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ANALYSIS OF THE TAPERING OF XYLEM CONDUITS AS A COMPENSATION MECHANISM FOR HYDRAULIC LIMITATIONS TO TREE GROWTH

Direttore della Scuola: Ch.mo Prof. Vasco Boatto
Supervisore: Ch.mo Prof. Tommaso Anfodillo
Co-Supervisore: Ch.mo Prof. Maurizio Mencuccini

Dottorando: Giai Petit

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Riassunto

Negli alberi, l'acqua si muove attraverso una rete di cellule xilematiche per mezzo di un gradiente negativo di potenziale idrico tra i siti di utilizzo (foglie) ed i siti di assorbimento (radici), la cui distanza può superare i 120 m negli individui più grandi del pianeta. L'architettura del sistema di trasporto deve garantire contemporaneamente un'adeguata efficienza idrica e la stabilità meccanica dell'individuo. Infatti, l'aumento dei vincoli di natura idraulica determina delle forti limitazioni alla crescita in altezza, che l'albero cerca di compensare attraverso delle modificazioni strutturali del sistema di trasporto, la più efficace delle quali sembrerebbe essere la rastremazione degli elementi di conduzione. Recenti studi sembrerebbero supportare l'ipotesi del modello WBE sull'universalità del grado di rastremazione dei condotti xilematici nelle piante vascolari: questo dovrebbe tendere ad un valore soglia che minimizzi l'effetto della lunghezza del percorso sulla resistenza idraulica totale del sistema, che quindi rimarrebbe all'incirca costante all'aumentare dell'altezza della pianta. Pertanto è stato ipotizzato che le limitazioni di natura idraulica che determinano il rallentamento del ritmo di crescita in altezza siano conseguenti ad un'insufficiente grado di rastremazione degli elementi xilematici, mentre l'altezza massima raggiungibile sarebbe sostanzialmente determinata dalla dimensione assoluta degli elementi di conduzione, in particolare quelli apicali, dove è concentrata la maggior parte della resistenza idraulica.

L'obiettivo di questo lavoro è di valutare l'effetto combinato delle dimensioni e della rastremazione degli elementi di conduzione nel compensare l'azione inibitrice dei vincoli idraulici sulla crescita in altezza. Tra gli argomenti discussi, il primo riguarda un'analisi approfondita della struttura matematica e logica del modello WBE, mentre gli altri si riferiscono ad esperimenti empirici. Il primo consiste in un'analisi delle modifiche alla struttura dello xilema durante l'ontogenesi in *Acer pseudoplatanus*. Sono state selezionati individui di due classi di età/dimensioni (giovani/piccoli e vecchi/alti). L'utilizzo di piante innestate (cloni delle piante selezionate in campo) come controllo ha permesso

di scindere l'auto-correlazione tra età e dimensioni e quindi determinare quale di questi due fattori sia responsabile delle eventuali modifiche strutturali.

Nel quarto e quinto capitolo viene discussa l'importanza della rastremazione degli elementi di conduzione in specie di conifere (*Pinus cembra*, *Larix decidua* and *Picea abies*) alla treeline, dove le basse temperature inibiscono la formazione di elementi di conduzione di grandi dimensioni e la crescita in altezza è fortemente limitata. In *P. cembra*, l'analisi della rastremazione è stata estesa anche alle radici, mentre gli individui di *P. abies* sono stati sottoposti ad un trattamento di riscaldamento della gemma apicale per due stagioni vegetative consecutive.

Nel capitolo 6, invece, viene elaborata un'ipotesi riguardante la formazione del durame in *Acer pseudoplatanus*, in cui la settorialità intra-anulare dei flussi idrici, testata attraverso l'iniezione di colorante nell'anello più interno di rami di 5-6 anni e lunghi circa 1 m, e la rastremazione degli elementi di conduzione potrebbero giocare un ruolo chiave.

In tutti gli esperimenti svolti, le analisi anatomiche hanno previsto l'estrazione di campioni legnosi lungo l'asse longitudinale del fusto/ramo. Questi sono stati inclusi in paraffina, tagliati con microtomo rotativo a 10-12 µm, le relative sezioni colorate con safranina e incollate su vetrini porta-oggetto. I vetrini sono stati osservati al microscopio, sono state misurate le ampiezze cellulari e determinato il diametro idraulico per ciascun punto di campionamento.

In *A. pseudoplatanus*, la complessiva riduzione del grado di rastremazione e l'aumento di dimensione dei condotti apicali negli individui vecchi/alti rispetto ai giovani/piccoli non è stata riscontrata nei relativi innesti. Il grado di rastremazione degli individui di piccole dimensioni, a prescindere dall'età dei loro meristemi, è risultata generalmente conforme al principio di minimizzazione e stabilizzazione della resistenza idraulica predetta dal modello WBE. Pertanto, tali modifiche strutturali sono risultate essere legate a cambiamenti dimensionali piuttosto che a fenomeni di invecchiamento. Inoltre, la safranina iniettata nell'anello più interno di rami della stessa specie è fluita fino all'apice rimanendo sempre confinata nell'anello più interno di ciascun internodo, senza cioè diffondersi radicalmente da un anello al successivio, dimostrando così una settorialità intra-annuale dei flussi idrici.

Negli alberi alla treeline, è risultato che il grado di rastremazione degli elementi xilematici è generalmente conforme alle previsioni del modello WBE. Inoltre, l'effetto del riscaldamento della gemma apicale a promosso la formazione di elementi di conduzione apicali di maggiori dimensioni e getti apicali più lunghi, supportando così l'ipotesi di una limitazione idraulica alla crescita in altezza anche negli alberi alla treeline.

Infine, è stato osservato che la rastremazione non è limitata alle parti aeree, ma continua anche lungo le radici, dove essa è risultata maggiore di quella del fusto.

Le analisi presentate in questo lavoro hanno dimostrato l'importanza della rastremazione degli elementi di conduzione come fondamentale meccanismo di compensazione per l'effetto limitante che i vincoli di natura idraulica esercitano sulla crescita in altezza, il cui massimo sembrerebbe dipendere sostanzialmente dalla possibilità di formare condotti di grandi dimensioni all'apice.

Summary

In trees, water flows throughout a network of xylem conduits by mean of a negative pressure gradient between sites of consumption (leaves) and absorption (roots), the distance of which can be longer than 120 m in the world's tallest specimens. The architecture of the transport system must guarantee an adequate water supply and the mechanical stability of the tree. In fact, increased hydraulic constraints cause strong limitations to height growth. Trees adopt a number of structural modifications to compensate for the effect of these constraints and the tapering of xylem conduits is probably the most effective. Recent studies seemed to support the WBE model's hypothesis about the universality of the degree of conduit tapering in vascular plants, which should converge to a threshold value that minimizes the effect of path length on the total hydraulic resistance, which hence would remain substantially constant with increasing tree height. Therefore, it has been hypothesized that hydraulic constraints, which lead to the decline in the rate of height growth, are consequent to a reduced degree of conduit tapering, whereas the maximum height is substantially set by the absolute dimensions of xylem conduits, in particular those at the apex, where the most hydraulic resistance is concentrated.

The aim of this work was to evaluate the combined effect of the dimension of xylem conduits and their tapering in compensating the inhibition-action of hydraulic constraints on height growth. Among the discussed topics, the first is a thorough analysis of the mathematical and logical structure of the WBE model, while the others describe empirical experiments. The first consists of an analysis of the structural modifications of the xylem transport system during ontogenesis in *A. pseudoplatanus*. Specimens from two age/size classes (young/small and old/tall) were selected and the use of grafted plants (i.e., clones of the parent plants selected in the field) as controls allowed to decouple the auto-correlation between age and dimensions, and thus to determine which of these two factors is responsible for the structural modifications.

In the fourth and fifth chapter, the importance is discussed of conduit tapering in coniferous species (*Pinus cembra*, *Larix decidua* and *Picea abies*) at the treeline, where low temperatures inhibit the formation of wide conduits and height growth is strongly limited. In *P. cembra*, the analysis of conduit tapering was extended to roots, whereas on the selected specimens of *P. abies* a heating system was applied to the developing apical bud for two successive growing seasons.

In chapter 6, a new hypothesis is elaborated regarding the heartwood formation in *A. pseudoplatanus*, where the intra-ring sectoriality of water flows, tested by a dye injection into the innermost ring of 5-6 year-old, 1 m long branches, and conduit tapering might play a key role.

In all the experiments, wooden samples were extracted at different distances along the longitudinal axis of each stem/branch. Samples were embedded in paraffin, cut with a rotary microtome at 10-12 µm and sections were stained with safranine and fixed permanently on slides. Slides were observed under the microscope, the lumen areas of conduits measured and the averaged hydraulically weighted diameter assessed for each distance.

In *A. pseudoplatanus*, an overall reduction in the degree of conduit tapering and an increase in width of the apical conduits of old/tall trees compared to young/small ones was not detected in the relative grafts. The degree of tapering in the small plants, irrespective of their meristematic age, resulted in agreement with the WBE model’s principle of minimization and stabilization of the total hydraulic resistance. These structural modifications were therefore connected to the size variations rather than to proper ageing. Moreover, the safranine injected into the innermost ring of branches of the same species flowed until the apex, remaining confined within the innermost ring at each internode, i.e., without any radial spread between successive rings, thus demonstrating a substantial intra-ring sectoriality of water flows.

At the treeline, trees showed a degree of conduit tapering on average in agreement with the WBE model’s prediction. Moreover, warming applied to the developing apical buds promoted the formation of wider apical conduits and apical shoots resulted as longer, thus supporting the hypothesis of a hydraulic limitation to height growth also in trees at the treeline.

Lastly, it was observed that the tapering of xylem conduits is not confined to the aboveground organs, but also continues along the roots, where the degree of tapering resulted as higher than that of the stem.

The analyses presented in this work demonstrates the importance of conduit tapering as a fundamental mechanism of compensation for the limiting effect of hydraulic constraints on growth in height, whose maximum seems to substantially depend on the possibility of forming wide apical conduits.

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1 Introduction

Trees are among the world's biggest organisms and their ontogenesis is accompanied by an enormous change in body mass. For example, the body mass of a giant sequoia may increase by 12 orders of magnitude from seedling to mature tree and its final root-to-leaves distance (i.e., the path length of sap flow) can be longer than 120 m. However, this three-dimensional increase in size is characterized by a rate of height growth which varies with the different ontogenetic stage and typically declines with increasing age and size (e.g., Sachs 1965; Lappi 1997). This phenomenon, which on an even-stands-scale leads to a decline of the forest net primary productivity, has long been studied. While some studies suggested that it is determined by the genetic effects of ageing (Yoder et al. 1994; Gower et al. 1996; Bond 2000; Day et al. 2001; Binkley et al. 2002), others hypothesized that it is driven by the increased physical constraints of raising water for increased root-to-leaves distances as trees grow taller (hydraulic limitation hypothesis: Mencuccini & Grace 1996; Ryan & Yoder 1997; McDowell et al. 2002b; Barnard & Ryan 2003; Phillips et al. 2003). Due to the autocorrelation between tree age and height, the conclusions in favour of age- or size related effects did not exclude one another. Instead, recent researches on clones of different age and height presented evidence in favour of size-mediated effects (Mencuccini et al. 2005, Matsuzaki et al. 2005; Bond et al. 2007; Mencuccini et al. 2007). However, although recent advances in tree physiology revealed the predominant importance of hydraulic constraints in setting the physical limit to tree height (Koch et al. 2004), the structural modifications to the transport system allowing trees to span such a huge size range from seedling to maturity are not yet clearly established (e.g., Mencuccini 2002; Midgley 2003; Niklas & Cobb 2006). In fact, the root-to-leaves xylem transport system can be unlikely simplified as a set of cylindrical tubes (Shinozaki et al. 1964a,b), the resistance of which linearly increases with its length (Hagen-Poiseuille formula; Tyree & Ewers 1991), and trees had been discovered to adopt a number of strategies to mitigate, but never completely eliminate, the effect of increased height on the total hydraulic resistance. For instance, tall trees were found to increase the allocation to fine roots, i.e., to increase the surfaces for water uptake (Magnani et al. 2000); to decrease the leaf area to sapwood area ratio, i.e., to increase the conduction area per unit-leaf (McDowell et al. 2002a); to increase sapwood permeability, i.e., to increase the xylem conductance per unit area (Pothier et al. 1989). This last compensation mechanism has recently received renewed attention after the publication of a theoretical model clarifying the potential significance of the increase in conduit size from apex to stem base (West et al. 1999: West, Brown and Enquist or WBE model). The key trait of the WBE model is that xylem conduits must taper enough to allow trees to uncouple the total hydraulic resistance from the path length (i.e., conduit tapering would keep the resistance to a nearly constant value). Looking beyond all the speculations surrounding the model, its indubitable merit was to invoke renewed interest in wood anatomy, from the actual effect of conduit tapering in minimizing the whole tree hydraulic resistance (Becker et al. 2000; Zaehle 2005; Petit et al. 2008) to its potential role as the fundamental driver for the evolution of vascular plants (Enquist 2003; Anfodillo et al. 2006; Weitz et al. 2006). That xylem conduits increase in size from the apex downwards has long been known (e.g., Zimmerman 1983; Gartner 1995; Meinzer et al. 2001; Nijsse et al. 2001; Martínez-Vilalta et al. 2002; McElrone et al. 2004; McCulloh & Sperry 2005), but investigations have only recently demonstrated that conduit tapering is a common feature among plants of different species and size, and typically follows a power function with a scaling exponent proximal to that predicted by the WBE model (Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007, Petit et al. 2008). Departures from this ideal pattern, which would keep the total hydraulic resistance at a nearly constant value, have been proposed as strategies for the minimization of the costs in carbon allocation while guaranteeing a sustainable amount of hydraulic resistance for the metabolism (Mencuccini et al. 2007; Petit et al. 2008). Nevertheless, since trees approaching their maximum height seem to be characterized by a reduced conduit tapering (e.g., Anfodillo et al. 2006; Petit et al. 2008), it has been speculated that the consequent increased hydraulic constraints would be the physical and physiological explanation for the reduced growth rate observable in tall trees (Anfodillo et al. 2006). The fundamental idea is that proposed also by Koch et al. (2004), i.e., that trees cannot sustain their metabolism when leaves experience too low water potentials. Actively growing trees would therefore be expected to have an optimized xylem transport system and, as they grow taller, the degree of tapering should converge to that threshold value allowing the independence of hydraulic resistance from path length (West et al. 1999; Becker et al. 2000), while maintaining the widest apical conduits compatible with the risk of cavitation (e.g., Hacke et al. 2001; Wheeler et al. 2005) and enlarging xylem conduits at the base. Since it's likely that a tree cannot indefinitely enlarge its basal conduits, once the maximum width is reached, further increases in height would lead to a consequent increase in total hydraulic resistance, whether because of an overall reduction in the degree of conduit tapering due to the formation of the so-called plateau of conduit diameters at the stem base or because of a formation of narrower apical conduits to maintain "optimal" conduit tapering. Typically, tall trees with monopodial branching showed reduced conduit tapering consequent to a basal plateau in the stem's longitudinal profile of the averaged conduit diameters (Becker et al. 2003; Anfodillo et al. 2006; Mencuccini et al. 2007) accompanied by wider apical conduits (Riondato, personal communication).

The aim of this work was to investigate the tapering of xylem conduits as a mechanism of compensation for the effect of increased tree height on total hydraulic resistance.

First, I made a thorough analysis of the WBE model (West et al. 1997; West et al. 1999). Since all the recent investigations on allometric relationships and particularly on conduit tapering gave empirical support to the WBE model (Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007, Petit et al. 2008), in spite of several criticisms regarding its mathematical appropriateness and biological relevance (Dodds et al. 2001; Kozlowski & Konarzewski 2004; Coomes 2006; Coomes & Allen 2007), our analysis aimed to highlight the importance of conduit tapering for the development of the model for vascular plants and the role that this feature of the transport system plays with respect to tree growth.

I conducted three different experiments aimed to clarify some possible important implications that the tapering of xylem conduits may have on the growth of trees.

In the first experiment, I analyzed possible changes in conduit tapering during the ontogenesis of an angiosperm (*Acer pseudoplatanus* L.) and evaluated whether they were determined by the effect of ageing or that of the increased dimensions. I also conducted specific hydraulic measurements to analyze the scaling of the total hydraulic resistance with the distance from the stem apex and compared hydraulic and anatomical data to evaluate the effect of conduit tapering on the distribution of resistances along the longitudinal axis.

Moreover, I analyzed the xylem anatomy of small trees growing at high altitude under extreme environmental conditions. At the treeline, growth in height is limited and low temperatures are known to affect either cell divisions or the formation of wide cells (Tranquillini 1979; Kirdianov et al. 2003; Oberhuber 2004; Danby & Hik 2007; Rossi et al. 2007). I evaluated whether conduit tapering may also be important for short distances when conduits are narrow, and whether a departure from the optimal degree of tapering could lead to an even more reduced growth rate and, ultimately, to a maximum height. I therefore analyzed the conduit tapering of trees growing at the treeline and also heated the apical shoot of some trees to evaluate the effects of the increased temperatures on the xylem anatomy and, ultimately, on tree growth.

Another important investigation was the first ever on the tapering of xylem conduits along the roots. I extracted the longest root of three specimens of *Pinus cembra* L. and analyzed some scaling equations between root diameter, root length and diameter of xylem conduits.

Lastly, I tested a new hypothesis on loss of conductivity of the innermost sapwood (i.e., the heartwood formation). Since tree age and height are autocorrelated, many phenomena that have been demonstrated to be controlled by the genetic effect of ageing, can also be discussed as size-related effects. Heartwood formation was commonly reported as an age-controlled phenomenon. Nevertheless, tree height was also found to be a good predictor and hydraulic limitations (e.g., reaction wood, drought) were often reported to promote heartwood formation (Stokes & Berthier 2000; Berthier et al. 2001; Climent et al. 2002). I tested the fundamental idea of this hypothesis, i.e., whether the fluid contained within a tree ring flows axially and directly towards the apex or spreads also to all the other rings forming the sapwood. I injected a dye into the innermost ring of branches of *Acer pseudoplatanus* L. If the dye always remained confined within the innermost ring irrespective of the number of rings of the internodes, it could be speculated that the innermost ring stops conducting water once its whole path hydraulic resistance becomes too high. This would occur because, when it was the new formed shoot, the tapering of xylem conduits could minimize the hydraulic resistance, whereas with the successive increments, a number of resistances (i.e., corresponding to the successive current shoots) would had added to it with the effect of a continuous increase of resistance year by year.

2 A re-examination of the WBE model

2.1 Introduction

During ontogeny, all living organisms increase in size and this variation can be up to even 12 orders of magnitude in the biggest trees such as Giant Sequoias. But, changes in size do not reflect proportional changes in the physiological processes and empirical measurements of metabolic rate revealed that it often scales with size as a power function, with a scaling exponent reported to be sometimes close to 2/3 (e.g., White & Seymour 2003; Speakman 2005), others to 3/4 (e.g., Schmidt-Nielsen 1984; Savage et al. 2004), yet others to be a species-specific variable (e.g., Kvist & Lindström 2001; Glazier 2005). However, allometric scaling laws have been often considered as more or less ubiquitous, i.e., they show a size-independent, intra- and inter-specific similarity. Thus, it is a common thought that there might be a fundamental reason explaining this common feature among organisms. An early attempted explanation was based simply on Euclidean geometry, which implies that the surface area of an object of a given shape must scale to the volume raised to the 2/3 power. Hence, by assuming that heat loss is proportional to the surface area of the body, it would follow that the power needed to maintain a constant body temperature is proportional to the body-volume raised to the 2/3 power. Instead, a general explanation in favour of the predominance of the 3/4 power laws in biology was proposed by West et al. (1997 and 1999, hereafter WBE model), based on the often observed self-similarity of natural structures (Mandelbrot 1982). As a general model, it had to stand on mathematical assumptions and was often criticised for its logical contradictions and biologically unrealistic constraints and assumptions (Dodds et al. 2001; Kozlowski & Konarzewski 2004; Makarieva et al. 2005; Chaui-Berlinck 2006; Coomes 2006). Nevertheless, in the last years the model has received huge attention from biologists and ecologists because of its new all-comprehensive perspective for the description of natural structures and phenomena.

In this chapter, the structure of the WBE model is thoroughly re-analysed and the two different versions compared (West et al. 1997, 1999), with particular attention paid to their fundamental assumptions, the implications and any possible contradictions for the logical development of the model.

2.1 WBE model I (West et al. 1997)

The WBE model proposed that the predominance of the 3/4 power laws in biology originates from the fractal architecture of the vascular transport system of organisms. The first version of the model (West et al. 1997) basically referred to pulsatile transport systems like the cardiovascular system of animals and only possibly extended to the tracheary system of insects and xylematic and phloematic transport systems of plants.

The WBE model is based on three fundamental assumptions:

1. the branching pattern of the transport system is *space filling*, i.e., it supplies the whole volume of the organism, and this condition is guaranteed by a fractal-like geometry;
2. the terminal elements are size-invariant;
3. the energy needed for the delivery of resources, i.e., the total hydrodynamic resistance, is minimised.

Besides these three fundamental assumptions, a number of others can be extrapolated. Firstly, given the fractal nature of the system, as the authors stated “the model should be viewed as an idealized representation in that I ignore complications, such as tapering of vessels, turbulence, and non linear effects”, it followed that the network is constituted of successive levels within which elements are identical cylinders (for which the linear flow can be described by the Hagen-Poiseuille formula, i.e., assumption 3) and, in order for the system to conserve the amount of fluid at each level, the total transversal area at every level must also be preserved (see equation 2.2).

As a self-similar fractal system, the number of branches (N_k) increases in geometric proportion with levels (k)

$$N_k = n_0 \cdot n_1 \cdot n_2 \cdot .....n_k = n^k \qquad \text{equation 2.1}$$

where n is the number of branching generations (i.e., a furcation factor).

Branches decrease in size from basal level (*0*) to terminal level (*N*) and the dimensional variations are characterized by specific ratios of radius (r_k) and length (l_k) that hold independently of the level *k*:

$$\beta = \frac{r_{k+1}}{r_k} = n^{-a/2} \qquad \text{equation 2.2}[1]$$

$$\gamma = \frac{l_{k+1}}{l_k} = n^{-1/3} \qquad \text{equation 2.3}$$

Equation 3 derives from the *space filling constraint* (assumption 1). The authors wrote: “A space-filling fractal is a natural structure for ensuring that all cells are serviced by capillaries. The network must branch so that a group of cells, referred to here as a *service volume*, is supplied by each capillary. Because $r_k << l_k$ and the total number of branching N is large, the volume supplied by the total network can be approximated by the sum of spheres whose diameters are that of a typical *k*th-level vessel, namely $4/3\pi(l_k/2)^3 N_k$. For large *N*, this estimate does not depend significantly on the specific level, although it is most accurate for large *k*. This condition, that the fractal be volume-preserving from one generation to the next, can therefore be expressed as…”

$$V_{TOT} \approx \frac{4}{3}\pi\left(\frac{l_k}{2}\right)^3 N_k \approx \frac{4}{3}\pi\left(\frac{l_{k+1}}{2}\right)^3 N_{k+1} \qquad \text{equation 2.4}$$

which gives equation 2.3.

