags with damp paper towels to prevent further water loss and transported to the laboratory. To avoid potential artefacts associated with open vessels, we collected the entire saplings.

For each sapling we selected one healthy leaf (three leaves in total) and used the optical method proposed by Brodribb *et al.* (2016b,a) to capture embolism events in the midrib. Images were taken with a reflex camera every minute for 24 hours. Due to the thickness of the midrib we performed the measurement using reflected light (see <http://www.opensourceov.org>).

Image sequences were analysed with ImageJ to identify embolism events, seen as changes in the reflection of the midrib. We used the toolbox “OSOV” (<https://github.com/OpenSourceOV>) to perform image subtraction of subsequent images to reveal rapid changes in light reflectance. Embolism events were thresholded, allowing automated counting of each event using the “analyse-stack” function. Noise was removed using the outlier removal. A map of embolism events, colour coded for time, was automatically generated by colouring the embolism area in each sequence, with the first event of cavitation at time 0.

Statistical analysis

Data were log₁₀-transformed in order to meet normality and homoscedasticity assumptions. We used the lmodel2 package in R (version 3.3.1, R Core Team, 2016) to perform type-II linear regressions with reduced major axis (RMA), which is appropriate for studying allometric relationships between co-dependent variables (Smith, 2009). We tested if Dh and vessel number varied with distance from the leaf tip (L). We tested how number of vessels scaled with the supplied leaf area (LA_{supp}) and whether there was a variation in the number of vessels per unit of leaf area from tip to base of the midrib. We tested how the total conductive area scaled with the supplied leaf area (LA_{supp}) and whether the exponent obtained was not different from 1.16 as predicted by WBE model.

Results

Xylem anatomical traits varied markedly along the midrib. Leaf area spanned from 82 to 217 cm² and midrib length from 9 to 15 cm. At the distal part of the midrib length, the xylem was composed of narrow vessels with a high number of vessel per unit of leaf area (V/cm^2). Moving from leaf tip to petiole base the general observed pattern was that vessels widen in diameter and decrease in number per unit of leaf area (Fig. 2, 3). No major changes in anatomical traits were observed at the junction between leaf lamina and petiole.

Dh widened rapidly from midrib tip to petiole base, where it was approximately four times wider than at the tip. At the leaf tip Dh was 5.5-6.5 μm and in the petiole it was 20-25 μm. The widening pattern was well fitted by a power law when plotted against distance from the leaf tip (L) and with an exponent of 0.42 ($R^2=0.88$, CI exponent=0.39;0.45, Table 1) (Fig. 2).

The number of vessels increased from 15-40 at 1 cm from the midrib tip, to hundreds (min 190 - max 383) at the midrib base and along the petiole. VNo scaled with LA_{supp} with an exponent of 0.66 (Fig. 3A) significantly <1 (slope C.I.=0.62; 0.70, $R^2=0.92$). Vessel number per unit of leaf area (V/cm^2 , number of vessels divided by the supplied leaf area) varied along the midrib. The highest vessel number per unit of leaf area was observed in the first few cms ($V/cm^2 > 50/cm^2$) of the leaf tip

and steeply decreased towards the leaf base ($V/cm^2 < 12/cm^2$) reaching a plateau along the petiole (Fig. 3B).

Total conductive area (calculated as the sum of vessel areas) scaled isometrically with the supplied leaf area with an exponent of 0.98, not different from 1 (exponent C.I. = 0.94; 1.03) ($R^2=0.97$, Table 1) but statistically different from 1.13, the one predicted by WBE model (Fig. 4).

The first cavitation event always occurred in the basal part of the midrib at about 7-10 hours after the sapling was excised (time=0). Cavitation at the leaf tip occurred around 150-200 minutes later, and continued for about 6-7 hours (500-540 minutes) (Fig. 5).

