

## XYLEM ANATOMY OF *BETULA PENDULA* ROTH SAPLINGS: RELATIONSHIP TO PHYSICAL VASCULAR MODELS

### *ANATOMIA DEL XILEMA DE JUVENILES DE BETULA PENDULA ROTH: RELACION CON MODELOS VASCULARES FISICOS*

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#### ABSTRACT

In conifers and diffuse-porous angiosperms, tracheary elements have an important role in structural support and water transport. In these plants it is expected that the conflict between the mechanic and hydraulic function will result in less efficient water transport at the whole plant level than the expected for plants whose conduits do not fulfil a mayor structural role (vines and ring-porous trees). Here we analyse the vascular architecture of *Betula pendula* saplings in order to examine predictions for diffuse-porous trees. Murray's law (conservation of  $\Sigma r^3$ ) was not supported in basal tissues where the mechanical demands are be greater. Although distal portions did not depart significantly from Murray's law, lower exponents better described the relationship between consecutive levels. We found a moderate acropetal increase in conduit number, in disagreement with the model of West, Brown and Enquist, but loosely approximating Murray's law optimum. Conductive area decreased acropetally, giving a roughly conical area profile that is mechanically stable but has low transport efficiency, as predicted for diffuse-porous species. However, this trend in conductive area was uneven, the strongest reduction occurring between terminal branches and petioles. The vascular architecture of woody plants seems to respond to different constrains depending on the branching level and the mechanical functions associated with it.

KEYWORDS: Murray's law, plant vascular models, water transport models, vascular anatomy.

#### RESUMEN

En las coníferas y las angiospermas difuso-porosas, los elementos traqueales tienen un importante rol en el soporte estructural y el transporte de agua. En estas plantas se espera que el conflicto entre las funciones mecánicas e hidráulicas resulte en un transporte de agua menos eficiente a nivel de planta entera que lo esperado para plantas cuyos conductos no cumplen un rol estructural importante (enredaderas y árboles con madera difuso-porosa). En este trabajo analizamos la arquitectura vascular de juveniles de *Betula pendula* con el fin de examinar predicciones para árboles difuso-porosos. Los tejidos basales, donde las demandas mecánicas son mayores, no se ajustaron a la ley de Murray (conservación de  $\Sigma r^3$ ). Aunque las porciones distales se ajustaron significativamente a la ley de Murray, exponentes menores describieron mejor la relación entre niveles consecutivos. Encontramos un incremento acropétalo moderado en el número de vasos, contrario al modelo de West, Brown y Enquist, pero aproximado al óptimo de la ley de Murray. El área de conducción disminuyó acropétalmente, resultando en un perfil de área cónico que es mecánicamente estable, pero de baja eficiencia de transporte, como se predice para especies difuso-porosas. Sin embargo, esta tendencia en el área de conducción fue desigual, donde la mayor reducción en área ocurre entre las ramas terminales y los pecíolos. La arquitectura vascular de las plantas leñosas parece responder de manera distinta a diferentes restricciones, dependiendo del nivel de ramificación y de las funciones mecánicas asociadas a éste.

PALABRAS CLAVES: Ley de Murray, modelos vasculares de plantas, modelos de transporte de agua, anatomía vascular.

## INTRODUCTION

Xylem anatomy is relevant to both water transport and support of the aerial portion of the plant (Tyree & Zimmermann 2002, Tyree 2003). Recent models of vascular systems which emphasize optimization of transport capacity (West *et al.* 1999, McCulloh *et al.* 2003) reflect the belief that natural selection has maximized hydraulic conductance of plant vascular systems for a given biomass investment. This high conductance could reduce the effect of the drop in water pressure that can limit carbon fixation by inducing stomatal closure (Meinzer & Grantz 1990, Sperry & Pockman 1993, Meinzer *et al.* 1995, Saliendra *et al.* 1995, Hubbard *et al.* 2001). Whereas the earlier pipe model (Shinozaki *et al.* 1964a, 1964b, Chiba 1998) predicts increasing resistance with increasing path length, more sophisticated recent models propose different mechanisms that would avoid this problem (West *et al.* 1999). On the other hand, it is quite possible that selective pressures have resulted in a more conservative design, minimizing risks of cavitation rather than optimizing performance. Some authors point out that the actual hydraulic architecture of trees would maximize the water transport efficiency within mechanical and developmental constraints (McCulloh & Sperry 2005).