I mention the significance of three different concepts already introduced in the model:

- *space filling*: the whole volume of the body is supplied by the network system;
- *service volume*: this is the unit volume which is supplied by each terminal element and it equals the volume of a sphere with the diameter equal to the length of the terminal element; because the system is *space filling*, it

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[1] This relationship originates from the scaling of the total transversal area between levels:

$$A_{k+1} = A_k^{-a} \quad \rightarrow \quad \pi \cdot r_{k+1}^2 = n \cdot \pi \cdot r_k^{2a} \quad \rightarrow \quad \frac{r_{k+1}}{r_k} = n^{-a/2}$$

where a=1 in case of *area preserving branching*.

follows that the sum of the service volumes equals the total volume of the body;

- *volume preserving*: this means that, at each level k, the sum of spheres with diameter equal to the length of the kth-element is approximately the same, independently of the kth-level.

Since the only units supplied by the network are the terminal ones and the energy is not dissipated throughout the system, given the size invariance of the terminal elements, the fluid is conserved at each level as it flows through the system and the flow rate (Q) at each level k is

$$Q_0 = N_k Q_k = n^k \pi r_k^2 v_k = n^N \pi r_N^2 v_N \qquad \text{equation 2.5}$$

where v_k is the fluid velocity at the *k-th* level. Consequently, because a space-filling fractal-like system with size-invariant terminal units imposes n^N proportional to the total body volume (Kozlowski & Konarzewski 2004), while the pressure drop (ΔP_N), r_N, l_N and v_N of the terminal elements are independent of body size, the pressure drop ΔP_k across each *k-th* element varies with n.

To derive the allometric equations, WBE model assumed that, since “fluid transports oxygen and nutrients for metabolism”, the flow rate (Q_0) is proportional to the metabolic rate (B). Here was one of the crucial reasonings of the model: if $B \propto M^a$, where M is body mass, then $Q_0 \propto M^a$ and, given equation 2.5,

$$n^N \propto M^a \qquad \text{equation 2.6}$$

It’s extremely important to underline that this holds true if and only if the body volume (V) is not proportional to M, otherwise it comes logically that $n^N \propto M$ (Kozlowski & Konarzewski 2004).

By solving equation 2.6 for N, it follows that the number of generations (N) scales logarithmically with body mass:

$$N = \frac{a \cdot ln(M / M_0)}{ln(n)} \qquad \text{equation 2.7}$$

where M_0 is a normalization scale for M. The total volume of fluid (V_F) in the system is

$$V_F = \sum_{k=0}^{N} N_k V_k = \sum_{k=0}^{N} \pi r_k^2 l_k n^k \qquad \text{equation 2.8}$$

whose geometric progression gives

$$V_F = \frac{\left(n\gamma\beta^2\right)^{-(N+1)} - 1}{\left(n\gamma\beta^2\right)^{-1} - 1} n^N V_N \qquad \text{equation 2.9}$$

Logically, since $V_F \propto n^N$ and $V \propto n^N$ (*space filling* assumption) so that $V_F \propto V$, again the notion that $n^N \propto M^a$ implies that, for the WBE model, V is not proportional to M.

However, the authors appeared to overcome this evidence by simply not considering it and continued in their reasoning.

Because $n\gamma\beta^2<1$ and N>>1, equation 2.9 can be well approximated as

$$V_F = \frac{V_0}{1 - n\gamma\beta^2} = \frac{V_N\left(\gamma\beta^2\right)^{-N}}{1 - n\gamma\beta^2} \qquad \text{equation 2.10}$$

Given the size invariance of the terminal units, it follows that

$$\left(\gamma\beta^2\right)^{-N} \propto V_{TOT} \propto M \qquad \text{equation 2.11}$$

and using this relation in equation 2.7, then gives

$$a = -\frac{ln(n)}{ln\left(\gamma\beta^2\right)} \qquad \text{equation 2.12}$$

that, given equations 2.2 and 2.3, equals 3/4. However, the last equation, on which all the scaling relationships predicted by the WBE model are based, appears simply inappropriate, because it assumes the proportionality between body mass and body volume, a condition that is excluded *a priori* because of the nature of the system (the *space filling constraint* with size invariant terminal elements implies $n^N \propto V$).

2.2 WBE model II (West et al. 1999)

With its original formulation (West et al. 1997), the WBE model could not be applied to the vascular system of plants, commonly simplified as a set of bundles containing cylindrical pipes running in parallel from roots to leaves (*pipe model theory*: Shinozaki et al. 1964a,b). In fact, without furcation, the number of elements at each generation would remain constant (Fig. 2.1), as well as the fluid velocity through each pipe.

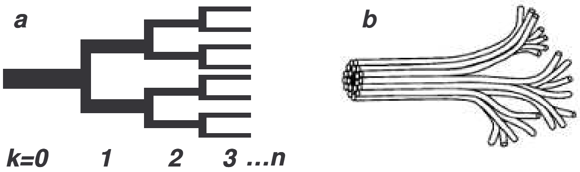

Fig. 2.1 One representation of a hypothetical branching network that satisfies the WBE model (from West et al. 1999). (a) Topology of plant branching network. (b) Symbolic representation of branch vascular structure, showing conducting tubes and non-conducting tissues (black).

Thus, the amount of fluid volume, although conserved throughout the system, would not be the same at each generation and, consequently, Q_0 in equation 2.5 varies with the number of branching generations, so the metabolism of the plant (B) cannot be proportional to the amount of fluid contained by the elements at the basal level 0 and the equations from 2.6 to 2.12 cannot be derived.

A new version of the WBE model was then proposed specifically for vascular plants (West et al. 1999), where, in order to derive the same fundamental scaling equations as the original model (West et al. 1997), and specifically to maintain the relation $Q_0 \propto B$, the important property has been introduced that conduit elements must taper among levels, i.e., their radii increase from the terminal to the basal level, and the ratio between levels is

$$\overline{\beta} = \frac{d_{k+1}}{d_k} = n^{-\bar{a}/2}$$

equation 2.13[2]

where d is the conduit radius. r_k became the branch radius and l_k the length of both conduit and branch of the *k-th* level.

A fourth fundamental assumption was introduced, that is

- the bio-mechanical constraints are uniform.

While in the first version of the model the condition of area preserving branching was essential for the principle of the conservation of fluid (equation 2.5), in the

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[2] This relationship originates from the scaling of the total transversal area of conduits between levels:

$$A_{k+1}^{CT} = \left(A_{k+1}^{CT}\right)^{-\bar{a}} \quad \rightarrow \quad \pi \cdot d_{k+1}^{2} = n \cdot \pi \cdot d_{k}^{2\bar{a}} \quad \rightarrow \quad \frac{d_{k+1}}{d_k} = n^{-\bar{a}/2}.$$

model for vascular plants it preserves the structure from buckling by maintaining the biomechanical constraints as uniform.

Because the rate of flow at the *k-th* level is $Q_k = n^N q_k$, where q_k is the conductance of the *k-th* element, the conservation of fluid among levels can be expressed as

$$Q_0 = N_k Q_k = n^N q_k = n^N q_N \qquad \text{equation 2.14}$$

Hence, the conductance, or its reverse, the resistance (R_k), of the *k-th* element must be conserved among levels, so, by using the Hagen-Poiseuille formula for laminar flows in cylindrical tubes (e.g., Tyree & Ewers 1991), it follows that

$$\frac{\pi d_k^4}{8\eta l_k} = \frac{\pi d_{k+1}^4}{8\eta l_{k+1}} \qquad \text{equation 2.15}$$

where η is the fluid viscosity.

Combining the last equation with equations 2.3 and 2.13, it follows that

$$\begin{aligned} \gamma &= \bar{\beta}^4 \\ n^{-1/3} &= n^{-2\bar{a}} \end{aligned} \qquad \text{equation 2.16}$$

which gives $\bar{a}$=1/6. This is an important feature of the whole transport system, because it strongly affects the behaviour of the whole-path resistance from the basal to the terminal level (R_{TOT}), which is given by

$$R_{TOT} = \sum_{k=0}^{N} R_k = \left[\frac{1 - \left[\left(n^{1/3} - 1 \right) \cdot l_{TOT} / l_N \right]^{(1-6\bar{a})}}{1 - n^{(1/3-2\bar{a})}} \right] \cdot R_N \qquad \text{equation 2.17}$$

where l_{TOT} is the total path length (i.e., tree height) and R_N is the hydraulic resistance of the terminal elements. When $l_{TOT} >> l_N$, the behaviour of R critically depends on the degree of conduit tapering, that is whether $\bar{a}$ is less than, more than or equal to 1/6. Particularly, in order to keep R constant with increased path length (l_{TOT}), $\bar{a}$ must be ≥1/6 (Fig. 2.2 left). However, the distance at which the contribution of conduit tapering actually becomes effective in minimizing the total hydraulic resistance strictly depends on the dimension of xylem conduits and the effect appears more relevant for shorter conduits (Fig. 2.3).

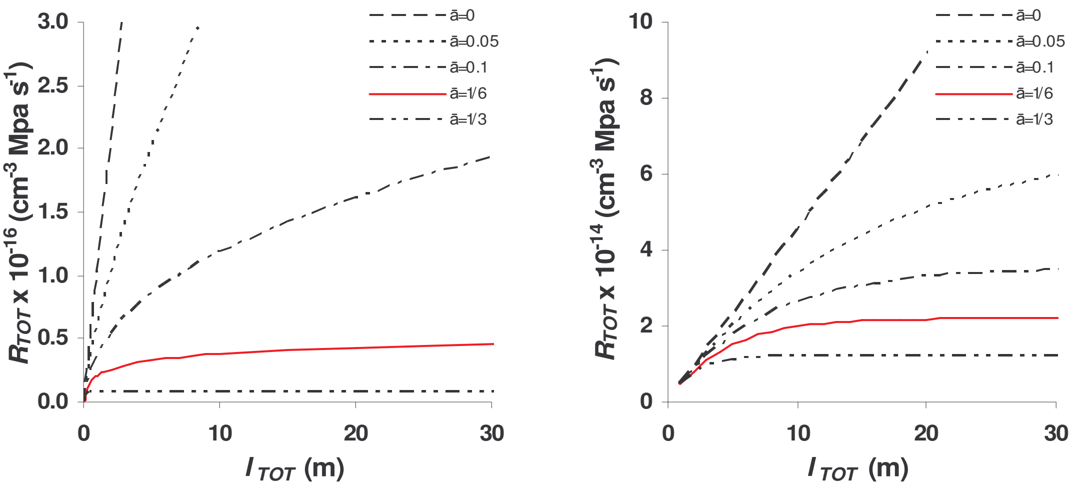


Fig. 2.2 Relationship between total hydraulic resistance (R_{TOT}) and total path length (l_{TOT}) for various degrees of conduit tapering ($\bar{a}$). (Left): length and conduit radius of elements scale with l_{TOT} as stated by the WBE model (West et al. 1999), with l_N=0.05 m and d_N=6 μm. (Right): the length of elements is kept constant (1-m-long) all along the pathway and d_N=6 μm (Becker et al. 2000).

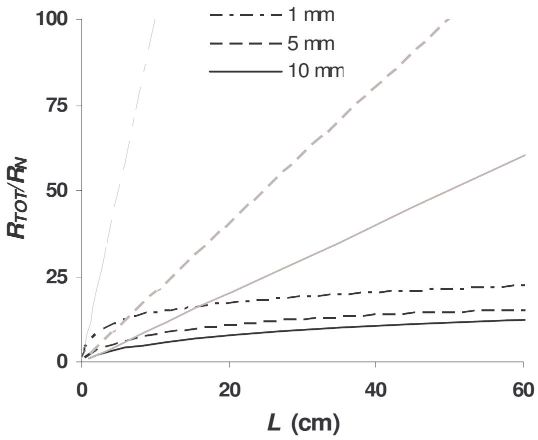


Fig. 2.3 The effect of conduit tapering in minimizing total hydraulic resistance (R_{TOT}) for different lengths of apical conduits. At a given distance from the apex (L), the difference between the relative total resistance (weighted to the resistance of the apical conduit, R_N) of a tapered tube (with $\bar{a}$=1/6) and an untapered tube with the same apical conduit dimension decreases with increasing length of the apical conduit (in the example, 1, 5, 10 mm).

Moreover, since the hydraulic resistance of a conduit is inversely proportional to its radius raised to the 4th power (cf. equation 2.15), conduit tapering can compensate for the effect of increasing path length even for fixed conduit lengths along the pathway. Becker et al. (2000) showed that the effect of $\bar{a}$ in making the resistance independent of path length is even more evident for fixed conduit lengths (Fig. 2.2 right), which seems quite reasonable as the conduit radii would increase exponentially from the terminal to the basal level.

Another consequence of the introduction of conduit tapering in the model is that, for a branching system to be area preserving, i.e., with *a*=1 in equation 2.2, there must be some non-conducting tissue compensating for the decrease in conduction areas with the increasing levels. Here, the question arising is: what is this non-conducting tissue? The authors wrote that “tubes are loosely packed in sapwood and there may be non-conducting heartwood providing additional mechanical stability”. This supposed more a realistic model of a tree, unlike the case of the *pipe model* (Shinozaki et al. 1964a,b) where all tubes have the same diameter ($\bar{a}$=0), hid a conceptual mistake, but was essential to the development of the model. Differently from the original version of the model (West et al. 1997), the introduced variable of conduit tapering ($\bar{a}$) hampered the proportionality between the amount of fluid contained in the system and body volume (equation 2.9). The logic of the new version (West et al. 1999) was simply that all the allometric relations can be derived by considering the total wood volume (V_W) of constant density, hence proportional to the total mass, *M*, so

$$V_W = \sum_{k=0}^{N} N_k V_k = \sum_{k=0}^{N} \pi r_k l_k n^k \qquad \text{equation 2.18}$$

and

$$V_W = \frac{\left(n\gamma\beta^2\right)^{-(N+1)} - 1}{\left(n\gamma\beta^2\right)^{-1} - 1} n^N V_N \qquad \text{equation 2.19}$$

The scaling relations could then be derived again starting from equation 2.6.

By re-examining the matter, a heavier tissue, which heartwood commonly is because of the higher concentrations of extractives compared to sapwood (e.g., Grabner et al. 2005), would counterbalance the effect of the overall increase in non-conducting tissues with increased levels that would cause an unstable top-heavy structure (McCulloh & Sperry 2005). The condition of homogeneous wood density was necessary to introduce the assumption that $V_W \propto V$.

Moreover, the amount of non-conducting tissues was also proposed as the key factor limiting tree growth. In fact, for a given total cross-section area

($A_k^{tot} = \pi r_k^2$,i.e., the preserved sum of transversal areas of the *k-th* branches), the conduction area (A^{CT}) is

$$A_k^{CT} = n_k \pi d_k^2 = A_N^{CT} \left(\frac{r_k}{r_N} \right)^{\frac{2(1+\bar{a})}{a}}$$ equation 2.20

where $A_N^{CT} = n_N \pi d_N^2$ is the conduction area of the terminal element. The proportion of conducting tissue relative to the total cross-section area is

$$f = \frac{A_k^{CT}}{A_k^{tot}} = n_N \left(\frac{d_N}{r_N} \right)^2 \left(\frac{r_k}{r_N} \right)^{\frac{2(1+\bar{a}-a)}{a}}$$ equation 2.21

This ratio must be ≤1 and limits to growth in both height and diameter are determined by the absence of non-conducting tissue at the basal level, i.e., f=0, and the maximum height (h^{MAX}) and basal radius (r_0^{MAX}) are given by

$$h^{MAX} = \frac{l_N}{1-n^{1/3}} \left(\frac{r_N^2}{d_N^2 n_N} \right)^{1/3(1+\bar{a}-a)}$$ equation 2.22

$$r_0^{MAX} = r_N \left(\frac{r_N^2}{d_N^2 n_N} \right)^{a/2(1+\bar{a}-a)}$$ equation 2.23

Equations 2.22 and 2.23 express the effect of the anatomical characteristics of the terminal elements and the tapering of xylem conduits ($\bar{a}$) in limiting tree growth without any respect to the possible limits imposed by the total amount of hydraulic constraints (i.e., the absolute value of the whole-path hydraulic resistance). Paradoxically, although the most fundamental assumption of the model is the minimization of hydraulic constraints, it predicts taller heights and larger basal diameters for trees hydraulically less efficient, i.e., with lower values of $\bar{a}$ (Fig. 2.4).

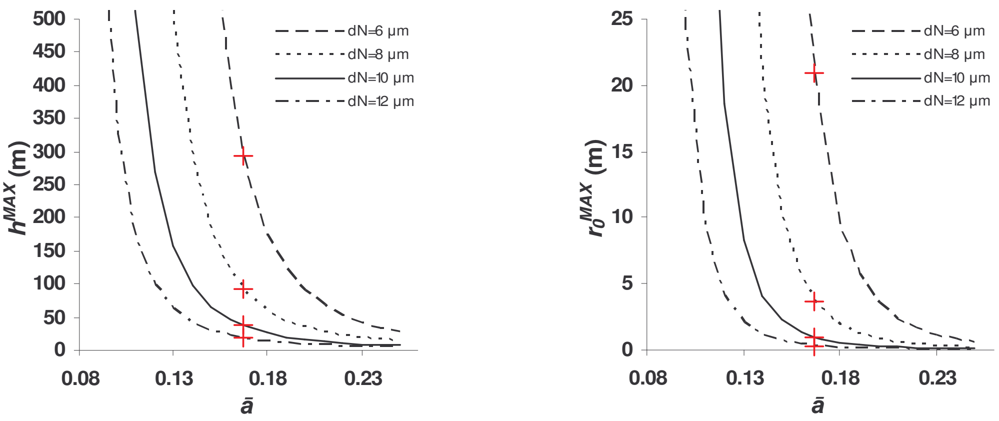


Fig. 2.4 WBE model predictions for the maximum height (h^{MAX}, left) and basal diameter (r_0^{MAX}, right) as functions of conduit tapering ($\bar{a}$) for various conduit radii (d_N) and given length (l_N) and radius (r_N) of the terminal elements. The red crosses are the prediction for the specific value of $\bar{a}_{WBE}$=1/6. In the example l_N=0.05 m ; r_N=0.5 mm, n=2 and N_N= 200, as in West et al. (1999).

To summarize, besides the constraint of the number of branching generations, the WBE model predicts limits to tree growth imposed by the tapering of xylem conduits under the availability of non-conducting tissues. Hence, conduit tapering has to be minimized while allowing the independence of hydraulic resistance from path length (tree height), regardless of the total hydraulic resistance sustained by the system, which could be elementary and arbitrarily stated as a species-specific feature.

2.3 Concluding remarks

This detailed review of the WBE theory highlights important controversies on which the model must stand. Besides the mathematical and logical weakness of some crucial points already discussed by others (Dodds et al. 2001; Kozlowski & konarzewski 2004; Makarieva et al. 2005; Chaui-Berlinck 2006), the novelty of our criticisms especially concern some conceptual and biological errors hidden behind the derived version for vascular plants, which are essential to derive the so-called universal quarter-power laws, and have never before been highlighted. However, although anatomically unrealistic, the WBE model for vascular plants had the huge merit of introducing the idea to plant ecologists that the tapering of xylem conduits can keep the whole-tree hydraulic resistance approximately constant as a tree grows taller. This concept conflicted with the widely-accepted notion that the increased hydraulic constraints with tree height limits tree growth (e.g., Ryan & Yoder 1997; Koch et al. 2004) and so the WBE model has frequently been ostracized and considered biologically irrelevant, especially because its restrictive assumptions do not permit the potential role of the hydraulic limitations to tree height to be considered.

3 Tapering of xylem conduits and hydraulic limitations in sycamore (*Acer pseudoplatanus* L.) trees

3.1 Summary

- Vertical conduit tapering is proposed as an effective mechanism to almost eliminate the increase in hydraulic resistance with increased height. Despite this potential role, very little is known about its changes during ontogeny.
- I analysed conduit tapering and stem morphology of young/small and old/tall individuals of *Acer pseudoplatanus* in the field as well as 3-yr-old grafted trees from both age classes. The distribution of hydraulic resistance along stems was also determined in a sub-sample of trees.
- Substantial conduit tapering was found in small trees (field-grown and grafted from both age classes), whereas values were lower in tall trees, indicating that tapering was a size-related, not an age-related process. Apical conduit diameters were larger in tall trees and were inversely correlated with the degree of tapering. Hydraulic resistance increased less than linearly with distance from the apex. Its scaling against distance was indistinguishable from the one predicted from anatomical measurements.
- Conduit tapering was an effective but partial mechanism of compensation for the increase in hydraulic resistance with tree height. Size-related changes in tapering and in apical conduit diameters may be explained by the combined need of reducing the build-up of hydraulic resistance while minimising the carbon costs of building vessel walls.

3.2 Introduction

The life history of trees is characterized by the enormous changes in body mass occurring during ontogeny. For example, the body mass of a giant sequoia may increase 12 orders of magnitude from seedling to large tree. The structural and physiological modifications that allow trees to achieve these dimensions while maintaining metabolism are currently the subject of research (e.g., Mencuccini 2002; Midgley 2003; Niklas & Cobb 2006). Trees typically display different rates of growth in height across ontogeny: the stem rapidly elongates during the first years or decades and thereafter its growth rate progressively declines (e.g., Sachs 1965; Lappi 1997). Recently, the old debate on whether the decline of growth rates of individual trees and that of forest net primary productivity is age-related or size-related (Yoder et al. 1994; Gower et al. 1996; Mencuccini & Grace 1996; Bond 2000; Binkley et al. 2002; Barnard & Ryan 2003; Phillips et al. 2003; Ryan et al. 2006; Martínez-Vilalta et al. 2007) has received renewed attention. While Day et al. (2001) suggested that reduced growth in ageing red spruce was primarily age-related, more recent papers (Mencuccini et al. 2005; Matsuzaki et al. 2005; Bond et al. 2007; Mencuccini et al. 2007) have instead presented evidence in favour of size-mediated effects. For instance, Mencuccini et al. (2005) showed that relative aboveground mass growth rates, net assimilation rates and several measures of leaf physiology and biochemistry declined in the field in trees of increased age and size, while the same parameters did not change significantly when measured across grafted seedlings of varying age but similar size. Because the grafts were obtained by grafting apical shoots taken from the same donor trees in the field onto a common rootstock, they putatively maintained the same meristematic age of the donor trees.

According to the hydraulic limitation hypothesis (Ryan & Yoder 1997; McDowell et al. 2002b), since the hydraulic resistance of a tube is proportional to its length (Hagen-Poiseuille formula; Tyree & Ewers 1991), the whole-plant hydraulic conductance should decrease as trees grow taller, because of the increased path length of water flow from roots to leaves. This would either result in lower water potentials, which would cause water stress conditions or elicit stomatal closure to prevent water stress. In either case, increased path length and resistance would lead to a reduced growth rate. Koch et al. (2004) suggested that the maximum height of 120-130 m achieved by coastal redwoods (*Sequoia sempervirens* Lamb.) is likely to be determined by the physical constraint of lifting water from roots to the top of the crown. Most tree species do not reach those heights, yet it is possible that hydraulic constraints play an important role in limiting their growth in height (Ryan & Waring 1992; Mencuccini & Grace 1996; Ryan et al. 1997).

Nevertheless, mechanisms of compensation are known to exist that help minimise the build-up of resistance with size, such as increasing allocation to fine roots (Magnani et al. 2000) decreasing leaf area/sapwood area ratios (McDowell et al. 2002a), or increasing sapwood permeability with age or size (Pothier et al. 1989).

According to Zimmermann's segmentation hypothesis (Zimmermann 1978; Zimmermann 1983), to preserve the whole organism from a hydraulic collapse, xylem conduits decrease in size from the stem base to the apices and are narrower at nodal zones in order to confine any embolism to the peripheral organs, which account for the most of the total hydraulic resistance and are subjected to the lowest xylem tension in the plant.