Discussion

In this study, we tested how vessel anatomical traits in the leaf varied with midrib length and how embolism spread along the midrib. We showed that midrib length allows predicting anatomical traits such as vessel diameter, vessel number and total conductive area. We also found new evidence supporting the idea that wider vessels are more vulnerable to embolism than narrow ones, with our results showing that the embolism events within the midrib occur first at the base where the conduits are wider.

The prediction of Murray's law that variation in vessel diameter should occur only among vein orders but not within the same order was not confirmed by our results. Vessel diameter significantly varied along the midrib of sycamore maple leaves. It increased along the midrib and petiole with a degree of widening more than double of that reported for the stem xylem (0.42 vs. 0.2) (Anfodillo *et al.*, 2006; Petit *et al.*, 2008; Olson *et al.*, 2014), but similar (non-statistically different) to the one reported in the literature for leaves of ash (Petit and Anfodillo, 2013). Our results show that also in the leaf, vessel diameter scales with distance from the apical part within the same vein order and that the widening exponent in the leaf is higher than the one reported for the stem.

Basipetal vessel widening is consistent with the prediction of equal flow to all the possible paths. Considering a tree, this means that leaves located at different heights within the crown will be supplied uniformly (West *et al.*, 1999). Similarly, in the leaf, stomata are located at different distances from the midrib base and the tip-to-base widening of the vessel along the midrib is probably involved in buffering the increase in hydraulic resistance due to the pathlength throughout the leaf lamina.

Vessel diameter in the distal part of the midrib (1 cm from the tip) is similar to the diameter reported for the minor vein vessel of *Populus* sp., where vessel diameter in the 7th order veins was ca. $5 \mu m \pm 1$ SE (Carvalho *et al.*, 2017). This value is in line with the intercept value calculated on

$Dh \propto L$ regression, 5.75 μm with confidence intervals of 5.37–6.16 μm . Natural selection might have favoured narrow vessels in the terminal parts of the transport system, because they maximize the diffusion surface per volume of water. It is then possible that the vessel diameter in the proximity to the terminal parts of the water transport, where the water diffuses into the mesophyll, might be similar among different species.

Recent studies have shown that wider vessels are more vulnerable to embolism than narrow ones (Scoffoni *et al.*, 2017b; Olson *et al.*, 2018; Liu *et al.*, 2019). Olson *et al.* (2018) showed that taller individuals with wider vessels in the stem are more vulnerable to embolism than the shorter conspecifics that have smaller vessels (Olson *et al.*, 2018) and Gleason *et al.* (2019) found taller species to be more vulnerable to embolism than smaller ones. In leaves of different species, embolism occurred first in the wider vessels of the petiole and midrib followed by the vessels in the 2nd order veins and lastly in the minor vein vessels (Scoffoni *et al.*, 2017b). Our results show that along the midrib, embolism initiated at the base where vessel diameter is wider, supporting the idea that wider vessels are more vulnerable than narrow ones. Brodribb *et al.* (2016a) also showed that 1st order veins (i.e. the midrib) are the first to embolize and that the time interval between the first and last embolism event within a vein order is longer in longer veins. Based on our results, we would suggest that longer veins have a greater difference in vessel diameter from the tip to the base. Long veins would embolize at different times, first at the base, where vessels are wider and then at the tip where vessels are narrower. In short veins, where the difference in vessel diameter from tip to base is less, the time interval between the first and last embolism events would be shorter. Vessel number (VNo) and vessel number per unit of leaf area (V/cm^2) varied along the midrib from tip to base. At the midrib tip vessels are narrow with a high number per leaf area and they widen and decrease in number per leaf area towards the midrib base. Variation in vessel diameter and number may affect hydraulic conductivity along the midrib. The Hagen–Poiseuille law predicts that hydraulic conductivity depends mainly on diameter and length of the conduits, and a small increase in diameter corresponds to a great increase in conductance (Tyree and Zimmermann, 2002). If a narrow conduit is favoured by selection at the terminal part of the transport system to maximize the water diffusion surface (West *et al.*, 1997, 1999; Sack *et al.*, 2012), the narrow diameter decreases its conductivity. An increase in vessel number per unit of leaf area at the tip of the midrib may favour conductivity to the substomatal cavities.