West *et al.* (1999) suggested a fractal design of the vascular system in their "WBE" model. This approach assumes an invariant conduit number along the vascular pathway, and a distal reduction in the total conducting area and individual conduit lumen size, implying water capacitance in the lower portions. This model assumes that biomechanical constraints are uniform along the pathway, and predicts that water transport resistance is independent of the tree height. This model has been applied in several fields, including allometric scaling across species (Enquist *et al.* 1999, Enquist & Niklas 2001, Enquist 2002, Niklas & Enquist 2002, 2003) and energetics and population dynamics (Enquist *et al.* 1998, Niklas *et al.* 2003). This model was developed to represent the vasculature up to (but excluding) the petiole level. A more recent version of the model was extended to included petiole level (Price *et al.* 2007, Price & Enquist 2007).

McCulloh *et al.* (2003) extended Murray's law, which was originally developed for animal vascular systems (Murray 1926), to plant xylem. Murray's

law predicts that hydraulic conductance per investment in vascular tissue is maximized when the sum of conduit radii raised to the third power ( $\sum r^3$ ) between successive branching levels is conserved. They assumed: (1) xylem Q (water flux) is constant along the path, except in roots and leaves; (2) water conductance obeys the Hagen-Poiseuille law, i.e. conductance is proportional to the conduit radius to the fourth power; (3) conduit wall volume is proportional to total conduit volume, and (4) conduits are not involved in mechanical support. This model involves acropetal increases in conduit number and total conduit cross-sectional area, exploiting the efficiency of few, large vessels in the base.

Although hydraulic architecture of several species has been shown to closely obey Murray's law, the applications of Murray's law to plants has so far been limited to distal (petiolules and petioles) and sub-distal (1-year branches) tissues. McCulloh *et al.* (2003) tested Murray's law in petiolules, petioles and young wood of four woody angiosperm species. In a more recent study McCulloh *et al.* (2004) tested Murray's law in a conifer species, a diffuse-porous and a ring-porous species tested in their previous work, but considered tissues up to four years old saplings. Murray's law was rejected in conifer wood but not in angiosperm wood: an expected finding, because conifer wood consists mainly of tracheids that function both as mechanical support elements and water conduits, therefore violating one of the assumptions of Murray's law (above). It seems that WBE model holds up to the last branches but not within the leaves and the opposites may be true for Murray's law.

Here we analyze the xylem anatomy of *Betula pendula* Roth. (Betulaceae) saplings, and compare the results with assumptions and predictions derived from Murray's law and the WBE model. We ask whether either of these models accurately predicts variation in conduction area and conduit numbers from petioles to the stem base, as well as explicitly testing for agreement with the predictions of Murray's law (Murray, 1926) about the conservation of  $\sum r^3$ . *B. pendula* has diffuse-porous wood, but it has large and relative scarce vessels (Fig. 1). It might be expected that these vessels contribute relatively more to mechanical support than those of ring-porous trees.

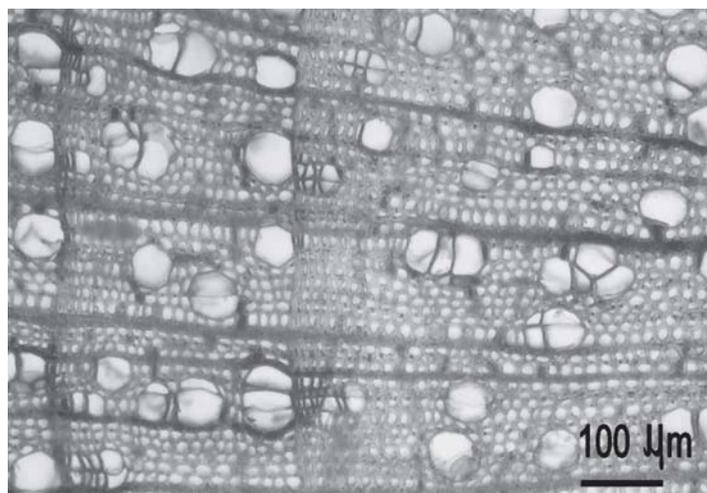


FIGURE 1. Xylem anatomy of *B. pendula*. 100X magnification.

FIGURA 1. Anatomía del xilema de *B. pendula*. Aumento de 100X.

## MATERIALS AND METHODS

### PLANT MATERIAL

Four healthy *Betula pendula* saplings (1.7 - 1.8 m tall), we obtained from a commercial nursery. This species was chosen because of its simple architecture and deciduousness (resulting in leaves being found only on terminal branches) which simplified the assignment of branching hierarchies. There are many cultivars and varieties of *B. pendula* available in greenhouses and gardens. This study does not pretend to address the anatomical variation between them nor to describe the wood anatomy of the species. Four individuals as a sample size is within the previously used for testing Murray's law (McCulloh *et al.* 2003).

### SAMPLES

During the growing season, plants were transplanted to large plastic pots (50 L) and transferred to a nursery with moderate natural lighting and abundant daily watering. To avoid embolism each plant was watered to field capacity the night before measurements were made. The saplings were cut at predawn at the root-stem interface and all leaves were removed and kept refrigerated and moist for area measurement and petiole sampling.