Recently, the architecture of the water transport system received renewed attention after the introduction of a theoretical model clarifying potential significance of the increase of conduit size from the apex to the stem base (West et al. 1999: West, Brown and Enquist or WBE model). According to the WBE model, evolution forced all plants to adopt a fractal-like body architecture to minimise the limitations imposed on water transport and to maximise their exchange surfaces with the external environment (Enquist 2003). The ideal WBE plant is composed of successive ideal branching levels and, as a consequence of its fractal-like geometry, its anatomical characteristics, such as conduit diameter (*d*), branch diameter (*D*) and branch/conduit length (*l*), scale among successive levels, *k* (proximal) and *k+1* (distal) as:

$$\frac{d_{k+1}}{d_k} = n^{-\bar{a}/2} \qquad \text{equation 3.1}$$

$$\frac{D_{k+1}}{D_k} = n^{-a/2} \qquad \text{equation 3.2}$$

$$\frac{l_{k+1}}{l_k} = n^{-1/3} \qquad \text{equation 3.3}$$

where $\bar{a}$, a and n are specific parameters independent of level k (see Tab. 3.1 for principal abbreviations).

CODE	MEANING
S1_4	S = Sycamore 1 = Age Class 1 4 = tree number
S4_98	S = Sycamore 4 = Age Class 4 98 = tree number
GS1_4	GS = Grafted Sycamore 1 = Age Class 1 4 = graft of tree number 4
GS4_98	GS = Grafted Sycamore 4 = Age Class 4 98 = graft of tree number 98
D	Stem diameter (cm)
Dh	Hydraulic diameter (µm)
L	Distance from the apex (cm)
b	Scaling exponent from: $Dh=a\cdot L^b$
d	Scaling exponent from: $R=c\cdot L^d$

Tab. 3.1 List of principal abbreviations and codes with relative meaning.

By simply assuming that (1) the network is volume filling (West et al. 1997), (2) the terminal branching unit is size invariant, (3) the biomechanical constraints are uniform and (4) the energy dissipated in fluid is minimised, the model predicts many general allometric scaling equations, which can be applied to all plants. Particularly, since the total hydraulic resistance (Z) is

$$Z = \sum\nolimits_{k=0}^{N} Z_k = \left[\frac{1-\left[\left(n^{1/3}-1\right)\cdot L/l_N\right]^{(1-6\bar{a})}}{1-n^{(1/3-2\bar{a})}} \right]\cdot Z_N \qquad \text{equation 3.4}$$

where L is total length of the connected k levels and l_N and Z_N are the length and the hydraulic resistance (calculated with the Hagen-Poiseuille formula: see Tyree & Ewers 1991) of the terminal unit-tube, it can be mathematically demonstrated that, for $L >> l_N$, the hydraulic resistance critically depends on $\bar{a}$ and becomes a nearly constant value independent of path length with $\bar{a}$ equal or higher than 1/6 (Becker et al. 2000). Moreover, approximating

$$L \approx \frac{l_0}{1-n^{-1/3}} \qquad \text{equation 3.5}$$

where l_0 is the length of the basal level, WBE model predicts:

$$d \propto L^{3\bar{a}/2} \qquad \text{equation 3.6;}$$

$$L \propto D^{2/3a} \qquad \text{equation 3.7;}$$

$d \propto D^{\bar{a}/a}$ equation 3.8.

The scaling exponents of equations 3.6 and 3.7 are known to be rather variable. Anfodillo et al. (2006) demonstrated that the estimates for these scaling exponents critically depended on the tree height, while Niklas (1995) reported the exponent for the equation 7 to vary during ontogeny with the value of 2/3 typically found only in actively-growing, mature trees.

Xylem conduits have often been reported to increase in size from the apex downwards and from the stem pith outwards (e.g., Zimmerman 1983; Gartner 1995; Meinzer et al. 2001; Nijsse et al. 2001; Martínez-Vilalta et al. 2002; McElrone et al. 2004; McCulloh & Sperry 2005), but only recently it has been found that the tapering of xylem conduits follows a similar trajectory in plants of different species (Anfodillo et al. 2006). This study, as well as others (Weitz et al. 2006; Coomes et al. 2007), have demonstrated that the degree of conduits tapering is, at least for juvenile trees, very similar to that predicted by the WBE model. What happens in older trees is less clear. Anfodillo et al. (2006) suggested that there may be a decline in the degree of conduit tapering with increased height. In other words, they suggested that the tapering coefficient $\bar{a}_{WBE}$ may decline from its optimal value of 0.167 in juvenile trees to smaller values in older trees. Particularly, at least for trees with columnar posture, the deviation from the optimal conduit tapering is typically given by a flattening of the profile of conduit dimensions towards the stem base (Becker et al. 2003; James et al. 2003), maybe a consequence of the achieved maximum conduit size (Anfodillo et al. 2006).

This is an interesting possibility, since it would provide a theoretical linkage between the hydraulic limitation hypothesis (Ryan & Yoder 1997) and the WBE model (West et al. 1999), whereby hydraulic constraints would begin to affect tree growth once the tapering of xylem conduits is reduced, such that negative effects of increased path length cannot be avoided any longer.

This hypothesis has not been systematically tested so far. Mencuccini (2002) reviewed published data and concluded that the hydraulic conductance of stem segments of maple and pine trees scaled with their diameter as stated by WBE model, while the whole-tree hydraulic conductance of the same species scaled with the diameter at breast height (*dbh*) with a smaller exponent than WBE model. Similarly, the hydraulic resistance of stem segments (Yang & Tyree 1993) was reported to decrease with their distance from the apex, in agreement with the WBE model (Enquist 2003). In addition to the lack of systematic tests of this hypothesis, complete analyses of the distribution of hydraulic resistance as well as anatomical characters along stems are also rare (e.g., Zimmermann 1978; Ewers & Zimmermann 1984; Tyree 1988).

In addition to the degree of tapering along a stem down from the apex, the hydraulic efficiency of a vascular system can be affected by additional variables. First, no matter how rapidly conduit diameter changes with distance from the apex, the absolute value of conduit diameter *in primis* will affect conductance. Secondly, McCulloh et al. (2003) and McCulloh & Sperry (2005) proposed that, to maximise the structural investment in xylem tissues, a network should have a minimum number of wide conduits at the base feeding an increasing number of narrowing conduits distally. These authors showed that conduit furcation occurs in some cases but also that, in cases in which the conduits provide mechanical support to the plant (e.g., in tree stems), conduit furcation is limited or absent.

The aim of this work is to provide an answer to some important questions regarding the tapering of xylem conduits. Firstly, by systematically measuring the conduit diameters in young and old trees, I tested the hypothesis that the degree of conduit tapering does not change during ontogeny in sycamore (*Acer pseudoplatanus* L.), a diffuse-porous angiosperm tree. Moreover, to understand whether changes in conduit tapering were size- or age-related, I also compared those donor trees in the field with grafted plants obtained from the same trees and grown outside our greenhouses. Lastly, I combined anatomical and hydraulic measurements to investigate the distribution of conduit diameters and hydraulic resistances along branches and stems to determine whether hydraulic conductance of these organs was primarily affected by conduit tapering, apical conduit diameters, conduit furcation or a combination of the three.

3.3 Materials and methods

3.3.1 Site and plant material

The study area was located at Cramond, Almond Valley, in the western part of Edinburgh (55^{o}58'42" N, 3^{o}16'09" W). The study woodland is comprised of two dominant species, i.e., sycamore (*Acer pseudoplatanus* L.) and ash (*Fraxinus excelsior* L.), and a number of minor species as well as active regeneration (sycamore and ash) and a dense understorey of shrubs and ground flora. Sycamore trees were located mainly in an area close to the coast at the bottom of the steep slope of the valley.

Nine sycamore trees, five young (age ≤ 5 years and height ≤ 5 m: hereafter *S1*) and four old (age > 130 years and height ≈ 25 m: hereafter *S4*) were selected from the area. Apical shoots of *S1* and shoots from lateral branches from the top third of the crown of *S4* were grafted onto 2-yr-old rootstocks (hereafter *GS1* and *GS4*) in 2003 to separate size- and age-related effects on plant structure and physiology. More details on grafting protocol and growing conditions are given in Mencuccini et al. (2005) and Hamid (2006). Analysed trees maintained the same codification as in Mencuccini et al. (2005) and Hamid (2006).

Wood from *S4* trees was sampled by extracting wooden cores along the main stem (two cores from diametrically opposite points every 3.5 m along the stem, from the top of the canopy to the tree base), paying attention to avoid zones with tension wood and knots. In addition, the whole topmost branch (≈ 3 m long) was also cut. *S1*, *GS1* and *GS4* trees were felled. Top branches and whole felled trees were sampled along the longitudinal axis in at least 8-10 points, where cross-sections were cut. For wooden disks with diameters larger than ≈ 2 cm, 2-3 sub-samples of the last 3-4 annual rings were cut from the whole cross-section, again avoiding zones with tension wood.

3.3.2 Anatomical measurements

For each plant, measurements of stem diameter (*D*) were taken at different distances from the apex, where cross-sections and cores were extracted. Wooden samples (either from cores or cross-sections) were then embedded in paraffin (Tab. 3.2, Anderson & Bancroft 2002). Slides of 10-12 μm were cut with a rotatory microtome and stained with safranine (1% in water; see Tab. 3.3) and fixed permanently with Eukitt (Bioptica, Milan, Italy).

Reagent	Length of time (min.)
Ethanol 70%	120
Ethanol 70%	120
Ethanol 90%	90
Ethanol 90%	90
Ethanol 95%	90
Ethanol 100%	90
Ethanol 100%	90
BioClear	90
BioClear	90
BioClear	90
Paraffin (65°C)	120
Paraffin (65°C)	120

Tab. 3.2 Stages of the embedding process. Samples were immersed in successive containers filled with ethanol solution with different concentrations in water (to provide a progressive dehydratation of samples), BioClear (an organic solvent) and liquid paraffin.

Reagent	Length of time (min.)
BioClear	20
Ethanol 100%	20
Safranine 1%	10
Water	10
Water	10

Tab. 3.3 Stages of the staining process. Samples were immersed in successive containers filled with BioClear (to eliminate the remaining paraffin inside the tissues), ethanol (to dehydrate the tissues), safranine (to colour the tissues) and water (to rinse tissues and eliminate superfluous dye).

Digital images of the sections were taken at 100x magnification using a light microscope (Nikon Eclipse80i). Lumen areas were measured by WinCell software (Régent Instruments Inc., Sainte-Foy, QC, Canada). In order to eliminate conduit endings, only those conduits with a lumen diameter greater than half the diameter of the largest conduit (James et al. 2003) were chosen to estimate the hydraulically weighted diameter, *Dh*, of the last annual ring (or the last few rings in the case of missing rings):

$$Dh = \frac{\Sigma d_n^5}{\Sigma d_n^4} \qquad \text{equation 3.9}$$

where d_n is the n^{th} conduit diameter (Sperry et al. 1994).

To determine the number of images necessary to obtain a certain confidence level in the hydraulic diameter, I measured all the cells in the last annual ring for a sample of wooden disks of different diameters. A Montecarlo re-sampling approach was then used to estimate the mean hydraulic diameter based on different numbers of analysed images. Using this approach I estimated that at least 25-30 images of the last annual ring must be analysed to estimate *Dh* with a standard deviation equal to 20% of the mean (Fig. 3.1). Therefore, I

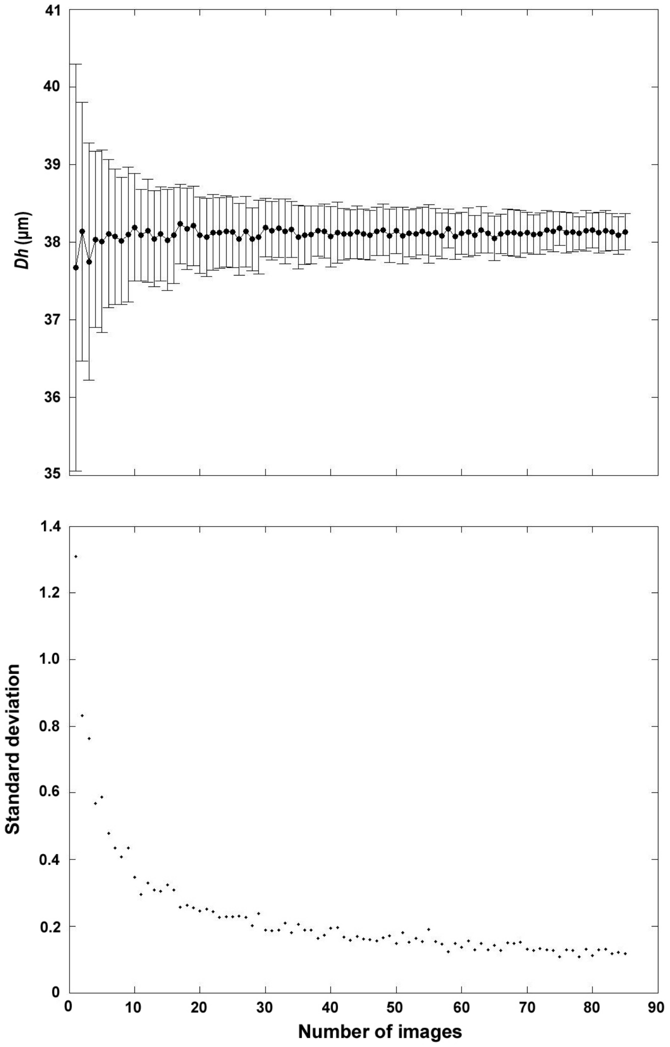


Fig. 3.1 Example of one Montecarlo analysis for the determination of the number of random pictures to be taken in order to estimate a reliable value of the hydraulically weighted diameter *Dh*. A) Changes in estimated mean *Dh* and standard deviation of the mean in relation to the number of images analysed; B) Standard deviation plotted as proportion of the mean in relation to the number of images analysed. The analysis refers to the last ring of a wooden disk of 21.1 mm of diameter taken at 86 cm from the apex of the top branch of S498 tree.

analysed the whole of the last ring for the small wooden disks and cores and 25-30 images of the last ring for the larger disks. On average, I measured ≈1,600 cells per section, ≈ 13,300 cells per plant and a total of ≈ 358,500 cells.

The parameter $\bar{a}$ was derived from equation 3.6. Since this estimate critically depends on the tree height and because of the WBE approximation for tree height (equation 3.5), I applied a correction factor of 0.794 to the scaling exponent of equation 3.6, i.e., for an ideal WBE tree of 20 levels (cf., Anfodillo et al. 2006). In practice, I multiplied by 0.84 (i.e., 2/3/0.794) the scaling exponent estimated from the relationship between the mean hydraulic diameter (*Dh*) and the distance from the tip (*L*) (Anfodillo et al. 2006). This protocol led to a conservative estimate of $\bar{a}$, that is, values of $\bar{a}$ were perhaps slightly underestimated, but surely never overestimated.

In addition, the vertical profiles of measured hydraulic diameters of each tree were used to estimate the hydraulic resistance R_{TOT} of a tapered pipe spanning the height of each tree, but using the actual measured values, as opposed to the theoretical scaling exponent derived by WBE. This approach assumes no conduit furcation (McCulloh et al. 2003) and an ideal pipe model system with tapered resistances in series. Because of the nonlinearity in the relationship of conduit diameter (and hydraulic resistance) with distance from the apex, calculations are sensitive to the length of the segments employed. Hence, segments of 0.1 cm length were employed in all cases.

3.3.3 Hydraulic measurements

I measured the water flow rate out of the apex, *F* ($\mathrm{cm^3\ s^{-1}}$), of nine plants (two *GS1*, two *GS4*, three *S1*, two *S4*), while applying a known pressure head, *P* (MPa), from the base to the cut apex (the cut was made ≈2 mm below the bud scar). Secondary apices were cut as well. Degassed distilled water was made to pass through the xylem under a positive pressure, *P*, generated as a water pressure head of 2.5 m above the stem. In order to achieve equal *P* from the base to every apex, the stem was immersed in a container, with the same water level as the water in the balance (c.f., Tyree et al. 1991). The hydraulic resistance, *R* ($\mathrm{MPa\ s\ cm^{-3}}$), was computed as *P*/*F*. The variation of *R* with the path length, *L* (cm), was estimated by progressively shortening the stem from its base after each measurement session and re-attaching the tubing to the new base.

3.3.4 Statistical analyses

The scaling parameters of the allometric equations were determined from pairwise comparisons of $\log_{10}$-transformed data. Using reduced major axis (RMA) analysis, the scaling exponents and allometric constants were identified as the regression slopes (αRMA) and *y*-intercept (βRMA), respectively. Regression coefficients, their 95% confidence and prediction intervals, were computed by standard methods (Sokal & Rohlf 1981) using a bootstrap procedure with 100,000 replications (Davison & Hinkley 1997).
Analyses of variance among treatments were carried out by using GLM procedure in *SAS 8.02* (SAS Institute, Cary, N.C., USA).

3.4 Results

3.4.1 Anatomy

The morphology of the stem was quite variable among plants. The analysis of stem tapering showed that the variation of stem length, i.e., the distance from the apex (*L*), with stem diameter (*D*) was well described by a power function (Tab. 3.4). *L* explained 80-98% of the total variance of *D*. The scaling exponent ranged from 1.555 to 5.446 and hence was always higher than the commonly reported exponent of 2/3 (Niklas 1995). However, in the tallest/oldest trees, the increase of *L* with *D* was lower than in the smallest ones, although *S4* and *S1* trees did not statistically differ from one another (overall ANOVA: F=2.78; p=0.077; *S4* was statistically different from *GS1* and *GS4*, but not from *S1*, Duncan test). Moreover, the stem tapering of the top branches of *S4* trees did not statistically differ from any of the other treatments (overall ANOVA: F=2.35; p=0.108).

		Model: $Log_{10}L=: Log_{10}a'+b' Log_{10}D$				Model: $Log_{10}Dh=: Log_{10}a''+b'' Log_{10}D$			
ID	***N***	**r^2**	***a'***	***b'***	***b' 95% CI***	**r^2**	***a''***	***b''***	***b'' 95% CI***
GS1_3	10	0.928	-1.063	3.339	2.143÷3.956	0.972	1.101	0.644	0.493÷0.710
GS1_4	9	0.946	-0.546	2.514	1.608÷2.926	0.798	1.338	0.337	0.117÷0.430
GS1_6	9	0.928	0.416	1.865	1.225÷2.141	0.889	1.324	0.382	0.222÷0.448
GS1_7	11	0.989	-0.515	2.423	2.214÷2.581	0.735	1.183	0.499	0.091÷0.645
GS1_8	10	0.887	-2.844	5.446	3.210÷6.681	0.822	0.902	0.868	0.455÷1.091
GS1_10	10	0.947	-0.422	2.524	1.753÷2.957	0.875	1.237	0.513	0.388÷0.635
GS4_7	10	0.943	-1.058	3.000	2.148÷3.538	0.927	1.116	0.505	0.359÷0.597
GS4_9	11	0.938	-0-083	2.183	1.684÷2.541	0.874	1.288	0.400	0.254÷0.479
GS4_98	10	0.956	-0.444	2.417	1.837÷2.687	0.823	1.119	0.5713	0.178÷0.720
GS4_99	10	0.953	-1.669	4.115	2.965÷4.794	0.870	1.136	0.5939	0.450÷0.735
S1_4	6	0.898	-0.111	2.007	1.538÷3.754	0.757	1.342	0.313	0.171÷0.766
S1_5	12	0.978	-0.186	2.062	1.774÷2.301	0.832	1.266	0..374	0.173÷0.480
S1_6	8	0.957	-0.199	2.170	1.537÷2.552	0.980	1.288	0.332	0.268÷0.366
S1_7	11	0.798	-0.972	2.643	1.620÷4.119	0.906	1.289	0.379	0.312÷0.525
S1_8	15	0.956	-0.511	2.200	1.659÷2.498	0.846	1.230	0.370	0.207÷0.441
S4_7	20	0.949	-0.500	1.692	1.430÷1.969	0.880	1.355	0.213	0.171÷0.254
S4_7 (branch 1)	15	0.928	-0.719	1.881	1.410÷2.218				
S4_7 (branch 2)	16	0.808	-1.098	2.428	1.460÷3.281				
S4_9	22	0.930	-0.151	1.586	1.377÷1.795	0.823	1.367	0.185	0.153÷0.211
S4_9 (branch 1)	17	0.897	-0.173	1.592	1.174÷1.913				
S4_9 (branch 2)	11	0.848	-0.998	2.404	1.488÷3.053				
S4_98	13	0.976	-0.208	1.592	1.406÷1.786	0.892	1.372	0.204	0.173÷0.245
S4_98 (branch	8	0.976	-0.344	1.708	1.386÷1.915				
S4_99	13	0.985	-0.357	1.555	1.451÷1.698	0.912	1.347	0.232	0.193÷0.287
S4_99 (branch	9	0.984	-0.313	1.510	1.322÷1.656				

Tab. 3.4 Anatomical and morphological features of xylem architectural scaling. Stem tapering, i.e., variation of path length (*L*) with stem diameter (*D*) (left panel), and variation of hydraulic diameter (*Dh*) with *D* (right panel): estimates for the log$_{10}$-transformed parameters *a'* and *b'*, *a''* and *b''*, and 95% confidence intervals for *b'* and *b''*.

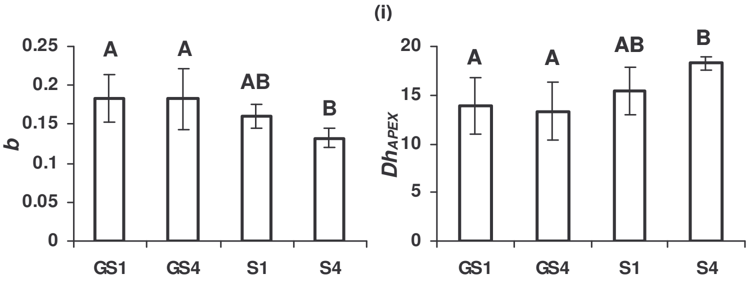
In all plants, the hydraulically weighted vessel diameter (*Dh*) increased from the apex to the stem base. The best fitting describing the variation of *Dh* with path length (i.e., distance from the apex, *L*) was obtained by using a power function, where *L* explained 75-98% of the total variance of *Dh*. The scaling exponents (*b*) ranged from 0.124 to 0.236. The mean value of $\bar{a}$ (i.e., the scaling of xylem conduit diameters among successive levels, West et al 1999) was on average smaller than the prediction of WBE model (mean $\bar{a}$=0.14; s=0.027), although only seven out of 19 values were significantly smaller than 0.167 (Tab. 3.5). Among these seven, four were *S4* trees. The smallest values of *b* (and $\bar{a}$) were those of the old/tall trees (*S4*), which significantly differed from those of the young and old grafted (*GS1* and *GS4* respectively), but not from those of the young/field (*S1*) (Fig. 3.2(i) left). All *S4* trees showed a discontinuity along their

Dh profiles: in fact, in the first few meters from the apex, *Dh* increased with *L* more smoothly and more slowly than further down along the stem to the base (Fig. 3.3).