The combination of a high degree of widening and decrease in vessel number per unit of leaf area from the leaf tip to petiole base resulted in an isometric relationship between the total conductive area and supplied leaf area. The exponent was statistically different from the one predicted by WBE model ($TCA \propto LA^{1.13}$). Isometry between total conductive area and leaf area is a trait found in stem,

branch and in leaf (Togashi *et al.*, 2015; Fan *et al.*, 2017; Petit *et al.*, 2018). The relationship between these two traits is used to understand the relationship between hydraulic capacity and photosynthesis (Petit *et al.*, 2018).

One of the most important predictions of the WBE model is that leaf specific conductance, meant as water flow per unit of leaf area, is invariant and independent of leaf position in the crown or, in other words, independent of conductive path length or tree size. This prediction can be tested by comparing scaling exponents of the relationship between theoretical flow rate (Q_{th}) with leaf area resulting from our study and the exponents predicted by WBE model. Theoretical flow rate can be estimated as the vessel diameter at the 4th power multiplied by number of vessels ($Q_{th} \propto Dh^4 VNo$) (Fan *et al.*, 2017). In the analysed leaves vessel diameter scales with leaf area as $Dh \propto LA_{sup}^{0.19}$ and vessel number scales with leaf area supplied as $VNo \propto LA_{sup}^{0.66}$. Theoretical flow rate would then scale with leaf area supplied as $Q_{th} \propto (LA_{sup}^{0.19})^4 LA_{sup}^{0.66}$ resulting in $Q_{th} \propto LA_{sup}^{1.42}$. Using the exponents predicted by WBE model and considering that in the measured leaves $LA \propto L^{2.13}$ due to 2-dimensional shape, then the Dh should scale with LA_{sup} with an exponent of about 0.1 and vessel number should scale isometrically with leaf area ($VNo \propto LA^1$). All these relationships leads that theoretical flow rate scales with leaf area as $Q_{th} \propto (LA_{sup}^{0.1})^4 LA_{sup}^1$ resulting in $Q \propto LA_{sup}^{1.4}$. Despite the exponents found for $Dh \propto L$ and $VNo \propto LA_{sup}$ being statistically different from the one predicted by the WBE model, the exponent for $Q \propto LA_{sup}$ is nearly identical to the predicted one, suggesting that WEB model prediction of independent leaf specific conductance can be considered valid also for the leaf hydraulic architecture.

In this study, we consider only one vein order and thus we cannot exclude that variation among vein orders follows the predictions of Murray's law, but we suggest that the position along the vein at which anatomical traits are measured has to be taken in to account. Our results show that vessel diameter and vessel number scales with midrib length and that vessel widening exponent found in the leaf are double than the ones predicted by the WBE model for the stem. Despite the difference in the predicted exponents, the efficiency of the vascular system as predicted by WBE model of independent leaf specific conductance with path length it might be valid also for the leaf hydraulic architecture but achieved with a different combination of scaling exponents. Further studies are needed to evaluate if such a pattern is consistent in different species and if also in leaf is possible to individuate universal hydraulic architectures. Finally, we can conclude that anatomical traits of the midrib vary with the distance from the terminal part of the water transport system and that wider vessels along the midrib are more vulnerable to embolism than narrower ones. We suggest that leaf length and/or supplied leaf area are key traits to be considered in leaf architectural models.