The xylem in the intact sapling (without leaves) was perfused with dye in order to identify active conduits. To do this the cut tree base was tightly

connected to a Tygon hose and perfused with safranin (25%). The staining of active vessels was conducted under a low pressure head of 1 m height to avoid refilling of non-functional vessels, similar to that described by McCulloh *et al.* (2003). Non-stained branches were cut and stained individually repeating the same procedure described above, or in some cases the column was replaced by pressure applied with a syringe.

Branches were assigned to levels. The main stem was assigned to "base", the branches emerging from it to "primary branches", one-year-old branches to "terminal branches" and leaf petioles to "petioles". Because branch number increases exponentially with successive levels, only 5-6 randomly selected terminal branches and 5-6 petioles were considered per individual, whereas all primary branches were sampled. Each branch was individually labeled, and transverse sections were cut at each level with a microtome. Sections were photographed with a digital camera (Nikon Coolpix 4300, Japan) connected to a microscope (Nikon, Japan) with a 100X and 400X magnifications.

### MEASUREMENTS

Photos were analyzed with SIGMASCAN (SPSS Inc. Chicago, IL, USA) and calibrated with a photographed microscope scale. On each image we measured conduit number, active xylem area and the vessel lumen area. Vessel radius ( $r$ ) was

estimated from vessel lumen area in cross-section, assuming a circular shape. With  $r$  we estimated  $\sum r^x$  for each branching level, i.e. the sum of the conduit radius to the  $x$  power. Total conduit area (conductive area) correspond to the sum of all the individual vessel lumen areas in a branching level. The previous parameters were measured in 2-3 radial sections per branching level to estimate values per active xylem area. The whole-level values were obtained by multiplying these values / area by total active xylem area of the level obtaining the whole level value for each parameter (level value = [section value estimate / section area] x total level area).

Plants were subsampled at petiole level. Five to six petioles per individual were randomly selected, transversally sectioned with a razor blade and stained with safranin for easy vessel measurement. We assumed that all xylem of petioles is active, as in McCulloh *et al.* (2003). Sections were photographed and analyzed as previously described for branches. We calculated petiole xylem area per unit leaf area, and then scaled to whole-plant values by multiplying by the ratio of total leaf area to subsample leaf area (petiole level value = sub-sample estimate value x [total leaf area / sub-sample leaf area]).

We then compared the sum of the radii raised to the  $x$ th power (for  $x = 0.5, 1, 1.5, 2, 2.5$  and  $3$ ) across branching levels (McCulloh *et al.* 2003). We tested

Murray's law (i.e. if  $\sum r^3$  is conserved across levels) for each pair of levels, and asked if conformity or otherwise depends on branching level.

The change in vessel number per level can be also expressed using the furcation number ( $F$ , Fig. 2), calculated as in McCulloh *et al.* (2003). This parameter also takes into account the branch furcation number ( $B$ ).  $F$  is calculated as follow:

$$F = [(F' - 1) / (B - 1)] + 1$$

Where  $F'$  is the raw conduit furcation number.

#### STATISTICAL TREATMENT

Vessel size, conductive area, conduit number and  $\sum r^x$  were compared between levels using a one-way ANOVA. In an exploratory analysis we found no individual or branch effect, as in McCulloh *et al.* (2004), so the branch measurements could be treated as independent. For conduit number, total conduit area and conduit size we used ANOVA and a Tukey test *a posteriori* to evaluate Murray's law. We compared  $\sum r^x$  between contiguous levels, recording the  $p$ -value of the ANOVA for different values of  $x$ . When the  $p$ -value at  $x = 3$  was lower than 0.05,  $\sum r^3$  was regarded as different between levels and Murray's law was rejected. The data were transformed to meet normality criteria: data for  $x = 0.5, 1$  and  $1.5$  were log-10, ln and log-10 transformed respectively. Vessel numbers per level was root-transformed.

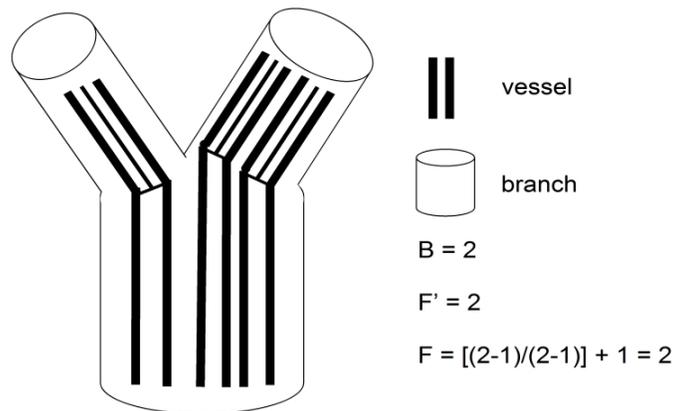


FIGURE 2. Explanation of the furcation number ( $F$ ), raw furcation number ( $F'$ ) and branch furcation number ( $B$ ).