		Model: $Log_{10}Dh=$: $Log_{10}a+b\ Log_{10}L$						
ID	***N***	**r^2**	***a***	***b***	***b* 95% CI**	***ā***	***ā* 95% CI**	***Dh$_{APX}$* (µm)**
GS1_3	10	0.974	1.306	0.193	0.175÷0.241	0.162	0.147÷0.202	24.416
GS1_4	9	0.938	1.411	0.134	0.069÷0.148	0.112	0.058÷0.124*	29.489
GS1_6	9	0.986	1.238	0.205	0.169÷0.217	0.172	0.142÷0.182	16.968
GS1_7	11	0.766	1.289	0.206	0.041÷0.257	0.173	0.034÷0.216	24.486
GS1_8	10	0.980	1.355	0.159	0.140÷0.175	0.134	0.118÷0.147*	22.479
GS1_10	10	0.914	1.323	0.203	0.171÷0.299	0.171	0.144÷0.251	26.247
GS4_7	10	0.968	1.294	0.168	0.155÷0.207	0.141	0.130÷0.174	22.651
GS4_9	11	0.821	1.304	0.183	0.104÷0.242	0.154	0.087÷0.203	25.257
GS4_98	10	0.906	1.224	0.236	0.107÷0.281	0.199	0.090÷0.236	21.252
GS4_99	10	0.905	1.377	0.144	0.119÷0.212	0.121	0.100÷0.178	25.355
S1_4	6	0.946	1.359	0.156	0.110÷0.218	0.131	0.092÷0.183	29.788
S1_5	12	0.915	1.300	0.182	0.099÷0.210	0.152	0.083÷0.176	22.444
S1_6	8	0.949	1.318	0.153	0.131÷0.201	0.129	0.110÷0.169	27.770
S1_7	11	0.893	1.429	0.143	0.116÷0.203	0.120	0.097÷0.170	28.950
S1_8	15	0.942	1.316	0.168	0.119÷0.189	0.152	0.100÷0.159*	23.034
S4_7	21	0.930	1.413	0.129	0.111÷0.145	0.108	0.093÷0.122*	24.794
S4_7 (below junction)	7	0.994	1.138	0.221	0.206÷0.235	0.186	0.173÷0.197	
S4_7 (branch 1)	15	0.871	1.446	0.106	0.071÷0.126	0.089	0.060÷0.106*	24.794
S4_7 (branch 2)	16	0.975	1.438	0.101	0.094÷0.111	0.085	0.079÷0.093*	21.762
S4_9	23	0.857	1.372	0.124	0.101÷0.150	0.100	0.085÷0.126*	30.790
S4_9 (below junction)	7	0.887	1.219	0.179	0.142÷0.327	0.150	0.119÷0.275	
S4_9 (branch 1)	17	0.715	1.438	0.081	0.059÷0.103	0.068	0.050÷0.086*	30.790
S4_9 (branch 2)	11	0.888	1.421	0.105	0.070÷0.123	0.088	0.059÷0.103*	22.266
S4_98	14	0.924	1.391	0.132	0.117÷0.161	0.111	0.098÷0.135*	29.244
S4_98 (below junction)	7	0.856	1.146	0.212	0.113÷0.267	0.178	0.095÷0.224	
S4_98 (branch)	8	0.829	1.440	0.099	0.035÷0.120	0.083	0.029÷0.101*	29.244
S4_99	14	0.963	1.403	0.148	0.133÷0.168	0.126	0.112÷0.141*	32.763
S4_99 (below junction)	6	0.775	1.332	0.172	0.040÷0.247	0.145	0.034÷0.207	
S4_99 (branch)	9	0.964	1.435	0.126	0.110÷0.150	0.106	0.092÷0.126*	32.763

Tab. 3.5 Scaling features of xylem conduits. Variation of hydraulically weighted diameter (*Dh*) with path length (*L*): estimates for the log$_{10}$-transformed scaling parameters *a* and *b*, the WBE parameter *ā* and 95% confidence intervals for *b* and *ā*. *Dh* of the most distal section is also reported.

Consequently, conduits of the top branches of *S4* trees tapered significantly less than *GS1*, *GS4* and also *S1* trees, over a similar distance of about 3-4 m (Fig. 3.2(ii) left). *Dh* at the apex was estimated for each plant from the relation $Dh=aL^b$ at the distance of 0.1 cm from the stem tip. *S4* trees significantly differed from *GS1* and *GS4* but not *S1* when the analysis was extended to the whole tree (Fig. 3.2(i) right; overall ANOVA: F=3.22; p=0.053; *S4* was statistically different from *GS1* and *GS4*, but not from *S1*, Duncan grouping test) but significantly differed from all small plants (i.e., including *S1*) when the analysis was limited to the top branches (overall ANOVA: F=13.89; p<0.001, Fig. 3.2(ii) right). Patterns between trees were similar for both *b* and *Dh*.



(ii) *analyses of S4 trees limited to top branches*

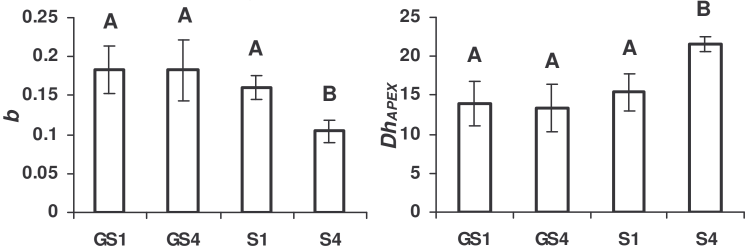


Fig. 3.2 Analyses of variance of the slope *b* of the relation $Log_{10}Dh=Log_{10}a+b\cdot Log_{10}L$ (left-hand side of the graph) and *Dh* at the apex (calculated from the same equation for a distance of 0.1 cm from the tip, right-hand side of the graph). Two different analyses were done: (i) considering the whole *Dh* profiles of all plants of all treatments (N=19; p(*b*)=0.037; p(Dh_{APEX})=0.053) and (ii) considering only the *Dh* profiles of the first 3 m of distance from the apex, ie., the whole profile for plants of treatments *GS1*, *GS4* and *S1* and the *Dh* profiles of the top six branches in *S4* (N=21; p(*b*)<0.001; p(Dh_{APEX})<0.001). Duncan grouping is shown for both analyses: groups with different letters differ significantly at 95%.

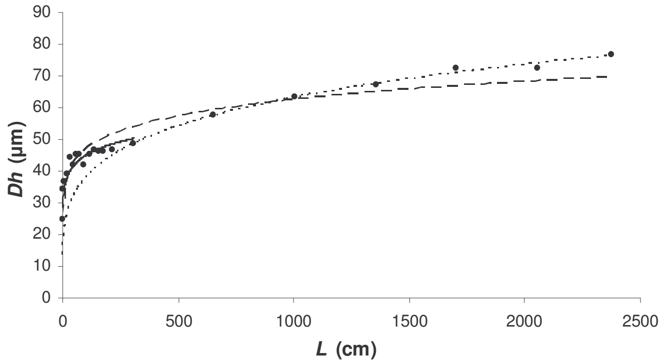


Fig. 3.3 Example of the variation of the hydraulically weighted diameter (*Dh*) with distance from apex (*L*) in a tall tree (*S4_7*). Dashed line describes the overall power-law tapering of xylem conduits from the apex to the bottom (RMA *b*=0.129); the continuous line describes the tapering of xylem conduits of the first 3 m from the top apex (RMA *b*=0.106); the dotted line describes the tapering along the main stem (from 3 m from top apex to the bottom of the stem; RMA *b*=0.221).

In addition, Dh_{APEX} was found to be inversely proportional to b (Fig. 3.4), suggesting a trade-off mechanism between tapering of conduits and size of xylem cells at the apex. This relationship remained when actual ,measured Dh_{APEX} data were regressed against b (data not shown).

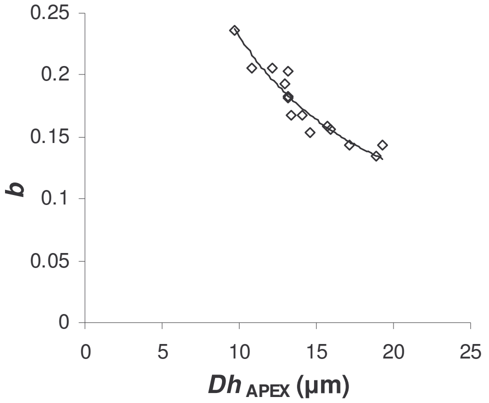


Fig. 3.4 Relationship between the hydraulically weighted diameter at the apex (Dh_{APEX}, estimated for each plant from the equation $Dh=aL^b$, with L=0.1 cm) and the scaling exponent b, describing the rate of tapering of xylem conduits. The equation of the fitting curve (RMA regression) is $b=1.648\cdot Dh_{petiole}^{-0.8525}$; r^2=0.897.

Moreover, the estimated R_{TOT} of the theoretical tapered pipe for the *S4* trees was significantly larger than for small trees (*GS1*, *GS4* and *S1*), which did not significantly differ from one another (Duncan grouping test; overall ANOVA: F=21.56; p<0.001).

3.4.2 Hydraulics

The whole-shoot hydraulic resistance (R) increased from the apex to the stem base. Most of the resistance was located towards the apex, where xylem conduits tapered more sharply. Consequently, R increased less than linearly with path length (L), and the best function describing this variation was again a power function (Fig. 3.5). L explained 83-98% of the total R variance and the scaling exponents ranged from 0.293 to 0.644, i.e., they were significantly lower than 1.00 (Tab. 3.6). The increase of R with L was reduced below isometry by tapering of conduits, i.e., the increased tapering of xylem conduits resulted in an increase of R with L lower than isometric (Fig. 3.6).

Moreover, I estimated the theoretical variation of *R* with *L* for an ideal tube (composed of successive 0.1-cm-long levels) with anatomical features obtained from the empirical measurements in the analysed plants (Dh_{APEX} and *ā*). The scaling exponents (*d*) of the fitting curve for the hydraulic measurements matched well the scaling exponents (*d'*) of the fitting curve for the theoretical *R* of ideal tubes of known anatomical characteristics (Fig. 3.7). The RMA slope of the linear relationship between predicted and measured scaling of resistances was 0.831 (with confidence limits including 1.00).

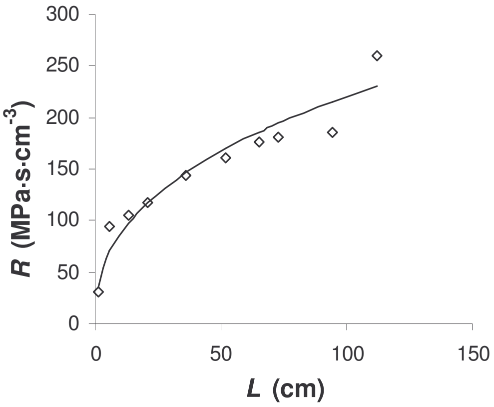


Fig. 3.5 Example of the variation of the whole-path hydraulic resistance (*R*) with path length (distance from tip, *L*) for one grafted plant (*GS4_99*). The continuous line is the fitting curve, whose RMA regression equation is: $R=35.075 \cdot L^{0.399}$; $r^2=0.954$. Each point represents an estimate of *R* obtained by measuring water flow through the branch or stem with a constant water pressure. The branch or stem was then shortened at the base and the measurement repeated in consecutive cycles.

ID	N	*Model:* $\mathbf{Log_{10}R=: Log_{10}c+d\ Log_{10}L}$				**Estimates from anatomy**
		r^2	*c*	*d*	*d 95% CI*	*d*
GS1_4	8	0.955	1.232	0.524	0.432÷0.729	0.486
GS1_8	10	0.841	1.153	0.467	0.223÷0.571	0.417
GS4_7	10	0.957	1.523	0.395	0.298÷0.517	0.380
GS4_99	10	0.954	1.545	0.399	0.266÷0.441	0.461
S1_4	12	0.969	1.045	0.423	0.370÷0.476	0.406
S1_5	10	0.978	1.371	0.319	0.286÷0.389	0.331
S1_8	7	0.829	1.320	0.293	0.169÷0.493	0.314
S4_98	10	0.907	1.213	0.644	0.525÷0.956	0.586
S4_99	8	0.975	1.288	0.463	0.420÷0.525	0.514

Tab. 3.6 Variation of whole-shoot hydraulic resistance (*R*) with path length (i.e., distance from apex, *L*). In the last column, estimates are given of the scaling exponent *d* for the hydraulic resistance of the same branches and stems estimated from anatomical measurements. Resistance was estimated assuming ideal tubes made by successive 0.1-cm-long levels, with *ā* and *Dh* at the apex as measured in the sample plants.

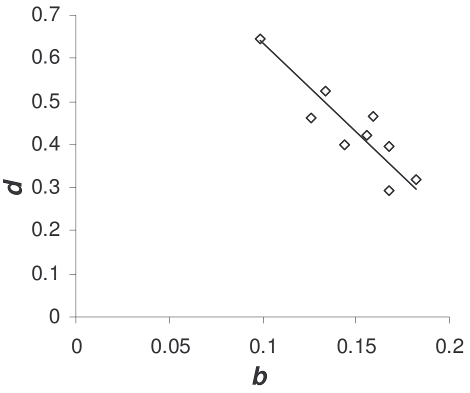


Fig. 3.6 Comparison between the scaling exponent *d* of the equation $R=c*L^d$ (resistance-distance relationship) and the scaling exponent *b* (hydraulic diameter-distance relationship) of the equation $Dh=a*L^b$. RMA regression: $d=-4.147\cdot b+1.052$; $r^2=0.789$. Each point represents a different branch or stem.

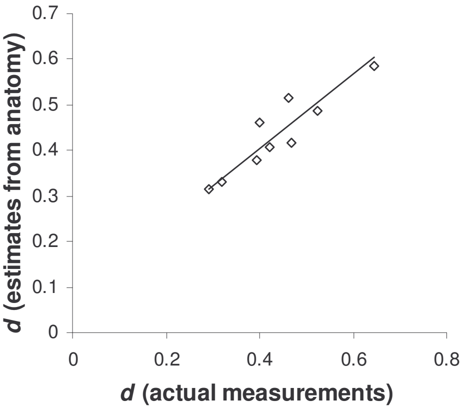


Fig. 3.7 Comparison between the scaling exponent *d* of the equation $R=c*L^d$ assessed by real measurements (*x*-axis; cf., Fig. 6) and the scaling exponent of the same equation but where *R* was estimated applying the Hagen-Poiseuille formula (Tyree & Ewers, 1991) to an ideal tube with the same length and anatomical features (Dh_{APEX} and $\bar{a}$) measured in real plants (*y*-axis). (RMA $y=0.07+0.831\cdot x$; $r^2=0.844$; 95% CI: $-0.071<\alpha<0.129$; $0.707<\beta<1.217$). Each point represents a different branch or stem.

3.5 Discussion

3.5.1 Age versus size

The results revealed that, in sycamore (*Acer pseudoplatanus* L.), the scaling of xylem anatomical features did not change as a consequence of increased tree age *per se*, since the scaling found in *GS4* did not differ from the one found in *GS1* but was significantly different form that of *S4*. In other words, the scaling relationships for the small trees (*S1, GS1* and *GS4*) did not differ from one another, despite differences in the growing conditions (forest for *S1* versus common garden for *GS1* and *GS4*) and meristematic ages (young for *S1* and *GS1* versus putatively old for *GS4*), whereas the scaling in *GS1* and *GS4* were found to be significantly different from that recorded in *S4*, suggesting that either size or environment, but not age, were responsible for these differences. When the analyses were limited to the distance of about 3 m from the apex, i.e., a distance similar to the total height/length of the field saplings, the grafted seedlings and the length of a major branch in the old trees, conduit tapering was found to be similar among all the analysed plants, except for the *S4* top branches, whose conduit tapering was significantly slower than in the small plants, irrespective of their meristematic age and whether grown in the field or the common gardens. Moreover, *S4* trees also had larger apical conduits than small trees. However, because the tall/old trees showed increased conduit tapering along the main stem, these differences tended to decrease when the analyses were carried out for the whole tree. The significance of this rather unusual tapering pattern will be discussed further on.

Moreover, *S4* trees showed greater stem tapering compared to the other treatments, most probably as a consequence of the increased mechanical constraints with height (Niklas 1995).

Compared to *S1 and GS1, GS4* trees showed similar scaling relations but also displayed higher variability in the estimated *b* (Fig. 3.2) This was likely a result of the variability in the recovering time from the grafting stress, which has been reported to affect growth and physiological processes after grafting (e.g., Mencuccini et al. 2007; Vanderklein et al. 2007).

3.5.2 Conduit tapering in tall trees

The analyses of conduit dimensions evidenced that the tapered nature of the xylem transport system was common among all the studied plants. Nevertheless, while previous studies found conduit tapering similar to WBE predictions, i.e., $\bar{a}\approx 0.167$, in these trees $\bar{a}$ was generally lower than 0.167, with the lowest values estimated for *S4* trees. Despite this variability, values of $\bar{a}$

were significantly different from WBE predictions only in seven trees, of which four *S4*, out of the 19 analysed. Previous authors attributed this reduction in tapering in the tall trees to the potential problems determined by excessive widening of xylem conduits at the stem base (Anfodillo et al. 2006), since the profile of conduit dimensions has often been found to form a plateau at the base of the trunk (Becker et al. 2003; James et al. 2003; Anfodillo et al. 2006; Coomes et al. 2007). However, our big trees (*S4*) showed a different *Dh* profile, with a point of discontinuity a few meters below the tree top, i.e., around the junction of the top branches with the main trunk. Below this point, conduit tapering was similar to the predicted WBE value, i.e., $\bar{a}\approx 0.167$, whereas in the top branches it was much lower ($0.068<\bar{a}<0.106$). It is interesting to observe that the treatment (*S4*) with reduced xylem tapering was also characterised by a different canopy architecture (i.e., sympodial with little apical dominance, as opposed to monopodial with high apical dominance, as in the other cases). The significance of these differences is presently unclear, but, as it will be discussed further on, it may be that, in the absence of apical dominance, lateral branches tend to widen their apical conduits (which consequently reduces conduit tapering further down the branch, i.e., trade-off in Fig. 3.4) to reduce the build-up of hydraulic resistance while maintaining a segmented architecture (i.e., the discontinuity of *Dh* profile) which would confine potential embolism to the peripheral-top branches (Zimmermann 1978; Zimmermann 1983). This hypothesis contrasts with the WBE assumption of the size-invariance of the terminal units (West et al. 1999), although it is consistent with the principle that all plant are forced to adopt a transport network that minimises the total hydraulic resistance (Enquist 2003).

3.5.3 Comparison of anatomical and hydraulic measurements

The hydraulic measurements confirmed that conduit tapering can compensate for the potential linear increase of hydraulic resistance with path length (Fig. 3.6). In fact, for theoretical tubes with the same tapering characteristics of the conduits of our plants, I estimated hydraulic scaling exponents against distance from the apex effectively identical to the ones obtained from direct hydraulic measurements (Fig. 3.7). While the scaling exponents were almost identical, the scaling constants of these relationships could not be directly compared, as anatomical characteristics such as number of conduits and end wall resistance will likely have varied from plant to plant (Sperry et al. 2005). Despite this, the close agreement between the two scaling exponents against distance from the apex brings about two important conclusions. First, the pathway conducting water to the top apex was not significantly affected by the presence of open pathways into the lateral branches kept at identical water potential. This suggests that, under these conditions, the water carrying capacity of each pathway was independent from the others, as also found by others (Larson et al. 1994; Brooks et al. 2003). Secondly, it is very unlikely that conduit furcation (*sensu* McCulloh et al. 2003) was present in our system. In other words, if pipes were not independent but were joined into fewer and fewer conduits basally (i.e., an aorta, as opposed to a pipe model, type of system) the relationship between the two scaling exponents would have systematically diverged from the 1:1 line. This result agrees well with previous conclusions by McCulloh & Sperry (2005), whose dataset also included a maple (*Acer negundo* L.), a diffuse-porous species. The maple stem showed no evidence of furcation and its degree of tapering was fairly close to the predicted WBE exponent, as also found here.

The scaling exponents for the measurements of hydraulic resistance versus distance from the apex were always lower than 1.0 (Fig. 3.5), suggesting a progressive saturation in the rate of increase in hydraulic resistance with distance from the apex. However, the length of the branches used for hydraulic measurements was too short to find a plateau in the hydraulic resistance profile along the stem (Becker et al. 2000). To determine whether conduit tapering completely compensated for the increased segment length (Zaehle 2005), i.e., if the hydraulic resistance tended to become independent of path length after a certain length, would have required directly measuring the variation of hydraulic resistance with path length along entire big trees, obviously not a straightforward operation.

3.5.4 Relationship Dh_{APEX} vs. $\bar{a}$

Another novel finding of this work was that conduit tapering ($\bar{a}$) was inversely related to the dimension of the apical conduits. This was not theoretically surprising given the sensitivity of hydraulic conductance to conduit diameter, but this trade-off has not been empirically demonstrated before, while the WBE model explicitly assumes size-invariance of the apical elements (e.g., West et al. 1999). Element size is not only related to hydraulic conductance (Tyree & Ewers 1991), but has significant implications also with regard to safety from embolism (e.g., Wheeler et al. 2005) and structural carbon investment (McCulloh & Sperry 2005). Hence, many different aspects are likely to be involved in this trade-off. Although tentative, I evaluated possible implications of this phenomenon on hydraulic properties and carbon costs. I calculated (i) the build-up of the total hydraulic resistance and (ii) the total carbon costs of the pipe walls, for different lengths (i.e., tree heights) and different combinations of Dh_{APEX} / b (Fig. 3.8). These combinations were chosen to represent the trade-off shown in Fig. 3.4 (representative only for small plants) for an ideal pipe, composed by successive cylindrical sections (1 cm long), whose wall thickness was estimated based on its resistance to implosion against xylem embolisms (Hacke et al. 2001). For a realistic range of apical conduit diameters between 10 and 25 µm (corresponding to tapering rates varying from 0.231 to 0.106, respectively), both the total hydraulic resistance and the total carbon costs were shown to be quite variable in small trees, although always much lower than in tall trees. This would suggest, as it should be, that neither the total hydraulic resistance nor the amount of carbon investment can limit growth in small trees growing in a favourable environment like our field site and our greenhouses. For tall trees, instead, the scenario was slightly different. Due to their segmented Dh profile and hence their unclear coupling of Dh_{APEX} and b, the trade-off in Fig. 3.4 did not include *S4* trees. However, whether one considers the whole tree profile or only the top branch, *S4* trees showed high values of Dh_{APEX} combined with reduced rates of conduit tapering (b), as predicted by the trade-off mechanism. Therefore, in taller trees total hydraulic resistance built up at rates inversely dependent on conduit tapering (i.e., low rates of tapering significantly increased the build-up of resistance, Fig. 8a, West et al. 1999; Becker et al. 2000), although at very low rates of tapering (i.e., b<0.09) the total build-up of hydraulic resistance was somehow compensated by having wider apical conduits. Moreover, wall carbon costs built up at rates directly dependent on conduit tapering (i.e., steep tapering significantly increased the build-up of carbon costs, Fig. 8b), whereas for low rates of tapering, carbon costs varied much less. Therefore, while in small trees carbon costs did not seem to be affected by the different combination of Dh_{APEX} and b, in tall trees fairly low rates of tapering coupled with large apical diameters (i.e., b≈0.10 and Dh_{APEX}≈25 um) seemed to produce the most efficient use of wall material for a similar hydraulic return, given the shape of the relationship in Fig. 3.4.