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Tables:**Table 1**

Parameters (y-intercept, a ; slope, b ; their 95% confidence intervals; R^2 and p -value) of the RMA linear regressions, where Dh is hydraulic diameter of the conduits, VNo is number of vessels, TCA is total conductive area, L is distance from leaf tip and LA_{sup} is supplied leaf area. Data are log–log transformed.

	a	b	R^2	p -value	a 95% CI	b 95% CI
$Dh \sim L$	0.76	0.42	0.88	<0.0001	0.73; 0.79	0.39;0.45
$Dh \sim LA_{sup}$	0.89	0.19	0.87	<0.0001	0.86; 0.92	0.17;0.20
$VNo \sim LA_{sup}$	1.44	0.66	0.92	<0.0001	1.38; 1.50	0.61;0.70
$TCA \sim LA_{sup}$	2.97	0.98	0.97	<0.0001	2.92; 3.02	0.94;1.03

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Figures:

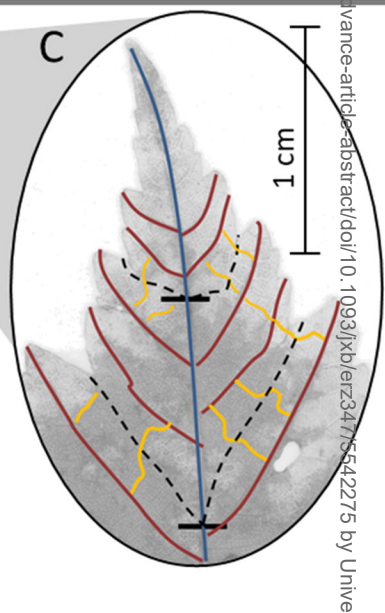
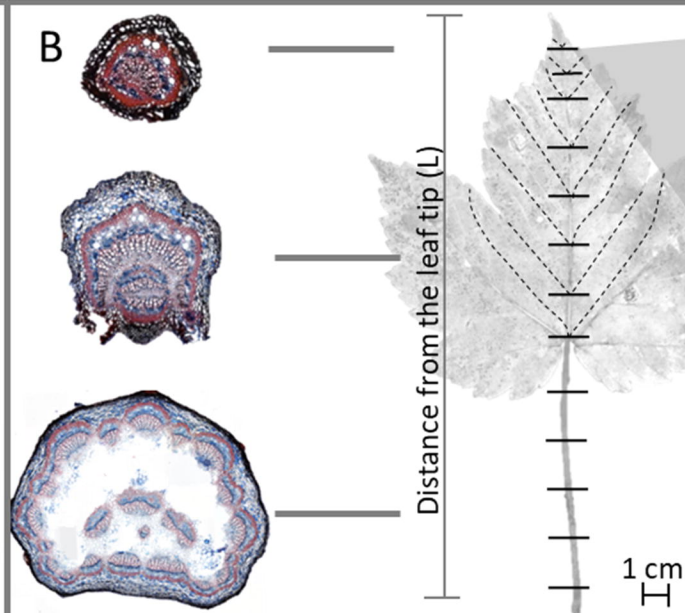
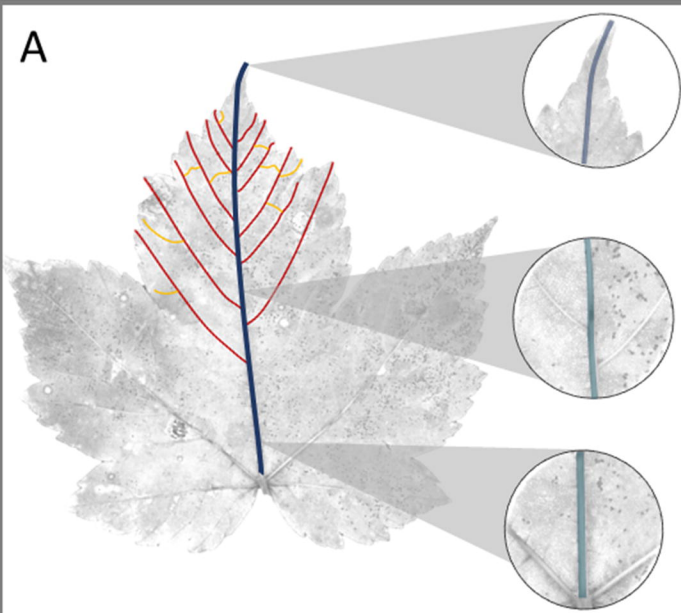
Fig. 1: (A) Schematic representation of the hierarchical subdivision of veins in orders: midrib in blue, examples of 2nd order veins in red and 3rd order veins in yellow. A zoom in of the leaf midrib at different distances from the leaf tip highlights differences in vein diameter. (B) Schematic representation of the different measurements taken along the midrib. Solid lines represent the points at which the anatomical sections were performed. Dashed lines represent the estimation of leaf area supplied for each section. The zoom-in (C) shows the estimation for the supplied leaf area delimited by the black dashed lines performed considering the 2nd (red) and 3rd (yellow) order veins and measured with ImageJ.