FIGURA 2. Explicación del número de división ( $F$ ), número de división interno ( $F'$ ) y el número de división de la rama ( $B$ ).

RESULTS

When comparing  $\sum r^x$  between levels we found contrasting results in the proximal vs distal pairs of levels (Fig. 3). We found no evidence that  $\sum r^3$  differed significantly between the terminal branches and petioles (Fig. 3). Therefore Murray's law cannot be rejected for the distal tissues, although the p-values from the ANOVAS were higher when the exponent "x" was < 3.  $\sum r^3$  did differ significantly between the stem base and primary branches (Fig. 3). Therefore Murray's law clearly did not hold for the basal tissues.

The number of active conduits changed significantly between levels (Tables I-II, Fig. 4). We found a consistent increase in the number of active vessels from main bole to petioles (Fig. 4), although increases were statistically significant only between the two basal levels and between the two distal levels. This increase between levels resulted in F values (McCulloh *et al.* 2003) always greater than 1, especially in the basal portion

where the branch furcation number (B) was low. The average F value for the base-primary branches comparison was 1.2, compared with ca. 1 for terminal branches vs petioles. The furcation number (considering the branching number) was greatest for the base/primary branches comparison and ca. 1 in the terminal branches/petiole comparison (Table II).

The total conductive area (sum of individual vessels lumen area) differed significantly between levels, decreasing from base to petioles (Table I, Fig. 4). The decrease in conductive area was significant only between base and petioles, thus producing funnel-shaped area profile.

Conduit radius decreased significantly from base to petioles (Tables I-II, Figs. 4-5), each level differing from the next. All levels showed an approximately normal distribution of vessels sizes (Fig. 5). Nevertheless in the petiole the size distribution of vessels was somewhat truncated toward the lower extreme.

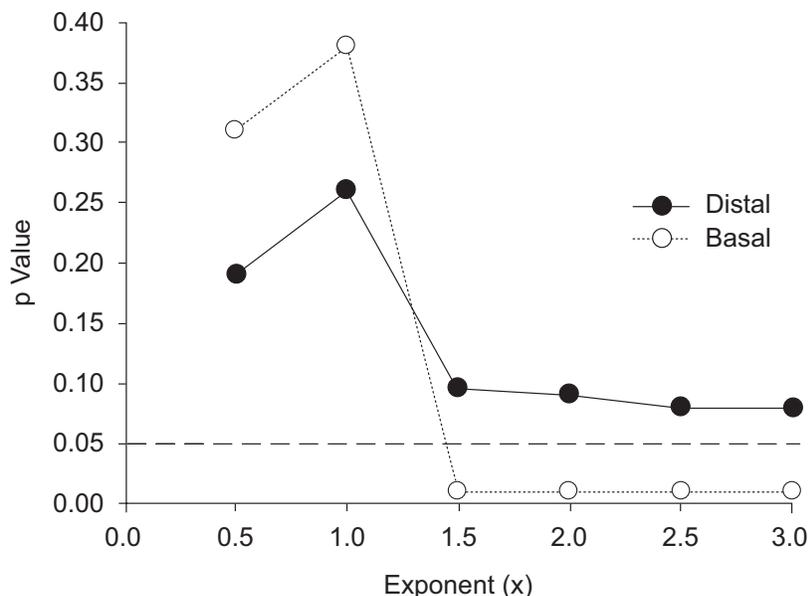


FIGURE 3. P value of the effect of level in ANOVA for different exponent (x) in  $\sum r^x$  with x=0.5 to 3.  $\sum r^x$  is compared between base-primary branches (proximal) and terminal branches-petioles (distal) pairs of levels.

FIGURA 3. Valor de p del efecto del nivel de ramificación en el ANOVA para diferentes exponentes (x) en  $\sum r^x$  con x=0.5 a 3.  $\sum r^x$  se compara entre la base del tallo y ramas primarias (proximal) y las ramas terminales y los pecíolos (distal).

TABLE I. Summary of ANOVA for overall effect of branching level on conduit number, total conduit area and conduit radii.

TABLA I. Resumen del ANOVA para el efecto del nivel de ramificación en el número de conductos, área de conducción y radio de conductos.

	Source	DF	MS	F	p
Conduit number	Branching level	3	3071.46	6.3809	0.0079
	Error	12	481.35		
	Total	15			
Total conduit area	Branching level	3	6.0163	4.5355	0.024
	Error	12	1.3265		
	Total	15			
Conduit radii	Branching level	3	0.0001	102.9222	<0.0001
	Error	12	0.0000		
	Total	15			

TABLE II. Relative change in average conduit number per level, total conduit area per level and vessel radius.