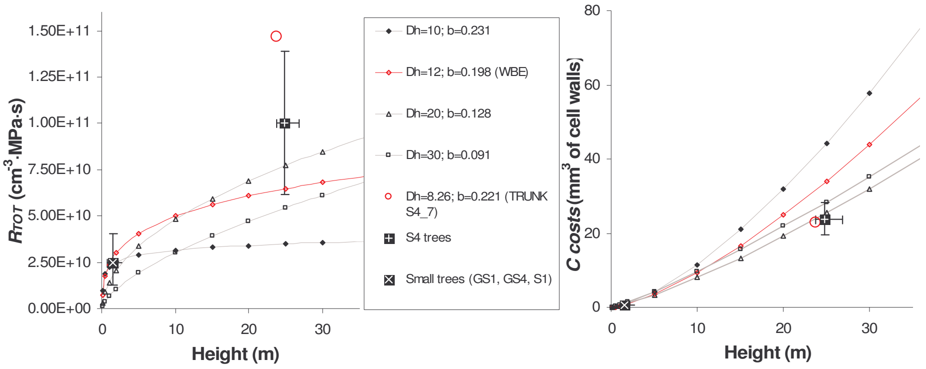


Fig. 3.8 Variation with height of (A) the total hydraulic resistance (R_{TOT}) and (B) the total carbon costs (estimated as the sum of the volume of cells walls) for one ideal tube (made up of consecutive 1-cm-long sections) with different dimensions of apical conduits (Dh_{APEX}), and conduits tapering (b) (represented by the different symbols). The combinations of values were taken to represent the trade-off between the two variables depicted in Fig. 3.4. The symbol ⊞ represents the averaged value for tall trees; the circle represents a tube of the same length, but with the same *Dh* profile of the main trunk of a tall tree (*S4_7*), extended until the apex; the symbol ☒ represents the averaged value for small trees (both field-grown and grafted).

The observed size-related decreases in b and increases in Dh_{APEX} (shown in Fig. 3.3, cf., the ⊞ symbol in Fig. 3.8) can probably be explained on the basis of a decline of hydraulic efficiency, perhaps driven by the need to maintain the efficiency in carbon investment (McCulloh et al. 2003). First, it is likely that *S4* trees reached their possible maximum conduit dimension at the stem base. In this case, maintaining an “optimal conduit tapering” while growing taller would have required to reduce the dimension of apical conduits, a biologically unrealistic situation which would have determined a very high total hydraulic resistance (see the circle in Fig. 3.8a), ≈1.5 times higher than real trees experienced (see + symbol in Fig. 3.8a). Moreover, if *S4* trees retained the small dimensions of apical conduits typical of the younger trees, and given the trade-off of Fig. 3.4, the conduits at the trunk base would have been very large (e.g., at 20 m, for a Dh_{APEX} of 10 μm, Dh_{BASE} would be ca. 100 μm; in our tall trees, 22-26 m of height, it never exceeded 79 μm). Beside the large increases in carbon costs (Fig. 3.8b), this would probably also impact on their vulnerability to cavitation (e.g., Hargrave et al. 1994; Pittermann & Sperry 2003).

Finally, it might be speculated that our tall trees (*S4*), due to their sympodial branching, developed lateral top branches with larger apical conduits and lower conduit tapering in order to grow still a few meters taller, and this new segmented *Dh* profile did not seem to negatively impact on the total amount of carbon investment (see Fig. 3.8b).

However, given the slopes in Fig. 3.8, it would appear that the best strategy to be adopted by a tree to grow taller is to structure the water transport network to best compensate for the build-up of hydraulic resistance with the increased height, i.e., the conduit tapering must be optimized.

I conclude that the observed size-related increase in hydraulic resistance in sycamore trees is likely to result from a late optimization of xylem structure in trees approaching their maximum height, mediated by a principle of efficiency of carbon allocation to conduit walls.

As noted (Fig. 3.8a), our big trees experienced a hydraulic resistance higher than the small trees (ca. 1-2 m tall), and this is likely why their physiological performances differed (Mencuccini et al. 2005; Hamid 2006). Importantly, a tapered xylem structure for, e.g, a 24-m-tall tree, limited the increase of total hydraulic resistance to only 3-5 times that of a 1-m-tall tree, as opposed to a factor of 24, as in the case of an untapered xylem structure. This means that the tapering of xylem conduits is likely the most important compensating factor reducing the effect of the build-up of hydraulic resistance with increased tree height.

As concluding remarks, I note that this study extends our knowledge on the mechanisms of compensation for the increase in hydraulic resistance during tree development and supports the notion that tapering of xylem conduits plays a crucial role in this respect. I demonstrated that vessel tapering accounted for the less than linear increase in measured branch hydraulic resistance with length and showed that these results indicated that vessel furcation was unlikely to occur in our branches. In addition, I showed the existence of a trade-off between vessel tapering and size of apical vessels, the significance of which needs to be explored further in the context of the carbon costs of vessel walls.

Moreover, the decline of tree performance previously reported (Mencuccini et al. 2005; Hamid 2006) was found to be accompanied by structural changes in the xylem driven by the need to minimise the increase of hydraulic resistance under limited carbon resources, a result independent of direct age-mediated processes and likely a function of size-related processes. Finally, while the observed ontogenetic trends were not entirely consistent with the WBE model, they highlight the significance of the basic principles of this model for a better understanding of the hydraulic limitations to tree height.

4 Degree of tapering of xylem conduits in stems and roots of small *Pinus cembra* L. and *Larix decidua* Mill. Trees

4.1 Summary

- Xylem conduits are known to increase in size from the apex downwards, but while the conduit tapering in the stem has been studied often and reported to converge towards a common pattern among all plants, information on conduit tapering in small plants and roots is extremely scarce.
- I selected 10 small trees (height<3m) along an altitudinal gradient and measured diameter and conduit dimensions along stems and roots in the last annual ring. Sections of 10 µm were cut from wooden disks taken at different heights in the stem and in the roots and then stained by safranine. Slides were observed under a microscope, the lumen areas of conduits were measured and mean hydraulic diameters (*Dh*) calculated.
- *Dh* increased from stem tip to base. In 9 out of 10 plants conduit tapering resulted as in agreement with WBE predictions. Despite trees at the treeline being older and smaller than in the sub-alpine forest, conduit tapering did not significantly differ between sites, evidencing that tree height is the main factor controlling the basipetal increase in conduit lumens. In the roots, the increase in conduit dimensions continued towards their tips, even more steeply than in the stem. The widest conduits were measured at the root tips.
- Conduit tapering resulted as a stable structural feature in small plants as well as in tall trees. It was found to continue along the roots, in agreement with the WBE principle of the optimization of water transport.

4.2 Introduction

Trees deliver resources from roots to leaves throughout a network of conduits, known as the xylem. This conducting system must remain efficient as the tree increases in size during ontogenesis. Nevertheless, phenomena of physiological decline have been commonly reported for old trees approaching their maximum height (e.g., Mencuccini & Grace 1996; Bond 2000; Hubbard et al. 2001; McDowell et al. 2002a, b; Phillips et al. 2003; Delzon et al. 2004; Ryan et al. 2006) and this reduced vigour was demonstrated to be more likely as the effect of the more restrictive physical constraints followed the increase in tree height rather than the effect of ageing (Mencuccini et al. 2005). According to the hydraulic limitation hypothesis (Ryan & Yoder 1997), as a tree gets taller, the increased roots to leaves distance (i.e., the path length of the water flow) and the consequent increase in hydraulic resistance (cf., Hagen-Poiseuille formula for laminar flows in cylindrical pipes: Tyree & Ewers 1991) would cause a reduced stomatal opening, thus reduced carbon assimilation. While hydraulic constraints have been demonstrated to be among the major determinants of the maximum height of trees (Koch et al. 2004), the physiological parameters rarely reflected a linear decline with tree height (e.g., Hubbard et al. 2001; McDowell et al. 2002a, b; Phillips et al. 2003; Delzon et al. 2004; Ryan et al. 2006) and some phenomena, such as the increase in allocation in fine roots (Magnani et al. 2000), decrease in leaf area to sapwood area ratio (McDowell et al. 2002a) and increase in xylem permeability (Pothier et al. 1989) were proposed to be mechanisms of compensation for the effect of the increased hydraulic resistance with tree height. The tapering of xylem conduits were recently hypothesized as the most effective strategy of mitigation for the effect of the increased tree height on the whole-path hydraulic resistance (West et al. 1999; Becker et al. 2000), and the empirical data available support the notion that the variation in hydraulic resistance with path length (i.e., the longitudinal axes of stem and branches) is substantially controlled by the tapering of xylem conduits (Petit et al. 2008).

The outcome of the WBE model (West et al. 1999) and its hypothesis that the whole-tree hydraulic resistance can become independent from the path-length for a degree of conduit tapering equal to or higher than a specific value (see also chapter 2) put new emphasis on the study of xylem anatomy and research started to focus on the longitudinal profiles of conduit dimensions. While the data collected by Anfodillo et al. (2006), Weitz et al. (2006) and Coomes et al.

(2007) suggested that the conduit tapering of all trees is ontogenetically stable and substantially as predicted by the WBE model (West et al. 1999), other studies reported different patterns in trees of different species and size (Becker et al. 2003; James et al. 2003). Moreover, Petit et al. (2008) reported reduced conduit tapering in sycamore (*Acer pseudoplatanus* L.) trees approaching their maximum height, whereas a wide range of variability in the degree of tapering was found among actively growing small trees of the same species. Although Becker et al. (2000) mathematically demonstrated that the effect of conduit tapering in minimizing the whole-tree hydraulic resistance becomes evident after the first 1-2 metres, this property might also be relevant for such distances if the dimension of the apical conduits are very small. So, where limiting factors affect the formation of wide conduits and trees are typically of a small size, the conduit tapering might be still very important to minimize the total hydraulic resistance. This might be the case of trees at the treeline, where low temperatures affect the metabolism, determining a reduced growth rate (Tranquillini 1979; Körner 1998; Paulsen et al. 2000; Körner 2003; Rossi et al. 2007).

The aim of this work, which is a meta-analysis of two recent degree theses (De Zan 2004, Petit 2004), was to provide some new information regarding the tapering of xylem conduits (i) in small coniferous trees (*Pinus cembra* L. and *Larix decidua* Miller, the most common high-altitude forest species in the north-eastern Italian Alps) growing under the limitations imposed by low temperatures and (ii) the anatomical characteristics of the xylem in the roots (analyzed only for *P. cembra* L.), where xylem conduits are known to increase in size with soil depth (McElrone et al. 2004), but no specific analyses of conduit tapering have yet been done.

4.3 Materials and methods

4.3.1 Plant material and sampling

Two conifer species growing in the Dolomites (Eastern Alps, Italy) were sampled in September 2003. Six *Larix decidua* Miller and four *Pinus cembra* L. specimens with no evident biotic or abiotic damage to stem or branches were selected along an altitudinal gradient (from 1500 to 2400 m a.s.l.; Tab. 1). For each tree, the stem was felled and the collar and longest root to the rootlets were fully extracted from the ground. Its root structure type and depth in the soil made root extraction more difficult for *L. decidua* than for *P. cembra* and so it was only possible to analyse the longitudinal trend of conduits diameter in nearly intact roots for this species.

Tree height was estimated as the path length of water flow along the stem, i.e., it was measured also taking into account any curve in the stem and not just as the straight distance between stem base and apex. Tree age was estimated by the number of annual tree rings at the stem base.

Wooden disks were taken at different distances from the stem tip to stem base, and along the longest root, trying to avoid nodal zones and the collar, where conduits are known to be narrower because of mechanical constraints (Spicer & Gartner 2001). Distance from the tip (*L*) and diameter (*D*) were carefully measured for each disk.

4.3.2 Anatomical measurements

Wooden disks (or at least two/three opposite portions of them comprising zones with no reaction wood, injuries or scars) were embedded in paraffin (Anderson & Bancroft 2002); transverse sections of 10-12 µm were then cut with a rotatory microtome, stained with safranine (1% in water) and fixed permanently on microscope slides with Eukitt (BiOptica, Milan, Italy). For a detailed description of the embedding process and microscope slides preparation see chapter 3.3.2. The sections were observed under a light microscope (Leitz, Laborlux S) at 200x. A specific software, WinCELL™ (Régent Instruments Inc., Sainte-Foy, QC, Canada), was used to analyze the digitalized images and measure the lumen area of conduits.

For each group of sections belonging to the same disk, at least five cell rows were analyzed (Pittermann & Sperry 2003) from early to late wood of the outermost annual ring. Between 7 and 14 disks per tree stem were analyzed (Tab. 3) and 9 to 15 from the longest root (Tab. 5), giving around 6,500 cells per tree (400 to 1,600 cells per disk).

Conduit diameter was calculated considering the lumen to be circular. I chose conduits with a diameter of more than half the diameter of the largest one, in order to eliminate those that may have been tapered, as suggested by James et al. (2003). Selected cells were averaged as the hydraulic diameter (*Dh*), so that cell diameters are weighted according to their hydraulic conductance (Sperry et al. 1994, Anfodillo et al. 2006):

$$Dh = \frac{\sum_{n=1}^{N} d_n^5}{\sum_{n=1}^{N} d_n^4} \qquad \text{equation 4.1}$$

where d_n is the diameter of the *n* cell.

4.3.3 Statistical analyses

The scaling parameters of allometric equations were determined from pairwise comparisons of $\log_{10}$-transformed data. Using a model type II regression analysis with the reduced major axis (RMA) protocol, the scaling exponents and allometric constants were identified as the regression slopes (αRMA) and *y*-intercept (βRMA), respectively. Regression coefficients, their 95% confidence and prediction intervals, were computed using standard methods (Sokal & Rohlf, 1981) and adopting a bootstrap procedure with 100,000 replications (Davison & Hinkley, 1997).

Comparisons of mean values of different parameters (t-tests) between the statistical populations of sub-alpine forest trees and at the treeline were performed using the *SAS 8.02* software (SAS Institute, Cary, N.C.).

4.4 Results

Sampled trees were very different in age, height (Tab. 4.1) and therefore in annual height increment (Tab. 4.2).

Trees at the treeline showed a significantly lower annual height increment compared to the sub-alpine forest (for *P. cembra* p=0.033; for *L. decidua* p=0.011), as reported in many other studies (cf., Tranquillini 1979; Körner 2003).

Tree code	Species	Altitude (m a.s.l.)	Exposure	Site	Age at stem base (years)	Diameter at collar (cm)	Height (cm)	Longest root (cm)
PC1	*P. cembra*	2185	N-E	Treeline	32	3.81	92	183
PC2	*P. cembra*	2190	S-E	Treeline	28	2.60	62	237
PC3	*P. cembra*	1520	N-E	Sub-alpine forest	26	3.42	166	
PC4	*P. cembra*	1640	S-E	Sub-alpine forest	39	5.08	274	355
LD1	*L. decidua*	2270	S-E	Treeline	30	2.96	75	
LD2	*L. decidua*	2200	E	Treeline	64	2.56	81	
LD3	*L. decidua*	2190	S-E	Treeline	70	2.07	60	
LD4	*L. decidua*	1590	E	Sub-alpine forest	19	2.54	128	
LD5	*L. decidua*	1380	N	Sub-alpine forest	23	2.06	166	
LD6	*L. decidua*	1730	N	Sub-alpine forest	18	1.68	114	

Tab. 4.1 Dendrometric parameters and site characteristics of the sampled trees.

Tree code	***ΔL*** (cm)	r^2	p
PC1	2.79	0.94	0.001
PC2	1.95	0.96	0.001
PC3	6.45	0.99	0.000
PC4	9.97	0.98	0.000
LD1	3.50	0.99	0.000
LD2	4.15	0.98	0.000
LD3	3.65	0.98	0.000
LD4	6.42	0.97	0.000
LD5	9.19	0.93	0.000
LD6	7.54	0.94	0.000

Tab. 4.2 Annual increment in height (*ΔL*). *ΔL* is assessed as the regression slope of the $\log_{10}$-$\log_{10}$ relationship between the year of development of the innermost ring measured at different distances along the stem and its height.

Stem tapering (i.e., variation of the main stem diameter, *D*, with the distances from stem tip, *L*) was generally low, i.e., $\alpha > 1$ (Tab. 3; see also Niklas 1995). Mean values of *Dh* ranged between 19.05 μm (s=2.04) at the stem apex and 27.93 μm (s=1.95) at stem base in *L. decidua* and between 16.12 μm (s=0.62) at stem apex and 25.03 μm (s=3.81) at stem base in *P. cembra* (Tab. 4.4).

Along the main stem, the scaling of the weighted hydraulic diameter (*Dh*) in the last annual ring with the path length (i.e., distance from the tip, *L*) showed similar patterns among the specimens of both *P. cembra* and *L. decidua*. The best fittings were obtained by using power functions (Tab. 4.3), which means that the *Dh* variations were not constant with *L*, but were higher close to the tip, as commonly reported (Becker et al. 2003; James et al. 2003; Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007; Petit et al. 2008).

		STEM				**95% Confidence Intervals**	
Tree code	**Model**	**N**	**Intercept**	**Slope**	r^2	**Intercept**	**Slope**
PC1	log *Dh* vs log *L*	7	1.019	0.156	0.83	0.741 to 1.079	0.113 to 0.314
	log *Dh* vs log *D*	7	1.014	0.194	0.67	0.805 to 1.155	0.091 to 0.332
	log *L* vs log *D*	7	-0.033	1.243	0.87	-0.415 to 0.738	0.697 to 1.539
PC2	log *Dh* vs log *L*	8	1.026	0.200	0.74	0.860 to 1.147	0.110 to 0.304
	log *Dh* vs log *D*	8	0.885	0.372	0.71	0.724 to 1.105	0.180 to 0.512
	log *L* vs log *D*	8	-0.709	1.860	0.92	-1.488 to -0.149	1.406 to 2.603
PC3	log *Dh* vs log *L*	8	0.863	0.259	0.92	0.673 to 0.951	0.213 to 0.358
	log *Dh* vs log *D*	8	0.826	0.403	0.92	0.676 to 0.919	0.331 to 0.519
	log *L* vs log *D*	8	-0.142	1.558	0.99	-0.258 to 0.083	1.394 to 1.647
PC4	log *Dh* vs log *L*	13	0.920	0.249	0.85	0.845 to 1.155	0.142 to 0.292
	log *Dh* vs log *D*	13	0.919	0.354	0.86	0.849 to 1.154	0.202 to 0.411
	log *L* vs log *D*	13	-0.003	1.421	0.99	-0.302 to 0.109	1.349 to 1.612
LD1	log *Dh* vs log *L*	9	1.190	0.112	0.87	1.053 to 1.219	0.084 to 0.194
	log *Dh* vs log *D*	9	1.226	0.135	0.93	1.199 to 1.251	0.110 to 0.160
	log *L* vs log *D*	9	0.317	1.209	0.93	0.158 to 0.795	0.769 to 1.459
LD2	log *Dh* vs log *L*	14	1.066	0.178	0.91	0.928 to 1.106	0.152 to 0.260
	log *Dh* vs log *D*	14	1.138	0.224	0.90	1.079 to 1.166	0.193 to 0.299
	log *L* vs log *D*	14	0.403	1.259	0.89	0.137 to 0.808	0.848 to 1.647
LD3	log *Dh* vs log *L*	10	0.970	0.244	0.76	0.713 to 1.093	0.169 to 0.400
	log *Dh* vs log *D*	10	1.180	0.186	0.90	1.116 to 1.219	0.150 to 0.249
	log *L* vs log *D*	10	0.855	0.760	0.94	0.709 to 1.040	0.576 to 0.937
LD4	log *Dh* vs log *L*	14	1.117	0.166	0.88	1.060 to 1.178	0.132 to 0.237
	log *Dh* vs log *D*	14	1.243	0.164	0.90	1.218 to 1.279	0.130 to 0.188
	log *L* vs log *D*	14	0.755	0.987	0.99	0.674 to 0.831	0.920 to 1.059
LD5	log *Dh* vs log *L*	9	1.013	0.208	0.92	0.958 to 1.164	0.135 to 0.237
	log *Dh* vs log *D*	9	1.246	0.179	0.95	1.228 to 1.300	0.129 to 0.199
	log *L* vs log *D*	9	1.116	0.860	0.99	0.996 to 1.170	0.808 to 0.986
LD6	log *Dh* vs log *L*	11	1.040	0.205	0.75	0.855 to 1.196	0.121 to 0.307
	log *Dh* vs log *D*	11	1.151	0.267	0.77	1.039 to 1.263	0.156 to 0.389
	log *L* vs log *D*	11	0.545	1.301	0.98	0.426 to 0.725	1.120 to 1.439

Tab. 4.3 Number of sampled disks (N), regression coefficients, r^2 and confidence intervals of the main relationships used to test the WBE model in the main stem. *Dh*: mean hydraulically weighted conduit diameter. *L*: path length (i.e., distance from stem tip); *D*: stem diameter.

In *P. cembra*, *b* spanned from a minimum of 0.156 (95% CI 0.113-0.314) for PC1 (2185 m a.s.l.; treeline) to a maximum of 0.259 (95% CI 0.213-0.358) for PC3 (1520 m a.s.l.; sub-alpine forest), whilst in *L. decidua* it was from 0.112 (95% CI 0.084-0.194) for LD1 (2270 m a.s.l.) to 0.244 (95% CI 0.169-0.400) for LD3 (2190 m a.s.l.), both at the treeline (Fig. 4.1). Considering the WBE ideal plant as constituted of 20 levels (West et al. 1999), nine of the ten trees analyzed showed conduit tapering consistent with the actual tapering predicted by the model, i.e., $b > 0.198$ without the approximation for tree height (Anfodillo et al. 2006) (see Fig. 4.1). The degree of tapering of both species was found to be inversely correlated to *Dh* at the stem tip (estimated as the *Dh* at 0.1 cm from the apex) (Fig. 4.2).

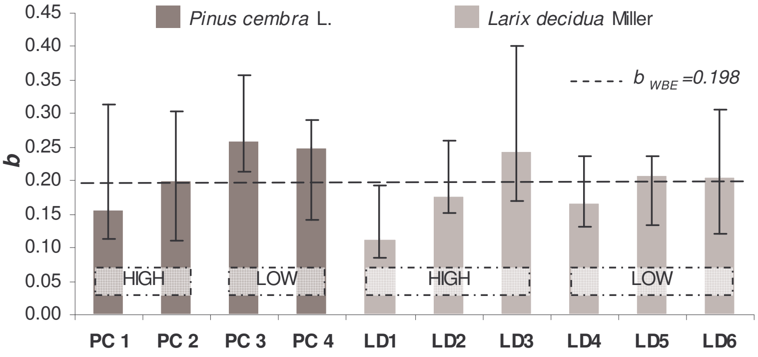


Fig. 4.1 Scaling exponents *b* for the stems of the sampled trees. Error bars represent 95% confidence limits and the dotted line the WBE threshold of 0.198, estimated as the actual *b* (i.e., without approximation for the tree height, see Anfodillo et al. 2006) for a WBE plant of 20 levels.

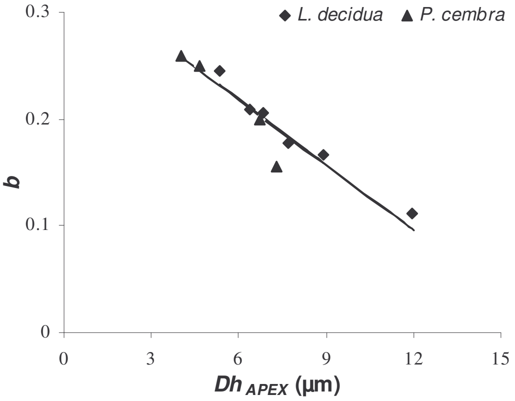


Fig. 4.2 Variation of scaling exponent *b* with the hydraulic diameter (*Dh*) estimated at a distance of 0.1 cm from the tip). The regression line is: b=0.3397-0.0203·Dh_{APEX} (r^2 = 0.908; p=0.0001).