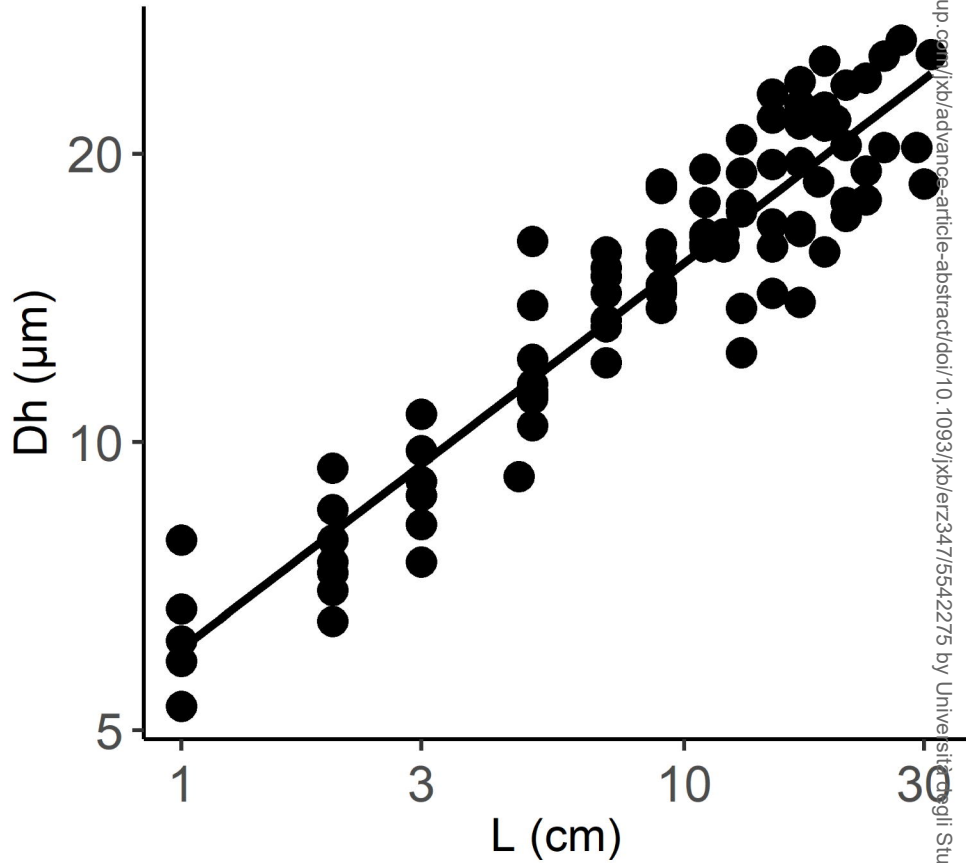
Fig. 2: Variation in hydraulic weighted diameter (Dh) of vessels with distance from midrib tip (L). Data were log–log transformed. The equation of the functions, the R^2 , p-values and 95% CI are reported in table 1.