TABLA II. Cambio relativo en el valor promedio por nivel del número de conductos, área de conducción y radio de los vasos.

Level	Relative change in conduit number	Branching number (B)	Furcation number (F)	Relative change in total conduit area	Relative change in vessel radius
Primary branches/ base	1.29	2.5	1.20	0.75	0.76
Petioles/ terminal branches	1.16	74.7	1.00	0.53	0.62
Petioles/ base	2.93	175.3	1.01	0.36	0.36

## DISCUSSION

Although xylem anatomy of *Betula pendula* saplings was not accurately predicted by either model examined here (West *et al.* 1999, McCulloh *et al.* 2003), both models correctly predicted some patterns. Murray's law was clearly not applicable to the design of the basal portion of the vascular system of *B. pendula* saplings (Fig. 3), as  $\sum r^3$  differed significantly between primary branches and the stem base. This result was expected because in the basal portion the vessels should be contributing to structural support despite their size and scarce number (McCulloh *et al.* 2004, 2005). Although  $\sum r^3$  did not differ significantly between levels in the distal tissues (terminal branches vs. petioles), lower exponents (e.g. conservation of  $x = 1.0$  between levels) better fitted for equality. The conservation of the radius across levels (i.e. when  $x = 1$ ) would be a closer fit to the WBE model. McCulloh *et al.* (2003)

and (2004) found a better fit to Murray's law when comparing  $\sum r^3$  between petioles and petiolules in compound-leaved species. Their comparison of first-year branches and petioles showed some deviation from Murray's law but not enough to be significant. Our data show even more deviation in the basal portion of saplings. The overall picture that emerges to date is therefore one of a reasonable fit to Murray's law in the distal tissues of woody plants, with deviation increasing basipetally. Mechanical constraints on vascular design, as discussed elsewhere (McCulloh & Sperry 2005), could account for such deviations.

Although the main elements of support in stems and large branches are fibres, the contribution of vessels to mechanical stability may also be important, especially in diffuse porous trees. In contrast, petioles and petiolules are mechanically supported primarily by turgor pressure and collenchyma (McCulloh *et al.* 2003). Structural factors could therefore constrain

vessel diameters in basal tissues, giving rise to increasing deviations from Murray's law optimum. As we shall see below, hydraulic safety considerations could also explain deviations from Murray's law.

The number of active conduits per level increased from base to petioles almost three times (Fig. 4). This violates the assumption of West *et al.* (1999), who envisaged the vascular system as "identical tubes of equal length running continuously in parallel from trunk to petiole". Our

data show a more gradual acropetal increase in conduit number between consecutive levels than that expected in a maximal efficiency scenario, especially in the distal portion. The low increase in conduit number between levels means a low furcation number (F in Table II). We found opposing results for F in the basal and distal portion. In the base, where a major deviation from Murray's law occurred, the vessels might be more involved in mechanical support; however, the basal F value was

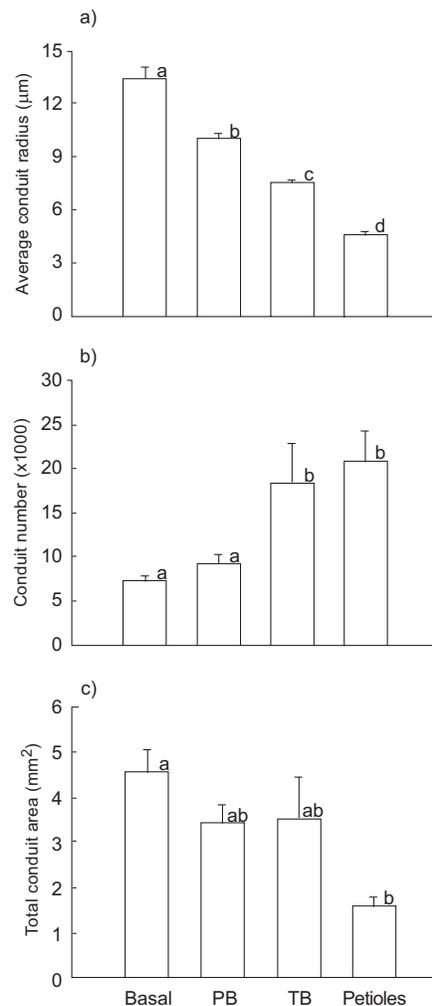


FIGURE 4. a) Average conduit radius ( $\mu\text{m}$ ), b) conduit number and c) total conduit area ( $\text{mm}^2$ ) in the base, primary branches (PB), terminal branches (TB) and petiole level of *B. pendula* saplings. Standard errors for each case are shown. Distinct letters correspond to significative differences (Tukey test).