No difference in the conduit tapering parameter (*b*) between trees at the treeline and the sub-alpine forest were found when the t-test was performed for both species together (p=0.192). Splitting the analysis, neither *P. cembra* nor *L. decidua* showed significant differences between sites (p=0.078 and p=0.729 respectively). Moreover, the scaling coefficient *a* (i.e., the intercept of the $\log_{10}$-transformed curve) between trees at the treeline and in the sub-alpine forest was different in *P. cembra* (p=0.045), but not in *L. decidua* (p=0.805).

In the roots, the hydraulic diameter (*Dh*) continued to increase with path length (*L*) and this change in size was continuous from stem to roots (Tab. 4.5 and Fig. 4.3). The best fittings were again obtained by using power functions. However, the scaling exponents of roots were always higher than those measured in the stem of the same specimen (see Tab. 4.3 and Tab. 4.5). In every measured plant, b_{ROOT} was significantly higher than 0.198 and hence substantially in agreement with the WBE model.

		ROOT				95% Confidence Intervals	
Tree code	**Model**	**N**	**Intercept**	**Slope**	**r^2**	**Intercept**	**Slope**
PC1	log *Dh* vs log *L*[(1)]	17	0.653	0.391	0.87	0.357 to 0.803	0.320 to 0.531
	log *Dh* vs log *L*	9	0.618	0.414	0.80	0.211 to 0.887	0.292 to 0.596
	log *Dh* vs log *D*	9	1.497	-0.163	0.81	1.447 to 1.514	-0.275 to -0.124
PC2	log *Dh* vs log *L*[(1)]	19	0.902	0.295	0.95	0.797 to 0.960	0.269 to 0.343
	log *Dh* vs log *L*	11	0.962	0.269	0.98	0.921 to 1.025	0.241 to 0.288
	log *Dh* vs log *D*	11	1.447	-0.245	0.91	1.372 to 1.463	-0.377 to -0.201
PC4	log *Dh* vs log *L*[(1)]	28	0.920	0.247	0.92	0.869 to 0.998	0.216 to 0.268
	log *Dh* vs log *L*	15	0.577	0.377	0.72	0.358 to 0.779	0.301 to 0.462
	log *Dh* vs log *D*	15	1.517	-0.165	0.69	1.479 to 1.532	-0.267 to 0.130

[(1)] Path length (*L*) = Stem length + Root length

Tab. 4.5 Number of sampled disks (N), regression coefficients, r^2 and confidence intervals of the main relationships used to test the WBE model in the longest root. *Dh*: mean hydraulically weighted diameter. *L*; path length (i.e., distance to stem tip); *D*: root diameter.

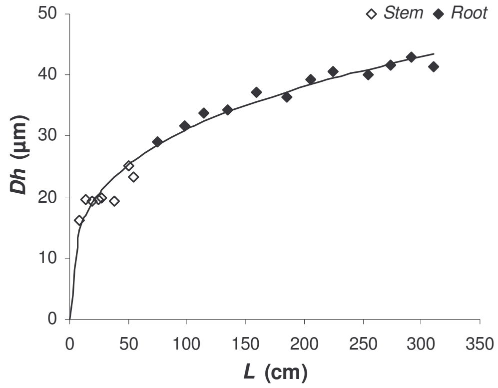


Fig. 4.3 Example (PC2) of the variation of the hydraulically weighted diameter (*Dh*) with path length (*L*) from stem tip to root tip. The fitting curve is: $Dh=7.976\cdot L^{0.295}$ ($r^2=0.955$; p<0.0001).

Lastly, *Dh* scaled with the diameter of the root cross-section (*D*) as a power function with a negative exponent, meaning that as the root grew longer and thinner, conduit dimensions increased (Fig. 4.4).

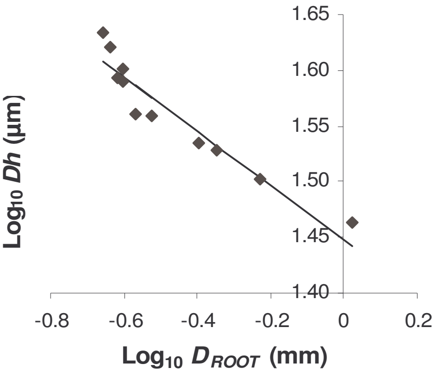


Fig. 4.4 Example (PC2) of the variation in hydraulic diameter (*Dh*) with diameter (*D*) in the longest root. $\log_{10}Dh=1.497-0.245\cdot\log_{10}L$ ($r^2=0.907$; $p<0.0001$).

4.5 Discussion

The results provide new evidence that the increase in conduit dimensions from stem tip to stem base is also a common feature in small plants. Importantly, I also found that this tapered structure of the transport network (i.e., characterised by a precise and gradual increase in conduit size basipetally) continues belowground, as previously reported (McElrone et al. 2004), with a slightly higher degree of tapering than those occurring in the stem, suggesting that the hydraulic resistance in the roots is stabilized even better than in the stem.

Although analyzed trees differed in age (from about 20 to 70 years old), tree height (i.e., path length) seemed to be the most relevant factor in controlling the increase in conduit dimension down to the stem base and the degree of conduit tapering was similar among plants, rejecting the possibility of an age-control over this xylem feature, as also demonstrated by Petit et al. (2008).

Despite sampling not being extremely wide due to time-consuming measurements, our results would suggest that trees are forced to maintain a nearly optimal tapered structure irrespective of site and environmental conditions. However, it is worth noting that, in both *P. cembra* and *L. decidua*, the trees showing the smallest annual increment in height were at the treeline and the same trees also showed the smallest degree of conduits tapering (PC1 and LD1 respectively). This simple observation might be important if seen in the light of the decline in tree growth at high altitude and the formation of the treeline (Tranquillini 1979; Körner 2003). Cambial activity is known to be very sensitive to low temperatures and xylogenesis appears strongly reduced when daily mean temperature drops below 6-8 °C (Rossi et al. 2007). The formation of wide conduits is also seriously hampered in temperature-limiting ecosystems, such as at high altitude and latitude (Kirdianov et al. 2003; Oberhuber 2004). In an arctic shrub species a heat treatment (5° C above ambient temperature) led to a significant increase in the proportion and diameter of the widest vessels (Gorsuch & Oberbauer, 2002). It could be speculated that any constraint affecting cell enlargement in the stem (e.g., low temperatures at high altitude) would cause an early stabilization of conduit dimension with distance from the stem apex (i.e., the so-called "plateaux effect": e.g. Becker et al. 2003; James et al. 2003), leading to a not optimised vascular structure (i.e., with conduit tapering lower than 0.167). Since the conduit tapering is likely to be very important to minimize the total hydraulic resistance also for small distances in case of small conduit dimensions, this reduced tapering would lead the overall resistance throughout the path to increase accordingly, thus decreasing the water transport efficiency and hence the potential growth in height. In a study on the effect of altitude on conduit tapering by Coomes et al. (2007), it was shown that the intercept of the log-log relationship *Dh* vs. *L* was significantly lower (i.e., smaller conduits at the apex) at high altitude. These authors also reported a reduction in conduit size when approaching the stem base in conifers at high altitude, suggesting that conduit tapering might go towards a reduced degree because low temperatures hamper the formation of wide conduits at the stem base.

This aspect is worth emphasising in order to encourage further analyses of those trees with an evident reduction in height growth in order to better focus on the limiting factors affecting xylem properties and growth in height and hence, perhaps, the formation of the treeline.

Another novel finding in this study was that *b* is strictly linked to *Dh* at the stem apex. Since it is likely that investment in enlarging apical conduits compensates for the increase of hydraulic resistance with path length better than maintaining an optimal conduit tapering (Anfodillo et al. 2006), and that wide conduits increase the risk of cavitation (Tyree et al. 1994; Pockman & Sperry 2000; Martínez-Vilalta et al. 2002), more specific investigations might clarify whether this relationship reflects a trade-off mechanism between efficiency and safety.

Scaling relationships of xylem characteristics of roots were comparable to those of stems. Indeed, our data showed that the increase in conduit dimensions along the roots, as also observed by others (Martínez-Vilalta et al. 2002; McElrone et al. 2004), scaled allometrically with distance from the stem tip. Moreover, despite their different degrees, stem and root conduit dimensions converged to the same value around the collar zone, evidencing a gradual and continuous increase of conduit size from the tip of the main stem to the root tips. In roots, *b* were even higher than in stems (always higher than the WBE threshold of 0.198). It is likely that the nearly absent mechanical constraints below ground and, more importantly, the higher water potential, might allow conduits to enlarge more than those in stems. The whole architecture of the root system was reported to follow the same area preserving branching rule as aboveground organs (Oppelt et al. 2001; Richardson & zu Dohna 2003), but, whilst for stems the WBE model predicts $Dh \propto D^{1/6}$ to guarantee mechanical stability (West et al. 1999), in roots the same relationship was even inversed and both the narrowest diameter and the widest conduits of the whole plant were found around the root tips. Therefore, root tips appeared as if they had developed under the lowest level of both mechanical and hydraulic constraints.

In conclusion, this study evidenced that conduit tapering is also an essential feature in small conifers and extends also belowground along the roots, where the rate of increase in conduit size is on average higher than that of the stem. The compensation effect of tapering for the increase in hydraulic resistance with increased tree height might be relevant in those environments where the formation of wide conduits is inhibited, such as at the treeline. However, I strongly encourage further investigations on xylem anatomy of trees at the treeline, because the extension of the hydraulic limitation hypothesis as a cause of the formation of this extreme environment can be tested.

5 Does the low-temperatures-inhibition to cell expansion induce a hydraulic limitation to tree height at the treeline?

5.1 Summary

- Recent investigation supported the hypothesis that the limit to tree height is determined by the effect of hydraulic constraints, whereas conduit tapering appeared as the most effective strategy to minimize the hydraulic resistance at given environmental conditions. Low temperatures are known to limit tree growth and their effect in reducing the sink activity of the developing tissues was proposed as a potential cause of the treeline formation. Here I tested a revised hypothesis, according to which this temperature-limited sink activity would rather determine an hydraulic limitation to the further growth in height.
- I selected 5 small spruces at a treeline site (≈2100 m a.s.l.) and 5 insede a closed forest at ≈1000 m a.s.l. and I treated the developing apical buds with an artificial warming during the growing season in 2006 and 2007. Another 5 specimens per site were selected as controls. I measured the increase in the current shoot length across the season, the final shoot length and the previous annual increments in height. Sections of 10 μm were cut from wooden disks taken at 2 cm from the apices and at different heights along the stem and then stained by safranine. Slides were observed under a microscope, the lumen areas of conduits were measured and mean hydraulic diameters (*Dh*) calculated.
- *Dh* at the stem apex resulted wider after the two years warming in trees at the treeline, whereas any significant variation was found in either controls either those at 1000 m a.s.l. On average, the assessed degree of conduit tapering in trees at the treeline was in agreement with the principle of stabilization of the hydraulic resistance.

- Results supported the hypothesis that the growth in height of trees at the treeline is limited by the effect of low temperatures on the sink activity of the developing tissue, which determines a production of narrow cells and, ultimately, an hydraulic limitation to tree growth.

5.2 Introduction

Mountainous regions are worldwide characterized by a striking vegetation gradient between the upper limit of the closed forest and the highest patches of upright trees (treeline). At these altitudes, the physiology of trees is hardly hampered by the extreme environmental conditions that characterize these cold environments. Low temperatures are responsible for the delayed cambial reactivation (Gričar et al. 2006; Rossi et al. 2007) and hence of an overall reduction in the duration of the growing season. Analyses on non structural carbon (NSC) pools revealed that low temperatures do not limit the source activity of the photosynthetic organs, yet the tree growth appeared more likely to be limited by the reduction of the sink activity in the developing tissues (growth or sink limitation hypothesis: Hoch et al. 2002; Körner 2003; Hoch & Körner 2005). In fact, the length of time needed for cell division and expansion seemed to increase with decreasing temperatures, approaching infinity at +1 to +2 °C (Körner 2003). Moreover, a thermal threshold at which the initiation of new roots and further lengthening is inhibited was often found at around 5-6 °C (e.g., Vapaavuori et al. 1992; Domisch et al. 2002; Alvarez-Uria & Körner 2007). In addition to this direct effect of low temperatures on the physiology of trees, other extreme environmental factors, such as frost, winter desiccation or prolonged snow cover can also induce stress conditions that alter their growth (e.g., Tranquillini 1979; Sakai & Larcher 1987; Havranek & Tranquillini 1995).

Experiments of artificial warming of trees at the treeline revealed an increase in both diameter and height growth (Danby & Hik 2007), as well as an overall enhancement of the efficiency of the xylem transport system, i.e. an increase in vessel density and conduit width and hence in the specific xylem conductivity (k_s) and stomatal conductance (g_s) (Gorsuch & Oberbauer 2002).

In the recent past, important contributions evidenced the key role played by the physical constraints of lifting water from roots to leaves in setting the limit to tree height (Koch et al. 2004) and that of the tapering of xylem conduits in mitigate the effect of height on the total hydraulic resistance (Petit et al. 2008). According to the hydraulic limitation hypothesis (Ryan & Yoder 1997; McDowell et al. 2002b; Ryan et al. 2006), as a tree gets taller, the increased roots to leaves distance (i.e., the path length of the water flow) and the consequent increase in hydraulic resistance (cf., Hagen-Poiseuille formula: Tyree & Ewers 1991) would cause a reduced stomatal opening, thus a reduced carbon assimilation and, ultimately, a reduced height growth. Systematic analyses on the longitudinal variation of conduit width along the stem (Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007; Petit et al. 2008) revealed that conduit tapering is a common feature among plants of different size and species, which seemed to converge to an optimal degree allowing the independence of the hydraulic resistance from the tree height, i.e., the resistance tend to an asymptotic value with increasing path length (West et al. 1999; Becker et al. 2000). However, the effect of tapering in substantially stabilizing the hydraulic resistance occurs after a distance that strongly depends on the length of the terminal element of the system (West et al. 1999). As it has been shown in Fig. 2.3 in Chapter 2, theoretically, the shorter is the terminal (apical) element, the sooner the hydraulic resistance is kept constant by the conduit tapering. Given that the hydraulic resistance of a tube is inversely proportional to the fourth power of its diameter (e.g., Tyree & Ewers 1991), widening apical conduits likely appears as the most effective strategy to minimize the hydraulic resistance of the whole system. Anfodillo et al. (2006) hypothesized that, once reached the maximum width of apical conduits, any departure from the optimal degree of tapering would hamper the growth in height.

The aim of this work was to investigate the potential role of conduit tapering in setting the height limit of trees at the treeline, i.e. small trees characterized by anatomical and morphological traits similar to those induced by hydraulic limitations such as small xylem conduits, low values of specific leaf area (SLA) and low rates of growth.

I compared the effect of a heating treatment applied to the developing apical shoot of specimens of *Picea abies* (Karst) at the treeline and 1000 m below in altitude at a closed forest site. Since it has been demonstrated that the stimulation effect on cell enlargement decreases with the temperature and trees at the treeline are not carbon limited (Körner 2003), the null hypothesis I tested was that the heating treatment applied to the most sensitive part of the tree to the total hydraulic resistance (i.e., the apex) should stimulate the developing cells to grow wider, resulting to an increased shoot elongation, whereas, at a corresponding heating, trees in the closed forest should not present any significant changes in xylem anatomy and growth rate.

5.3 Materials and Methods

5.3.1 Site and plant material

The study focused on one of the most common species growing at high altitude in the Dolomites (Easter Alps, Italy), the spruce, *Picea abies* Karst. The study area comprised two sites, one at Monte Rite (≈2100 m a.s.l., Cibiana di Cadore, BL, Italy) and one outside the Research Center for Alpine Ecology of the University of Padova (≈1000 m a.s.l., San Vito di Cadore, BL, Italy). In each site, I selected five specimens and I applied a heating system to the stem's apical developing bud (H1 to H5). Another five specimens with growing features apparently similar to the others were selected as controls (C1 to C5). The apical developing buds were placed inside a transparent polycarbonate cylinder (length 250 mm, diameter 50 mm, thickness 3 mm) provided with 20 spires of fine wire resistance tight to its inner side. Two sensors of fine wires of copper and constantane (type T), were connected to a Campbell CR10X datalogger to switch on/off the low voltage power supply (0-30 V, 0-10 A, with 35 W for each heated tube) to regulate the difference between the air temperature inside and outside each cylinder at 4-6℃ at Monte Rite and, to take into account the effect of elevation on the air temperature, at a corresponding 9-10℃ at San Vito (Fig. 5.1). Instead, cylinders without resistance were applied to control trees, also to evaluate their possible greenhouse effects.

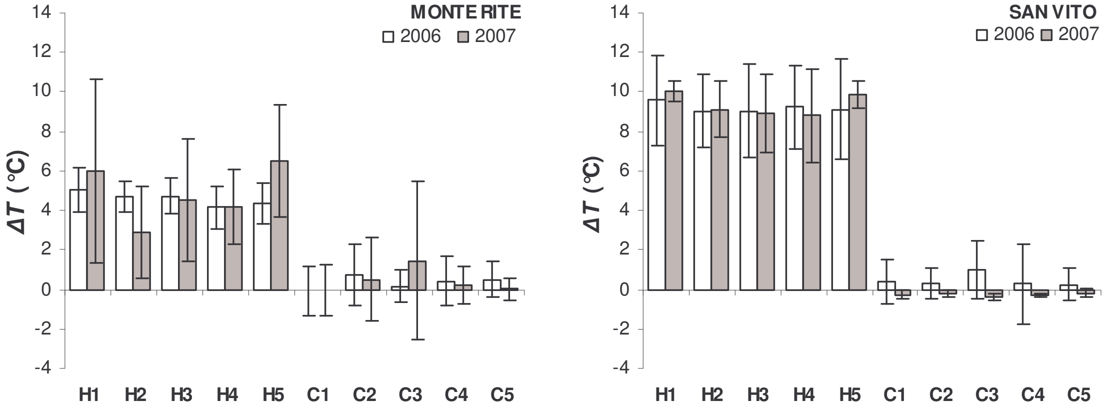


Fig. 5.1 Daily averaged difference of air temperature (*ΔT*) between inside and outside the polycarbonate cylinder of all trees of both sites, at high (Monte Rite) and low altitude (San Vito). H1-H5 are heated trees, C1-C5 are controls. Error bars represent standard deviations..

The heating treatment was repeated for two years to better evaluate its effect on tree growth, which is known to be very sensitive to the environmental conditions of the previous year, especially in coniferous species (e.g., Jalkanen & Tuovinen 2001). At Monte Rite, the heat was supplied from the 19th of May to the 12th of July in 2006 and from the 10th of May to the 16th of August in 2007, while at San Vito from the 28th of April to the 13th of September in 2006 and from the 19th of April to the 21st of August in 2007.

All trees were felled at the beginning of September 2007.

5.3.2 Anatomical analyses

For each tree, the total height, basal diameter and stem annual longitudinal increments were measured. Along the stem, wooden disks were carefully extracted at 2 cm for the distal node of the last four internodes. Other 6-8 disks were extracted lower down at different distances along the stem. For each sampling point, the stem diameter and relative distance from the apex were measured.

Wooden disks (or at least two/three opposite portions of them comprising zones with no reaction wood, injuries or scars) were embedded in paraffin (Anderson & Bancroft 2002); transverse sections of 10-12 µm were then cut with a rotatory microtome, stained with safranine (1% in water) and fixed permanently on microscope slides with Eukitt (BiOptica, Milan, Italy). For a detailed description of the embedding process and microscope slides preparation see chapter 3.3.2. Digital images of the sections were taken at 100x magnification using a light microscope (Nikon Eclipse80i). Lumen areas were measured by WinCell software (Régent Instruments Inc., Sainte-Foy, QC, Canada). Conduits were considered to be circular and, in order to eliminate their endings, only those with a lumen diameter greater than half the diameter of the largest conduit (James et al. 2003) were chosen to estimate the hydraulically weighted diameter, *Dh*, of the annual rings relative to 2005 and 2007:

$$Dh = \frac{\Sigma d_n^5}{\Sigma d_n^4} \qquad \text{equation 5.1}$$

where d_n is the n^{th} conduit diameter (Sperry et al. 1994).

5.3.3 Statistical analyses

The scaling parameters of the allometric equations were determined from pairwise comparisons of $\log_{10}$-transformed data. Using reduced major axis (RMA) analysis, the scaling exponents and allometric constants were identified as the regression slopes (αRMA) and y-intercept (βRMA), respectively. Regression coefficients, their 95% confidence and prediction intervals, were computed by standard methods (Sokal & Rohlf 1981) using a bootstrap procedure with 100,000 replications (Davison & Hinkley 1997).

Analyses of non linear regression and of variance were performed using *SAS 8.02* (SAS Institute, Cary, N.C., USA).

5.3 Results

5.3.1 Phenology

The development of the current apical shoot was well described by a logistic function (Fig. 5.2 and Tab. 5.1 – Tab. 5.2). The parameter *c*, which is a coefficient specifying the shape of the curve, i.e., the steepness of the line between the two inflection points, was considered as an index of the developmental speed of the terminal shoot. It resulted as significantly higher in heated trees at San Vito (t=3.58, p=0.007 in 2006; t=2.82, p=0.042 in 2007), whereas statistics did not reveal any difference between treatments at Monte Rite (t=1.86, p=0.099 in 2006; t=2.32, p=0.075 in 2007).

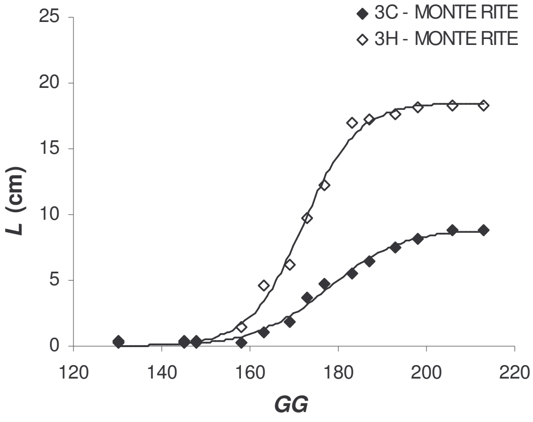


Fig. 5.2 Two examples of the development of the current apical shoot during the growing season. The logistic function describing the variation of shoot length (*L*) with time is $L = x + \frac{a}{1 + e^{b-c(GG-x)}}$, where *x* is the day of the first measurement (unclosed buds) and *GG* is the Julian day.