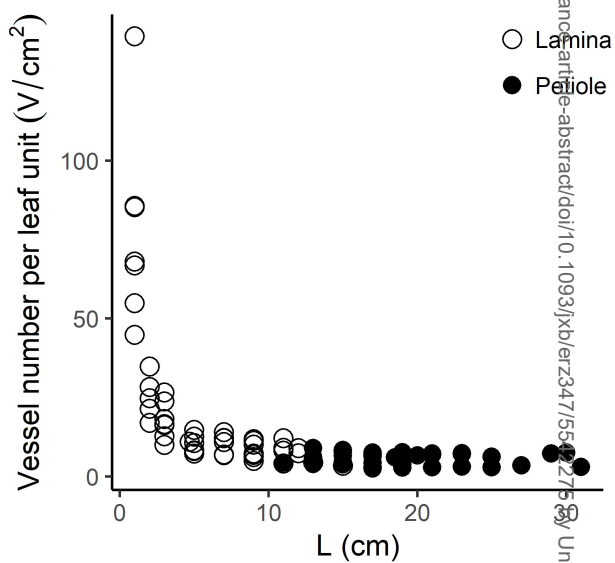
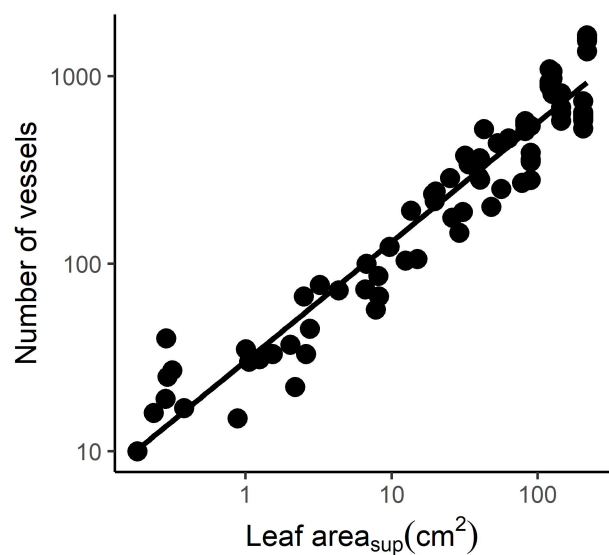
Fig. 3: (A) Variation in number of vessels (VNo) with the supplied leaf area (LA_{sup}) for log–log transformed data. The equation of the function is $y=27.54x^{0.66}$. The regression has an R^2 of 0.92, (CI exponent =0.61; 0.70) (Table 1). (B) Variation for untransformed data of vessel number per unit of leaf area (V/cm^2) along the midrib to highlight the steep decrease at the tip (1-5 cm) and the plateau reached at the midrib base and along the petiole. Empty circles correspond to measurements on the midrib, filled circles measurements on the petiole.

Fig. 4: Variation in total conductive area (TCA) with the supplied leaf area (LA_{sup}) for log–log transformed data (TCA = cumulative area of all vessels for each section). The equation of the function is $y=933x^{0.98}$. The regression has an R^2 of 0.97 (Table 1).

Fig. 5: The graph represents the time at which embolism events occurred along the midrib and report the regression line fitting the vessel diameter variation from the tip to the base of the midrib. The coloured horizontal segments represent the embolism events occurring at different times (left y-axis: time in minutes) along the midrib (L , leaf tip = 0) and the black line represents the power function describing vessel diameter widening along the midrib (right y-axis; for *Acer pseudoplatanus* midrib: $Dh=5.57*L^{0.42}$, table 1). The first cavitation event was set at time = 0. Below the graph a colour map reports all observed embolisms recorded along the midrib during desiccation.







Total conductive area (μm^2)

$1\text{e}+05$

$1\text{e}+04$

$1\text{e}+03$

1

10

100

Leaf Area_{sup}(cm^2)