FIGURA 4. a) Radio promedio de los vasos ( $\mu\text{m}$ ), b) número de conductos, y c) área total de conducción ( $\text{mm}^2$ ) en la base, ramas primarias (PB), ramas terminales (TB) y pecíolos de juveniles de *B. pendula*. Se muestran los errores estándar en cada caso. Letras diferentes corresponden a diferencias significativas (test de Tukey).

higher (1.2) than that observed distally (ca. 1). Higher values of F are usually found where the conduits do not participate in the mechanical stability of the stem as in vines and compound leaves (McCulloh *et al.* 2003, 2004). Nevertheless, in *B. pendula* we found lower F values at the petiole level, similar to those found in conifer wood (McCulloh *et al.* 2004). The F value found at the base is comparable with values observed for wood of other ring-porous trees (McCulloh *et al.* 2004).

Conduits tapered progressively from base to petioles (Figs. 4-5), as assumed by recent model (West *et al.* 1999) and evidenced in several studies (Zimmermann 1978, 1983, James *et al.* 2003, McCulloh *et al.* 2004, Niklas 2006, Weitz *et al.* 2006). Becker *et al.* (2000) showed that tapering of conduits can buffer against increasing resistance as path-length increases when resistance is limited by the smallest conduits. McCulloh *et al.* (2004) also pointed out that conduit tapering allows a stable

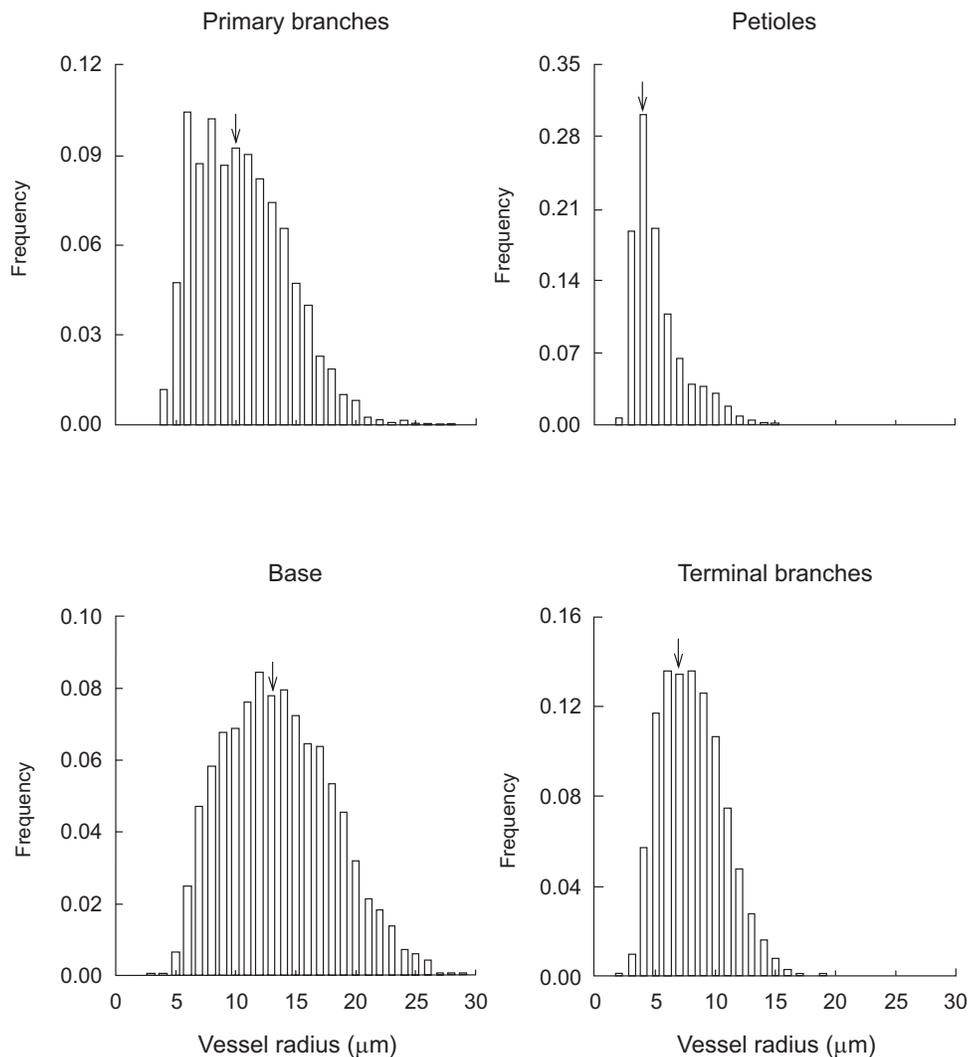


FIGURE 5. Distribution of lumen diameters ( $\mu\text{m}$ ) of active vessels at each branching level in *B. pendula* saplings. Arrows indicate the class that contains the average diameter.