MONTE RITE 2006

ID	r^2	*F*	*p*	*a*	*b*	*c*
1C	0.995	1231	<0.0001	14.99	4.51	0.17
2C	0.971	236.61	<0.0001	6.58	4.01	0.15
3C	0.999	1610.54	<0.0001	9.01	4.97	0.18
4C	0.988	624.12	<0.0001	7.86	4.76	0.20
5C	0.975	278.78	<0.0001	4.91	4.66	0.18
1H	0.990	733.82	<0.0001	16.10	4.48	0.19
2H	0.989	679.88	<0.0001	10.63	5.35	0.24
3H	0.997	3232.54	<0.0001	12.74	4.74	0.24
4H	0.977	368.69	<0.0001	5.61	4.34	0.20
5H	0.980	609.65	<0.0001	4.24	2.60	0.17

MONTE RITE 2007

ID	r^2	*F*	*p*	*a*	*b*	*c*
1C	0.976	472.13	<0.0001	11.07	5.02	0.12
2C	0.990	885.00	<0.0001	5.78	6.17	0.13
3C	0.992	1209.69	<0.0001	8.88	5.96	0.12
4C	0.980	596.19	<0.0001	7.17	4.61	0.10
5C	0.993	1642.72	<0.0001	4.47	5.95	0.13
1H	0.993	1657.94	<0.0001	24.89	7.10	0.17
2H	0.999	11132.10	<0.0001	6.90	5.65	0.17
3H	0.995	1925.04	<0.0001	18.49	7.10	0.16
4H	0.994	1903.55	<0.0001	17.27	5.05	0.12
5H	0.998	7557.23	<0.0001	8.41	7.80	0.26

SAN VITO 2006

ID	r^2	*F*	*p*	*a*	*b*	*c*
1C	0.991	1892.48	<0.0001	22.99	2.44	0.11
2C	0.976	524.42	<0.0001	14.01	3.46	0.12
3C	0.989	1157.12	<0.0001	27.30	3.30	0.11
4C	0.992	1089.12	<0.0001	21.59	5.57	0.14
5C	0.998	4449.44	<0.0001	40.14	4.53	0.12
1H	0.999	55446.60	<0.0001	27.06	2.42	0.26
2H	1.000	1285681.00	<0.0001	13.01	1.79	0.36
3H	0.999	24716.80	<0.0001	18.85	3.05	0.27
4H	1.000	97978.10	<0.0001	17.39	2.15	0.20
5H	0.996	5414.52	<0.0001	20.20	2.48	0.15

SAN VITO 2007

ID	r^2	*F*	*p*	*a*	*b*	*c*
1C	0.989	1273.74	<0.0001	6.18	4.28	0.15
2C	0.987	1224.36	<0.0001	11.32	3.38	0.14
3C	0.994	1836.24	<0.0001	10.73	5.14	0.12
4C	0.997	3638.10	<0.0001	15.91	7.06	0.16
5C	0.995	2205.23	<0.0001	24.11	5.79	0.14
1H	0.999	29424.80	<0.0001	26.96	3.28	0.26
2H	0.992	5867.47	<0.0001	3.51	3.47	0.17
3H	0.999	24984.20	<0.0001	12.77	4.39	0.26
4H	0.986	1679.90	<0.0001	13.91	2.42	0.15
5H	0.998	9834.14	<0.0001	20.36	5.87	0.30

Tab. 5.1 Variation in current apical shoot length (*L*) with time (days from the first measurement) in 2006 and 2007 at both sites, Monte Rite and San Vito. Estimates for the parameters of the fitting logistic equation: *a* is the upper asymptote (i.e., apical shoot length); *b* is the translation coefficient along the *x*-axis; *c* is the shape coefficient describing the steepness of the curve between the two inflection points.

The current longitudinal increment (i.e., the length of the apical shoot after the second year of heat treatment, 2007), compared to the averaged increment of the 5 years before the treatment (2001-2005), was significantly higher in heated trees at Monte Rite (Paired t-test: t=3.56, p=0.024), while control trees did not present any significant changes in the current shoot length (Paired t-test: t=1.80, p=0.146), nor did trees at San Vito, either the heated ones (Paired t-test: t=1.38, p=0.239) or those provided with unheated cylinders (Paired t-test: t=1.07, p=0.346) (Fig. 5.3).

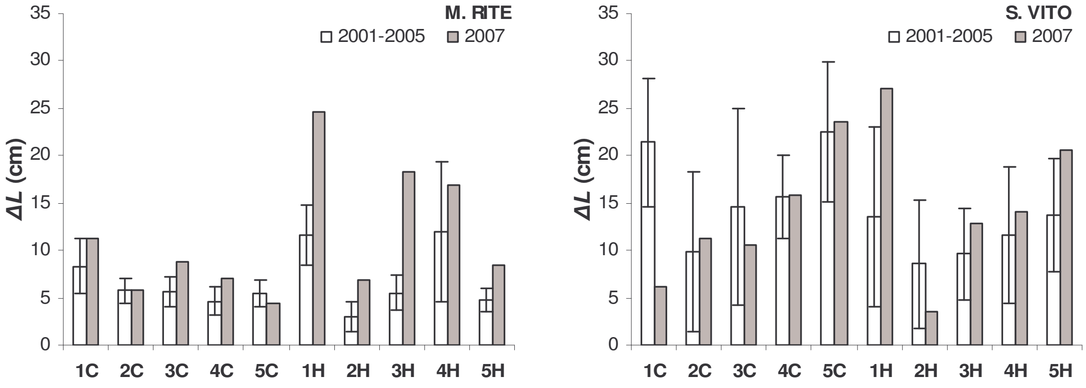


Fig.5.3 Mean longitudinal annual increment (*ΔL*) over the period 2001-2005 (white; error bars are the standard deviations) and the annual increment of 2007 (grey) of all trees of both sites, Monte Rite (left) and San Vito (right).

At Monte Rite, the apical conduits (at 2 cm from the apex) of heated trees were significantly wider in 2007 (i.e., after the two-years treatment) than 2005 (Paired t-test[3]: t=6.88, p=0.006), while controls did not show any significant difference (Paired t-test: t=0.26, p=0.808). At San Vito, instead, heated trees had similar apical conduits in both 2005 and 2007 (Paired t-test: t=0.51, p=0.637), whereas controls showed a significant reduction in width of lumina of apical cells in 2007 (Paired t-test: t=3.78, p=0.019) (Fig. 5.4).

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[3] This paired t-test was performed only on four trees out of five, because the most distal part of the apical shoot of 1H at Monte Rite had burnt after its accidental and prolonged contact with the heated internal resistance of the cylinder.

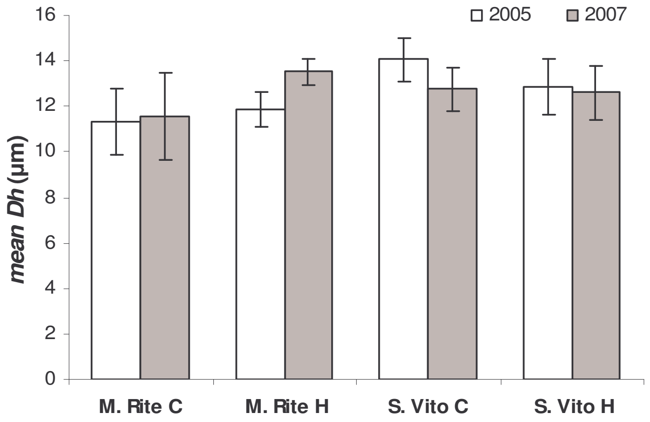


Fig. 5.4 Values of hydraulically weighted diameters (Dh) of the apices of 2005 (white) and 2007 (grey) averaged for treatment (cold, C, and heated, H) and site (M. Rite, high altitude, and S. Vito, low altitude). Error bars represent standard deviations.

In eight trees out of the ten at Monte Rite, the anatomical analyses revealed that the variation in *Dh* with the distance from the apex (*L*) is well described by a power function, with *L* explaining 80-95% of the total *Dh* variance (Tab. 5.4). The $\log_{10}$-transformed scaling coefficient *a* varied from 0.965 to 1.072, while the scaling exponent *b* varied from 0.132 to 0.202 and was significantly smaller than the WBE predicted value of 0.198 in four cases out of the 10 analyzed. Of these four, two concerned the same control tree (i.e., the *Dh* profiles of 1C relative to both 2005 and 2007), whereas the other two cases were relative to *Dh* profiles of 2005 of heated trees. However, this result is certainly affected by the less significance of the power function in 2007 than 2005.

ID	***Year***	r^2	***a***	**95% CI *a***		***b***	**95% CI *b***		$\bar{a}$	**95% CI $\bar{a}$**	
				min	***max***		***min***	***max***		***min***	***max***
1C	2005	0.87	1.07	1.04	1.22	0.15	0.05	0.17	0.12	0.05	0.15
1C	2007	0.86	1.06	1.03	1.11	0.14	0.12	0.17	0.12	0.10	0.14
1H	2005	0.91	1.07	1.04	1.19	0.16	0.08	0.19	0.13	0.07	0.16
1H	2007	0.80	1.01	0.93	1.13	0.16	0.10	0.21	0.14	0.08	0.18
3C	2005	0.91	1.00	0.89	1.05	0.18	0.15	0.27	0.15	0.13	0.22
3C	2007	0.87	0.96	0.76	1.01	0.18	0.15	0.31	0.15	0.13	0.26
3H	2005	0.95	1.04	1.00	1.12	0.16	0.11	0.19	0.13	0.09	0.16
3H	2007	0.87	1.06	0.93	1.10	0.14	0.11	0.21	0.11	0.09	0.18
4C	2005	0.95	1.02	0.93	1.06	0.19	0.16	0.24	0.16	0.13	0.20
4C	2007	0.91	1.05	0.95	1.09	0.16	0.13	0.22	0.13	0.11	0.18
4H	2005	0.98	0.99	0.95	1.03	0.20	0.18	0.23	0.17	0.15	0.19
4H	2007	0.89	1.04	0.92	1.08	0.13	0.11	0.20	0.11	0.09	0.17

Tab. 5.4 Variation of hydraulically weighted diameter (*Dh*) with path length (*L*): estimates for the $\log_{10}$-transformed scaling parameters *a* and *b*, the WBE parameter $\bar{a}$ and their 95% confidence intervals.

Moreover, we found that the *Dh* at the apex is strictly correlated with the degree of conduit tapering (Fig. 5.6). In our heated trees, in four cases we verified an increase in the dimension of apical conduits coupled with a simultaneous reduction in the degree of conduit tapering. Instead, in H1 we estimated a decrease in *Dh* at the apex accompanied by an increase in *b*, but the actual measurements of lumen areas of conduit at the apex were not possible as the most distal part of the apical shoot had burnt against the internal resistance.

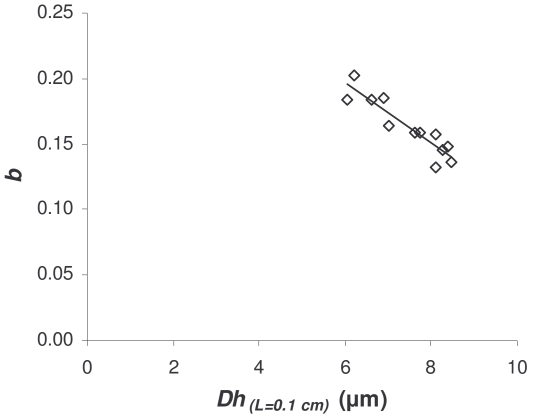


Fig. 5.6 Relation between the hydraulically weighted diameter (*Dh*) at the apex (estimated for each plant from the equation $Dh=aL^b$, with L=0.1 cm) and the scaling exponent *b*, describing the tapering of xylem conduits. The equation of the fitting curve (RMA regression) is b=0.347-0.025·$Dh_{(L=0.1\ cm)}$; r^2=0.842..

5.5 Discussion

The preliminary results presented here showed that the artificial warming applied to the developing buds of the stem's current apical shoot promoted cell enlargement and an increase in longitudinal growth at the treeline, in agreement with previous observations (Gorsuch & Oberbauer 2002; Danby & Hik 2007), whereas at the closed forest site we registered no patterned variation either in the dimension of apical xylem cells or in height growth.

It has been hypothesized (Aloni & Zimmermann 2001; Aloni 2001) that the tapered xylem architecture is controlled by a phytohormone (auxin), which is produced by the developing shoots. Auxin would promote cell lignification, hence would interrupt the distension phase, the length of which has recently been demonstrated as the most fundamental determinant for the final width of xylem conduits (Tedoldi 2004; Anfodillo et al. in preparation). The basipetal free diffusion of the phytohormone would determine a longitudinal gradient of concentration, the effect of which is the formation of a tapered xylem network.

These data supported the notion that the time for cell doubling rapidly decreases with increasing temperature towards an asymptotic value (Körner 2003). In fact, in those trees limited by low temperatures (at the treeline), the artificial warming promoted a substantial enlargement of conduits, whereas the increased temperature had no effects on the wood anatomy of trees growing in the closed forest.

Overall, the still-partial-analysis of the conduit tapering of the sampled trees suggested that the xylem architecture of those at the treeline is optimally or nearly optimally tapered, i.e. the degree of tapering theoretically minimizes the hydraulic resistance, whose variation with the distance from the apex approaches a nearly constant value (West et al. 1999; Becker et al. 2000). Since low temperatures seemed to limit cell widening in the same trees, it can be speculated that those trees are at their maximum hydraulic efficiency compatible with such cold temperatures, i.e. their whole-tree hydraulic resistance is the minimum possible. The artificial warming applied to the apical shoots enhanced the hydraulic efficiency of the hydraulically most constrained part of the stem (i.e., the apical part) by promoting cell enlargement, with the consequence of an increase in stem elongation.

To conclude, these results add new important information on the effect of artificial warming on the xylem anatomy, suggesting a possible revised hypothesis for the actual cause of the formation of the treeline. We point out that our observations are strongly in agreement with the hypothesis of a sink limitation in trees at the treeline. Nevertheless, we speculate that this might set a hydraulic limitation to the further growth in height, as well as in tall trees (Ryan & Yoder 1997; McDowell et al. 2002b; Koch et al. 2004). However, the conclusion of the data analysis of all the sampled trees will hopefully add clarifying information. Data on a possible limitation to conduit width at the base of the stem induced by low temperatures would seem particularly important to verify whether trees at the treeline are actually at their maximum hydraulic efficiency and hence whether their limit to height is determined by the effect of hydraulic constraints (Koch et al. 2004) imposed by the reduced sink activity (Körner 2003).

6 Hydraulic limitations and heartwood formation in sycamore (*Acer pseudoplatanus* L.)

6.1 Abstract

- Recent advances in tree physiology revealed that, at least in *Acer* spp., (i) the pathways of sap flow are substantially independent within the xylem, (ii) conduit tapering is the most effective mechanism of minimization for the effect of the increased tree height on hydraulic constraints and (iii) size-mediated modifications of the xylem anatomy are typical of trees approaching their maximum height. We tested the hypothesis of a size-mediated determinant of heartwood formation under the effect of increased hydraulic constraints with increased tree height of the most internal sapwood rings.
- We injected a safranine solution into the innermost ring of the basal cross-cut of four branches of *Acer pseudoplatanus* L and examined the spread of the dye once the apical apices were stained. Also, in other two branches we estimated the radial flow by injecting water into the innermost ring with different pressure heads than the pressure head applied to the remaining cross-cut. For one of the branches, we analyzed the longitudinal profile of the conduit widths of the innermost and the outermost ring and estimated the increase in hydraulic resistance with increasing heights.
- Results showed that the dye substantially flowed only through the innermost ring all along the branch and radial flow is determined by radial gradients of water potentials. The anatomy of this pathway revealed a nearly cylindrical profile of conduit widths, while that of the outermost ring was more tapered, yet the degree of conduit tapering was low, as commonly reported for lateral branches. So, the estimated total hydraulic resistance for the innermost ring increased more steeply than that for the outermost ring, and this difference is likely to be even more evident where the conduit tapering is optimized, like in the stem.
- Overall, the experiment supported the hypothesis of the independence of pathways and that there may be a direct connection with a reduced

resistance around the last node zone, where the xylem of the previous year and that of the current year are directly connected through an apical resistance, which must be lower than the radial resistance.

- We conclude by proposing a size-mediated hypothesis for the heartwood formation as a consequence of the loss of conductivity of the most internal sapwood rings due to the increase in the hydraulic resistance of their pathways as a tree gets taller.

6.2 Introduction

Trees deliver water from roots to leaves throughout a network system composed of dead cells, known as the xylem. The ontogeny of the xylem architecture has always been modelled as successive annual additions of a new xylem cone over the cone formed during the previous year (Fig. 6.1a). Until the xylem conducts water, it is known as sapwood, while the xylem portion become non-conducting is called heartwood. The amount of heartwood within the trunk has long been an object of study, especially by wood technologists because of its better mechanical properties and higher natural durability compared to those of sapwood (e.g., Taylor et al. 2002). Despite many hypotheses having been proposed to explain the heartwood formation, it is still unclear what is the actual physiological mechanism controlling the transition from conductive to non-conductive xylem. According to some authors, this process should be somehow under a genetic mechanism of control: trees would deposit toxic and excretory substances in the internal part of the trunk, which would hence become non-conductive (e.g., Stewart 1966; Bamber 1976), while useful mineral nutrients would be recycled from senescing sapwood (e.g., Meerts 2002; Magel et al. 1994). However, these appear to be weak proof that genetics controls the heartwood formation, because the mobilisation of compounds from the external sapwood inwards can be either the cause of the heartwood formation due to the occlusion of the more internal conductive cells, or the effect of a xylem tissue becoming no longer conductive for other reasons and hence becoming a sort of trash bin of the tree. Moreover, it is well known that in many species two different types of heartwood can be distinguished: the internal heartwood, which typically presents different chemical compounds and colour, and the external heartwood, which looks like the sapwood and differs from this only because it is simply non-conductive.

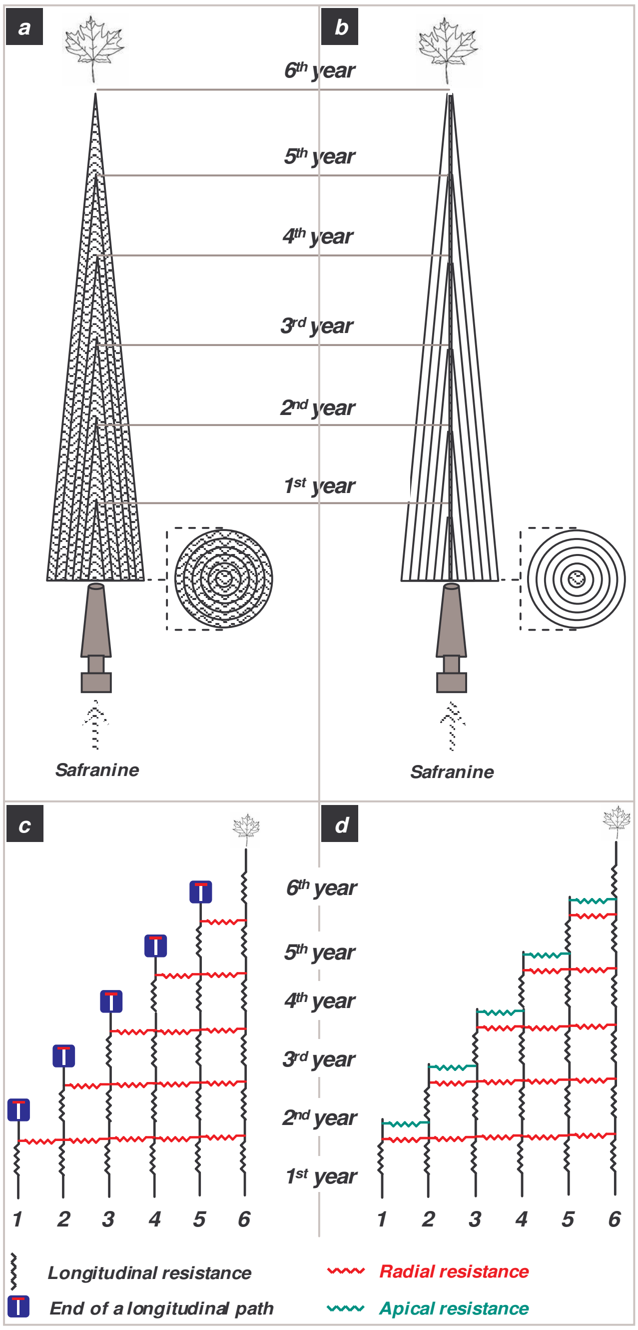


Fig. 6.1 Two comparative models for tree growth and water transport system functioning. In box *a*, a new xylem cone is added every year on the cone formed the previous year. Only the last cone delivers water directly to the leaves, while the water contained in the internal xylem cones needs to flow laterally through radial resistances (typically pit membranes; box c). If the innermost ring had stained, all the xylem would be coloured. In case b/d, apical resistance would guarantee a direct connection between each xylem cone and the leaves (each xylem cone would be opened at the top); if the innermost ring had stained, only the innermost ring at every internode would be coloured.

Following the appearance of the pipe model theory (Shinozaki et al. 1964), which states that the amount of sapwood should be proportional to the amount of leaves it serves (the model considers a tree as a set of conducting, cylindrical pipes running from roots to leaves), it has been hypothesised that the development of heartwood serves to regulate the amount of sapwood to a physiological optimum level, i.e., an optimal water supply to the leaves (Bamber 1976). Several studies seemed to agree with this hypothesis. For instance, Sellin (1994) found that, in *Picea abies* (L.) Karst., the amount of sapwood at the stem base becomes constant after a certain age and, consequently, the increment in diameter becomes proportional to the heartwood formation. Similarly, Gjerdrum (2003) and Knapic & Pereira (2005) found that in *Pinus sylvestris* L. and *Pinus pinaster* Ait., respectively, heartwood develops at rates (number of annual rings per year) that increase with age and get close to one ring per year in old trees.

Parallel to this way of thinking that heartwood formation is an age-related phenomenon, other researches demonstrated that either biomechanical or hydraulic constraint can affect its development (Stokes & Berthier 2000; Berthier et al. 2001; Climent et al. 2002).

In the recent past, conspicuous advances in tree physiology have substantially improved the knowledge on some phenomena, which have been long debated as to whether they were age- or size-related. For instance, the reduced growth rate with increased age and/or dimensions and, on a stand scale, the reduction in forest productivity, seem to have been solved in favour of the size-related hypothesis by using grafts of donor trees of different age/size classes (Mencuccini et al. 2005; Matsuzaki et al. 2005; Bond et al. 2007; Mencuccini et al. 2007a; Vanderklein et al. 2007). The same technique was also used to demonstrate that reduced tree growth is accompanied by a less efficient hydraulic transport system (Petit et al. 2008). Also, Koch et al. (2004) highlighted the central role played by hydraulic constraints in dimensioning trees. According to the fractal-like model of West et al. (WBE, 1999), a tapered structure of the water transport system is essential to the biological functioning of trees and their dimensions and shapes origin from allometric scaling laws connected with it. Despite this model appearing controversial in its assumptions and in the logical and biological derivations of its relationships (e.g. Dodds et al. 2002; Kozlowski & Konarzewski 2004), several empirical measurements agreed with its predictions (Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007, Petit et al. 2008). Although other studies also reported deviations from WBE predictions, Petit et al. (2007) clearly demonstrated, at least for sycamore (*Acer pseudoplatanus* L.) trees, that the tapering of xylem conduits substantially compensates for the increase in hydraulic resistance with path length.

In this study, we conducted a simple experiment to test a hypothesis for a size-related mechanism inducing heartwood formation, driven by the increase in hydraulic constraints with increased tree height. Each of the successive annual cones that compose a tree typically presents that minimum degree of conduit tapering (the WBE $\bar{a}\approx 1/6$: Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007, Petit et al. 2008) allowing the minimisation of the effects of the hydraulic constraints (Becker et al. 2000). Moreover, although the apical conduits are known to slightly change in dimensions during ontogeny (Petit et al. 2008), we can speculate that the minimisation of the hydraulic resistance is assured by the increase in conduit dimensions at the stem base (Anfodillo et al. 2006), where the increase in conduit dimension from the pith outwards is well documented (e.g., Zimmermann 1983).