FIGURA 5. Distribución del diámetro del lumen ( $\mu\text{m}$ ) de los vasos activos en cada nivel de ramificación en juveniles de *B. pendula*. Las flechas indican la clase de diámetro que contiene el promedio del nivel.

mechanical configuration at  $F=1$ . Such  $F$  values and conduit taper result in an acropetal reduction in conduit area thus producing a more stable area profile (see below).

The total conductive area decreased from the base to the petiole level (Fig. 4), as the acropetal increase in conduit number (Fig. 4) was not enough to compensate for the conduit taper. The WBE model (West *et al.* 1999) assumes a progressive acropetal decrease in conductive area, in this case as a result of a constant conduit number and a progressive vessel taper from base to apex. In contrast, McCulloh *et al.* (2003) proposed that the total transversal section area of the conduits should increase acropetally when following Murray's law. In this sense, our data approximate more closely to the WBE model, although the decrease in conductive area is not constant, with a drastic reduction at the distal-most (petiole) level. McCulloh *et al.* (2004) also found an acropetal conductive area reduction in distal branches up to 4 years old, this reduction being small in ring-porous species and more evident in conifers. This is in keeping with the proposal that if conductive area is proportional to stem cross-sectional area, it should not increase with height because this would lead to a mechanical instability (McCulloh *et al.* 2004). This area profile with an acropetal reduction of conduit area corresponds with the shape of the tallest free-standing column for a given volume of a determinate material in mechanical analysis (Keller & Niordson 1966). An acropetal increase in conduit area has been observed in distal tissues of vines and in compound leaves where the vascular system has little or no mechanical function (McCulloh *et al.* 2003). The area constriction also is in agreement with an increase in sap velocity as found in other diffuse-porous species in the tropics (Andrade *et al.* 1998) and in conifers (McDonald *et al.* 2002). This is due to the relationship between flow rate ( $Q$ ) and the sap velocity ( $v$ ) and conduits area ( $A_c$ ), namely

$$Q = vA_c$$

So, in order to keep a constant flow rate across ranks, a constriction in the conduits area would require an increased sap velocity.

Selection for traits promoting survival under water stress might at least partly explain the empirical deviations from current models emphasising optimization of hydraulic performance (West *et al.* 1999, McCulloh *et al.* 2003). Neither the WBE model

(West *et al.* 1999) nor the model based on Murray's law (McCulloh *et al.* 2003) explicitly incorporate traits associated with survival of water stress. A high  $F$ , as considered under Murray's law, optimizes the efficiency of the system but affects the safety margin (McCulloh *et al.* 2004). If an embolism is produced in a basal conduit in a plant with low  $F$ , this would affect only one or few distal vessels. On the other hand, a basal embolism in one conduit of a plant with high  $F$  would affect many distal vessels, compromising water supply to a large leaf area. So for a given conduit area it is safer to have numerous smaller conduits in parallel that do not divide frequently over few large conduits that divide each level. In plant vascular systems, the optimization of water transport per biomass unit (few, wide vessels in the base) can be in conflict with the optimization of protection against embolism and survival under water deficit (functionally redundant and smaller vessels). The actual vascular design could reflect an environmentally-mediated compromise between optimisation of performance, minimization of construction costs and survival under fluctuating water conditions.

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#### BIBLIOGRAPHY

- ANDRADE, J.L., F.C. MEINZER, G. GOLDSTEIN, N.M. HOLBROOK, J. CAVELIER, P. JACKSON & K. SILVERA. 1998. Regulation of water flux through trunks, branches, and leaves in trees of a lowland tropical forest. *Oecologia* 115: 463-471.
- BECKER, P., R.J. GRIBBEN & C.M. LIM. 2000. Tapered conduits can buffer hydraulic conductance from path-length effects. *Tree Physiology* 20: 965-967.
- CHIBA, Y. 1998. Architectural analysis of relationship between biomass and basal area based on pipe model theory. *Ecological Modelling* 108: 219-225.
- ENQUIST, B.J. 2002. Universal scaling in tree and vascular plant allometry: toward a general quantitative theory linking plant form and function from cell to ecosystems. *Tree Physiology* 22: 1045-1064.

- ENQUIST, B.J. & K.J. NIKLAS. 2001. Invariant scaling relations across tree-dominated communities. *Nature* 410: 655-660.
- ENQUIST, B.J., G.B. WEST, E.L. CHARNOV & J.H. BROWN. 1999. Allometric scaling of production and life-history variation in vascular plants. *Nature* 401: 907-911.
- ENQUIST, B.J., J.H. BROWN & G.B. WEST. 1998. Allometric scaling of plant energetics and population density. *Nature* 395: 163-165.
- HUBBARD, R.M., V. STILLER, M.G. RYAN & J.S. SPERRY. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* 24: 113-121.
- JAMES, S.A., F.C. MEIZNER, G. GOLDSTEIN, D. WOODRUFF, T. JONES, T. RESTOM, M. MEJIA, M. CLEARWATER & P. CAMPANELLO. 2003. Axial and radial water transport and internal water storage in tropical forest canopy trees. *Oecologia* 134: 37-45.
- KELLER, J.B. & F.I. NIORDSON. 1966. The tallest column. *Journal of Mathematics and Mechanics* 16: 433-446.
- MCCULLOH K.A. & J.S. SPERRY. 2005. Patterns in hydraulic architecture and their implications for transport efficiency. *Tree Physiology* 25: 257-267.
- MCCULLOH K.A., J.S. SPERRY & F.R. ADLER. 2003. Water transport in plants obeys Murray's law. *Nature* 421: 939-942.
- MCCULLOH, K.A., J.S. SPERRY & F.R. ADLER. 2004. Murray's law and the hydraulic vs mechanical functioning of wood. *Functional Ecology* 18: 931-938.
- MCDONALD, K.C., R. ZIMMERMANN & J.S. KIMBALL. 2002. Diurnal and spatial variation of xylem dielectric constant in Norway spruce (*Picea abies* (L.) Karst.) as related to microclimate, xylem sap flow, and xylem chemistry. *IEEE Transactions on Geoscience and Remote Sensing* 40: 2063-2082.
- MEINZER, F.C. & D.A. GRANTZ. 1990. Stomatal and hydraulic conductance in growing sugarcane: Stomatal adjustment to water transport capacity. *Plant, Cell and Environment* 13: 383-388.
- MEINZER, F.C., G. GOLDSTEIN, P. JACKSON, N.M. HOLBROOK, M.V. GUTIÉRREZ & J. CAVALIER. 1995. Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic properties. *Oecologia* 101: 514-522.
- MURRAY, C.D. 1926. The physiological principle of minimum work. I. The vascular system and the cost of blood volume. *Proceedings of the National Academy of Sciences, USA* 12: 207-214.
- NIKLAS, K.J. & B.J. ENQUIST. 2002. On the origin of vegetative biomass partitioning of seed plant leaves, stems, and roots. *American Naturalist* 159: 482-497.
- NIKLAS, K.J. & B.J. ENQUIST. 2003. An allometric model for seed plant reproduction. *Evolutionary Ecology Research* 5: 79-88.
- NIKLAS, K.J. 2006. Scaling the paths of resistance. *New Phytologist* 169: 219-222.
- NIKLAS, K.J., J.J. MIDGLEY & B.J. ENQUIST. 2003. A general model for the mass-growth-density relations across tree-dominated communities *Evolutionary Ecology Research* 5: 459-468.
- PRICE C.A. & B.J. ENQUIST. 2007. Scaling mass and morphology in leaves: an extension of the WBE model. *Ecology* 88: 1132-1141.
- PRICE C.A., B.J. ENQUIST & V.M. SAVAGE. 2007. A general model for allometric covariation in botanical form and function. *Proceedings of the National Academy of Sciences, USA* 104: 13204-13209.
- SALIENDRA, N.Z., J.S. SPERRY & J.P. COMSTOCK. 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta* 196: 357-366.
- SHINOZAKI, K., K. YODA, K. HOZUMI & T. KIRA. 1964a. A quantitative analysis of plant form-the pipe model theory: I. Basic analysis. *Japanese Journal of Ecology* 14: 97-105.
- SHINOZAKI, K., K. YODA, K. HOZUMI & T. KIRA. 1964b. A quantitative analysis of plant form-the pipe model theory: II. Further evidence of the theory and its implications in forest ecology. *Japanese Journal of Ecology* 14: 133-139.
- SPERRY, J.S. & W.T. POCKMAN 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* 16: 279-287.
- TYREE, M.T. 2003. The ascent of water. *Nature* 423: 923.
- TYREE, M.T. & M.H. ZIMMERMANN. 2002. *Xylem structure and the ascent of sap*, 2nd ed. Springer-Verlag, Berlin, Germany.
- WEITZ, J.S., K. OGLE & H.S. HORN. 2006. Ontogenetically stable hydraulic design in woody plants. *Functional Ecology* 20: 191-199.
- WEST, G.B., J.H. BROWN & B.J. ENQUIST. 1999. A general model for the structure and allometry of plant vascular systems. *Nature* 400: 664-667.
- ZIMMERMANN, M.H. 1978. Hydraulic architecture of some diffuse porous trees. *Canadian Journal of Botany* 56: 2286-2295.
- ZIMMERMANN, M.H. 1983. *Xylem structure and the ascent of sap*. Springer-Verlag, Berlin, Germany.