We carried out a staining experiment to test the existence, between two successive annual cones, of an apical connection characterised by lower hydraulic resistance than the radial resistance between the two adjacent rings, i.e., the resistances in parallel of the pits connecting the conduits of the two adjacent rings. This would lead the water of the internal rings to flow upwards through a preferential path and not laterally towards the external rings. As a tree grows taller, above the internal tapered cones, these preferential paths would become untapered because distally it is composed essentially of the apical conduits of the newer annual cones. Consequently, under the same difference of water potential (from roots to leaves), the increasing values of hydraulic resistance from the outermost ring inwards would cause a decrease in water flow from the outside to the inside of the trunk, as often reported (e.g., Nadezhdina et al. 2002; Ford et al. 2004). The heartwood would hence be formed because the hydraulic resistance of these paths would increase with the increased height until the water stops flowing.

6.3 Materials and methods

We pruned five branches of around 1 m at the base of the crown of a ca. 15-m-high sycamore (*Acer pseudoplatanus* L.) tree outside the laboratory of the School of GeoSciences (University of Edinburgh) at the end of February 2007, i.e., a period of the year when the physiological processes are reduced to a minimum, hence the best condition to avoid possible effects of cambial activity on the distribution of the water flow within the branch. The base of each branch was uniformly flattened under water with a razor blade to eliminate those first millimetres from the cut that may have been cavitated and all the possible pathways were opened by cutting all the buds. A liquid injector was then inserted into the innermost ring, while the rest of the cross-section was connected to a source of distilled and degassed water. The source of water was lifted up to ca. 1 m from the basal cut of the branch to create a pressure drop. Then, on three branches (SB1, SB2 and SB3), a bowl filled with a safranine solution (1% in distilled and degassed water) was connected to the injector and lifted to the same height (i.e., to the same water potential) as the water source. Lastly, each of theese branches was accurately placed upside-down to facilitate the water flow along the longitudinal axis and remained connected to the sources of both water and safranine solution until the most distal apex was coloured (ca. 48 hours). Then, for three branches out of four, the safranine supply was substituted by distilled water for an extra 48 hours of rinsing, in order to flow apart all the remaining safranine inside the lumina and hence substantially avoid radial expansion of the coloured solution once the branch was cut, as happened in the first trial carried out without rinsing (Fig. 6.2 left). Each branch was then cut at different distances from the apex, to check which part of the section was stained by the dye. Moreover, for the other two branches (SB4 and SB5), the injector was connected to a second water supplier, which was lifted to different height to create different pressure gradients between the two water sources and hence a radial gradient between the innermost ring and the rest of the xylem. In other words, we considered the boundary between two successive rings as the radial resistance controlling the rate of water flow. The water flow was measured at the source of both supplies (for the innermost ring and for the rest of the xylem) for all the pressure gradients applied.

Lastly, the anatomy of branch SB3 was analyzed: at each cut along the main axis, wooden disks were sampled, embedded in paraffin (Anderson & Bancroft, 2002) and cut with a rotary microtome to obtain sections of 10-12 μm to be stained with safranine (1% in water) and fixed permanently with Eukitt (Bioptica, Milan, Italy). Digital images of the sections were taken at 100x using a light microscope (Nikon Eclipse80i) and lumen areas relative to the innermost and last annual rings were measured by WinCell software (Régent Instruments Inc., Sainte-Foy, QC, Canada). For a more detailed description of sample preparation see chapter 3.3.2. In order to eliminate conduit endings, only those conduits with a lumen diameter greater than half the diameter of the largest conduit (James et al. 2003) were chosen to estimate the hydraulically weighted diameter *Dh* of the innermost and last annual ring (or the last few rings in the case of missing rings):

$$Dh = \frac{\Sigma d_n^5}{\Sigma d_n^4} \qquad \text{equation 6.1}$$

where d_n is the n^{th} conduit diameter (Sperry et al. 1994).

Then, for both the innermost and outermost ring, *Dh* was plotted against the distance from the current apex (*L*) and the scaling was determined from pairwise comparisons of $\log_{10}$-transformed data. Using reduced major axis (RMA) analysis, the scaling exponents and allometric constants were identified as the regression slope (αRMA) and *y*-intercept (βRMA), respectively. Regression coefficients, their 95% confidence and prediction intervals, were computed by standard methods (Sokal & Rohlf 1981) using a bootstrap procedure with 100,000 replications (Davison & Hinkley 1997).

A theoretical estimate of the total hydraulic resistance (*R*) relative to both the innermost and the last annual ring was assessed as the sum of resistances in series of an ideal pipe of the same length as the branch, constituted of tapered cylindrical segments of 1 cm length, whose diameter was derived from the longitudinal *Dh* profile. This approach assumes no conduit furcation (Petit et al. 2008; cf. McCulloh et al. 2003)

6.4 Results

6.4.1 Staining experiments

At the end of each staining experiment, at each cross-cut along the longitudinal axis it could be seen that the safranine flowed mainly through the innermost ring. Immediately after the cut, there was a radial spread of liquid outwards, so the first staining trial relative to branch SB1, which did not comprise the rinsing with water after the dye injection, was characterised by a relevant lateral spread of safranine from the innermost ring outwards (Fig. 6.2 left). Moreover, in branch SB2 the injector was not inserted properly inside the innermost ring, so the injection area spanned across 2-3 rings and the safranine stained the 2-3 innermost rings all along the branch (Fig. 6.2 right).

Fig. 6.2 Cross-sections at different distances along the branch after the safrenine injection in SB1 (left) and SB2 (right). In SB1, after the cross-cuts the staining solution contained inside the conduits spread radially. In SB2, the rinsing reduced the radial spread, but the area of dye injection was not confined within the innermost ring, determining an asymmetric colouration, which extended also to the next 1-2 rings.

Instead, the injector was well inserted inside the innermost ring at the base of branch SB4, and the dye flowed substantially through the innermost ring all along the longitudinal axis, i.e., at each cross-cut it could be seen that the dye flowed substantially within the innermost ring, irrespective of the number of rings in that part of the shoot (Fig. 6.3). However, in some cross-cuts there were traces of radial spread. All the open apices were stained, except a few lateral ramifications originated from adventitious buds.

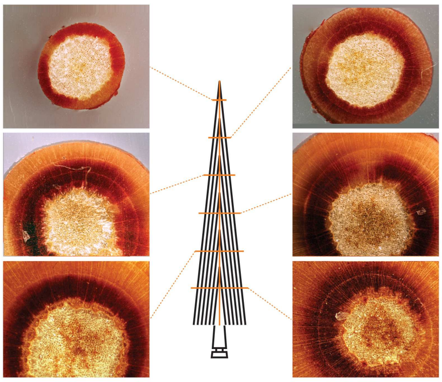
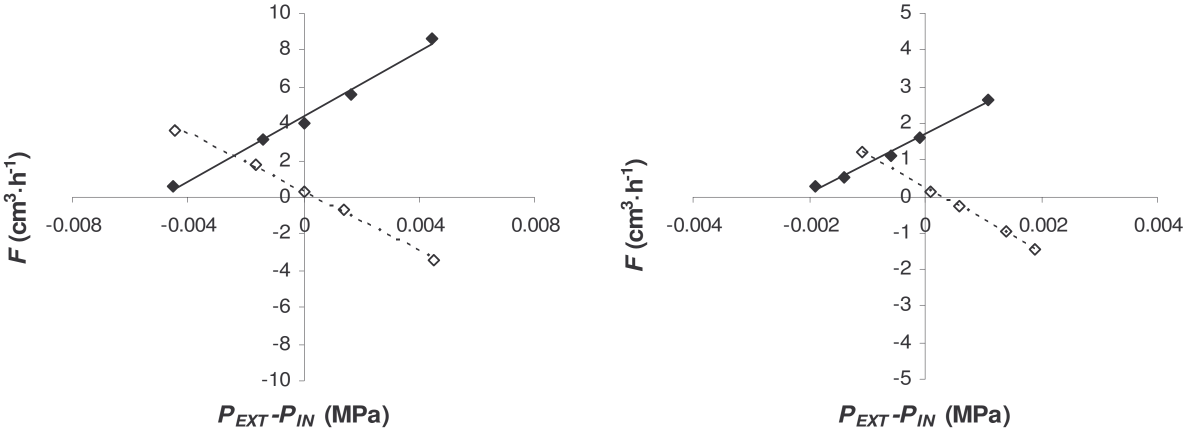


Fig. 6.3 Cross-sections at each internode along the branch after the safrenine injection and rinsing in SB3. The dye remained substantially confined within the innermost ring all along the branch, irrespective of the number of rings at each internode.

6.4.2 Flow originated from radial pressure gradients

The water flow measured at the source increased linearly with the pressure gradient applied between the sources of the innermost ring and the rest of the cross-sectional area (Fig. 6.4).



ID	*a*	*a (95% CI)*	*b*	*b (95% CI)*	*r^2*
SB5_in	0.27	0.14 to 0.47	-796.40	-861.60 to -723.86	0.995
SB5_ext	4.36	3.98 to 4.55	889.35	769.00 to 1028.00	0.993
SB6_in	0.25	0.23 to 0.29	-886.63	-920.00 to -845.74	0.999
SB6_ext	1.69	1.49 to 1.75	814.09	660.80 to 899.02	0.993

Fig. 6.4 Measured radial flows for different pressure gradients between the innermost ring (P_{IN}) and the remaining sapwood (P_{EXT}). The flow is measured at the water supplier for both the innermost ring (dashed line) and the remaining sapwood (solid line) while varying the pressure head of the other.

Without radial pressure gradient (ΔP=0), the estimated flow of the innermost ring did not significantly differ between the two branches, while the flow of the remaining xylem was higher in SB5 (cf. coefficients *a* in the table in Fig. 6.4). Instead, the absolute values of the regression slopes (*b*) were significantly similar (table in Fig. 6.4), suggesting that the radial resistance did not discriminate for the direction of flow. Moreover, the water flow of the innermost ring of both branches converged to 0 for a pressure gradient of around 0.0003 MPa.

6.4.2 Anatomy and hydraulic properties of the innermost and outermost ring

Along the outermost ring, the dimensions of the xylem conduits increased from the apex downwards following a power function (Fig. 6.5), as commonly reported in recent studies on allometric scaling laws for wood anatomy (Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007; Petit et al. 2008). The scaling of the hydraulically weighted diameter (*Dh*) of the outermost ring with the distance from the apex (*L*) was 0.104 (95% C.I. 0.091-0.174; r^2=0.934), significantly lower than the WBE predicted value of 0.20. *Dh* of the innermost ring also increased with *L* and the scaling exponent equalled 0.061 (95% C.I. 0.042-0.114; r^2=0.863).

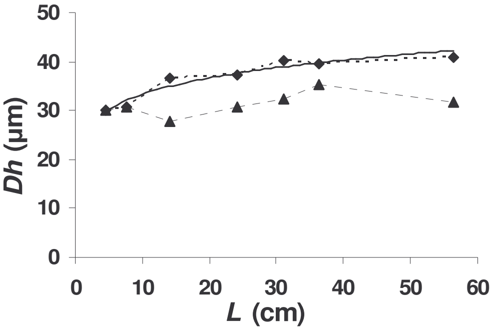


Fig. 6.5 Variation of the hydraulically weighted diameter (*Dh*) with distance from the apex (*L*) for the outermost ring (rhombi; scaling equation: $Dh=27.669 \cdot L^{0.104}$; r^2=0.934) and innermost ring (triangles; scaling equation: $Dh=25.235 \cdot L^{0.061}$; r^2=0.863).

Due to the different anatomical scalings, the estimated total hydraulic resistance (R_{TOT}) of the innermost ring revealed a steeper increase with *L* compared to the outermost ring (Fig. 6.6 left) and, for a length of 60 cm of SB3 (i.e., the length for which the scaling of *Dh* with *L* was estimated), the R_{TOT} of the former was 1.6 times higher than the latter and this factor increased with *L* (e.g.,1.8 at 120 cm; 2.1 at 300 cm; 2.3 at 500 cm).

Fig. 6.6 (right) shows the rate of increase in R_{TOT} with the increased path length for the innermost ring (dashed line) and for the annual new outermost ring (solid line) with reference to the resistance of the outermost ring estimated at 60 cm, kept as control unit. For instance, for a hypothetical branch length of 10 m, the calculated resistance of the outermost ring was six times higher than the control unit, whereas that of the innermost ring was higher by a factor of 16.

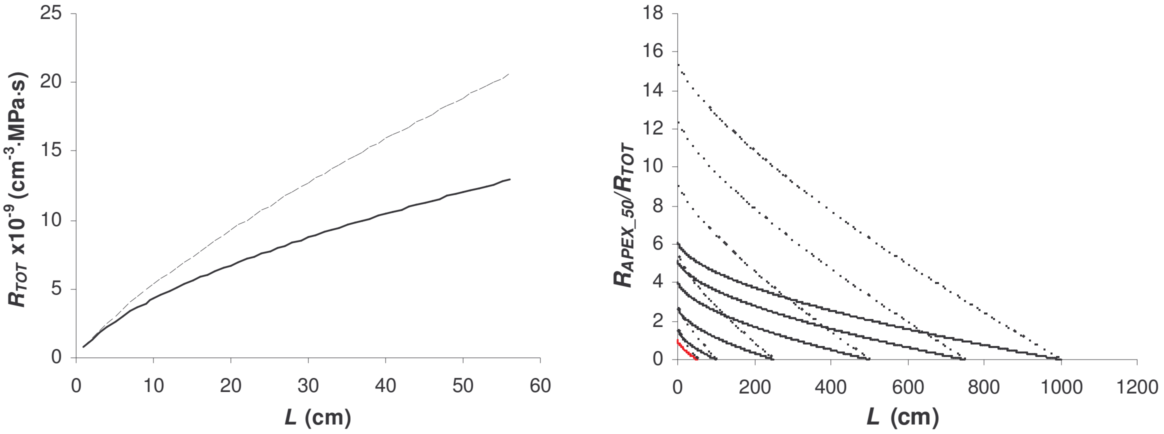


Fig. 6.6 Left: variation of total hydraulic resistance (R_{TOT}) with distance from the apex (*L)* for the outermost ring (solid line) and innermost ring (dashed line). Right: comparison of the simulated increase in hydraulic resistance experienced at the branch apex for the innermost ring (dashed line) and the annual new outermost ring (solid line) with different branch lengths up to 10 m

6.5 Discussion

As a premise, we are aware of the complexity and weakness of the experiments we conducted. Nevertheless, the results seem to be consistent with the starting hypothesis rather than rejecting them. So, as a first trial of a new perspective analysis of a known phenomenon such as heartwood formation, it appears to be worth discussing the novelty of the results and possible enhancements that can be made to permit a stronger research.

Firstly, the staining experiments revealed some intrinsic procedural difficulties. In three cases out of four we had a spread of the dye from the innermost ring outwards. After the cross-cut, the liquid inside the xylem likely moved because of a radial potential gradient, determined by the faster evaporation from the wider conduits (i.e., lower hydraulic resistance) of the more external rings (cf., Zimmermann 1983). In fact, after having introduced the rinsing with water after the safranine injection, we substantially obtained no more radial spread of the dye. Nevertheless, in other two attempts, the dye partially coloured the innermost ring plus some adjacent rings. The likely explanation was the difficulty in precisely centring only the innermost ring with the injector (see the basal cross-cut in Fig.6.2b). When the innermost ring was precisely centred, the dye injected remained substantially confined within it all along the branch and the partial radial expansion can be explained (i) as a free diffusion following the cease of flow and the cross-cuts of the branch, and (ii) as a natural consequence of the interconnection between the latewood and earlywood of the following annual ring (Umebayashi et al. 2007).

Overall, the staining experiments added new information about the independence of water flow pathways in *Acer* species: while McCulloh and Sperry (2005) and Petit et al. (2008) reported no conduit furcation and hence radial sectoriality, we also evidenced a substantial intra-ring sectoriality, i.e., the existence of a low-resistance connection between the apical shoot of the previous year and the apical shoot of the current year. Hence, a tree would be more realistically described as a pile of successive cones with open vertices (Fig. 6.1b/d).

The tapering of xylem conduits (i.e., the increase of *Dh* with *L*) of the outermost ring of the analyzed branch followed a power function with a lower scaling exponent than the WBE prediction (West et al. 1999) and those recently obtained by empirical measurements (Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007; Petit et al. 2008). However, it was reported that the *Dh* profile does not always follow the WBE trajectory (Mencuccini et al. 2007) and branches typically show a less tapered xylem structure than that of the trunk (Petit et al. 2008).

The simulation of increase in the total hydraulic resistance with the increased height for both the innermost and outermost ring revealed a substantially steeper increase for the former than the latter. Nevertheless, this simulation was strongly affected by the estimated tapering of the innermost ring. We argue that this is unlikely to happen and, while the width of the conduits of the last annual ring simply depend on their distance from the current apex, the longitudinal profile of the width of conduits of the innermost ring strongly depend on the sampling point at each internode (i.e., the current shoots of the previous years). Consequently, given a substantial constancy of apical conduit dimensions and conduit tapering (Anfodillo et al. 2006; Weitz et al. 2006), the *Dh* values of the innermost ring along the longitudinal axis should have a “saw-blade” shaped profile (i.e., the innermost ring is similar at each internode, because it simply reflects the anatomical characteristics of the successive annual shoots). Hence, while the build-up of hydraulic resistance of the outermost ring is minimized by the effect of conduit tapering, i.e., the increase in *R* with *L* is less than linear (cf. Petit et al. 2008), for the innermost ring the increase in *R* with *L* tends to linearity, because of the repetition of successive similar moduli of *Dh* internodal profiles (cf. Fig. 6.6 right and Fig. 6.1b/d). Given a physiological threshold for the hydraulic resistance that a tree can experience, it might be the case that the loss of conductivity of the most internal sapwood and its transformation into heartwood is the consequence of the reaching of this limit by the most internal pathways.

To conclude, we highlight the importance of this study in proposing a new perspective on the physiological determinants of heartwood formation. As a tree grows taller, while the newly formed xylem is appropriately tapered to compensate for the effect of height on the total build-up of hydraulic resistance, the innermost rings would progressively experience higher and higher hydraulic resistances, because of the addition at the top of the trunk/branch of a new series of similar resistances (i.e., the resistance of the new annual apical shoot; cf. Fig. 6.6 right and Fig. 6.1b/d) that will finally cause a complete inefficiency of the whole pathway, i.e., the transformation of sapwood (conductive xylem) into heartwood (non-conductive xylem).

Lastly, this work might open up a new frontier for a better understanding of whether heartwood formation is a size-mediated rather than age-mediated phenomenon. Importantly, information on the scaling of heartwood content with tree height rather than tree age would be highly desirable, but future investigations are also required on intra-ring sectoriality in other species and to identify species-specific maxima of hydraulic resistance that a plant can experience, beyond which a hydraulic pathway would collapse.

7 Discussion and conclusions

The investigations presented with this work highlighted the fundamental importance of the tapering of xylem conduits for the ontogenetic development of trees from seedlings to mature specimens. Although this hypothesis was already proposed in the context of the WBE model, i.e., with natural selection, vascular plants had evolved to minimize hydraulic resistance (Enquist 2003), we evidenced a crucial paradox of the model, which is the prediction of higher heights for more hydraulically constrained trees, unless the physical strain of raising water from roots to leaves had been proposed and demonstrated to determine a decline in growth rate and set the limit to the maximum height reachable (Ryan & Yoder 1997; Hubbard et al. 2001; McDowell et al. 2002b; Koch et al. 2004; Mencuccini et al. 2005; Ryan et al. 2006; Martínez-Vilalta et al. 2007). Nevertheless, the outcome of the model had the huge merit of stimulating ecologists to investigate conduit tapering within the context of the hydraulic limitations to tree height and our data agreed with recent analyses evidencing a general convergence to that degree of conduit tapering allowing the independence of the hydraulic resistance from path length (West et al. 1999; Anfodillo et al. 2006; Weitz et al. 2006; Coomes et al. 2007). However, departures from this pattern had been also shown and proposed as conservative strategies of carbon costs without much affecting the whole tree hydraulic efficiency (Mencuccini et al. 2007).

One of the most relevant findings of this work was that, at least in *Acer pseudoplatanus* L., the scaling of the hydraulic resistance with distance from the stem apex is substantially controlled by the tapering of xylem conduits, which hence resulted as the most effective mechanism of compensation for the effect of increased path length on the whole tree hydraulic resistance. We also demonstrated that those trees approaching their height limit are characterized by wide apical conduits and by a non optimized conduit tapering, i.e., no longer effective in minimizing the effect of increasing height on the total hydraulic resistance. The comparison of anatomical features of these tall trees with their 3-year-old grafts revealed that the observed structural changes in xylem anatomy were substantially size-related rather than age-related. In fact, both graft treatments, i.e., from small/young and tall/old parent trees, presented no significant differences in either apical conduit dimensions or conduit tapering, irrespective of their meristematic age, hence rejecting any age-related effect on these features of xylem anatomy.

The analyses on the tapering of xylem conduits in small coniferous trees at the treeline revealed that this is a also very important feature for small path distances at extreme environment sites. We showed that the degree of tapering of trees limited by low temperatures is substantially in agreement with the principle of the stabilization of hydraulic resistance (West et al. 1999; Becker et al. 2000). The heating experiment also supported the hypothesis of a hydraulic limitation to tree height at the treeline induced by the reduced sink activity of the developing tissues caused by low temperatures (Hoch et al. 2002; Körner 2003; Hoch & Körner 2005). In fact, after the warming treatment applied to the developing apical buds, we observed an increase in conduit width in trees at the treeline (temperature limited), in agreement with previous observations (Gorsuch & Oberbauer 2002), but no effects in trees 1000 m below, where growth appeared to be not limited. The whole-tree hydraulic resistance is very sensitive to the size of apical elements (Tyree & Ewers 1991; West et al. 1999; Becker et al. 2000), so the observed increase in conduit width at the apex of the heated trees at the treeline likely demeans the hydraulic resistance, promoting a longer stem increment (cf., Danby & Hik 2007).

Another novel finding was that conduit tapering also extends belowground, where the degree of tapering was on average higher than that in the stem, probably because of nearly absent mechanical constraints. The transition zone at the collar from stem to roots was characterized by a continuous increase in conduit width and the maximum width was measured around the root tips.

Altogether, our anatomical analyses supported the hypothesis proposed by Anfodillo et al. (2006) that the conduit tapering of trees close to their height limit would converge to an optimal degree (West et al. 1999; Becker et al. 2000) to maintain a nearly constant hydraulic resistance with increasing height, whose maximum is essentially set by hydraulic constraints, which are substantially determined by the width of apical conduits.

We are aware that considering the xylem transport system as a set of independent tapered tubes relative only to the last annual growth can appear too simplistic, nevertheless the analyses on *Acer pseudoplatanus* L. revealed a substantial sectoriality of the water transport system. We evidenced through the dye injection into the innermost ring that the water flow remained confined in it all along the internode and merged into the innermost ring of the successive internode and so on until the current apex. Also, the fact that the theoretical scaling of hydraulic resistance with path length estimated from the anatomical features perfectly matched the scaling assessed from actual hydraulic measurements revealed the independence of the stem pathways from those of lateral branches. Moreover, the radial spread of water across different annual rings was demonstrated to be determined essentially by radial gradients of water potentials, in the absence of which the pathways relative to the innermost sapwood rings would directly flow towards the apices (i.e., leaves). We hypothesized that this intra-ring sectoriality can be the cause of the loss of conductivity of the innermost sapwood (i.e., the heartwood formation) because of the increase in the whole pathway resistance due to the serial addition of constant, annual resistances (i.e., apical shoots). A fascinating implication of this hypothesis would be that the amount of sapwood rings would be maintained if the resistance of the annual, apical shoot is maintained as well (i.e., through maximum apical conduit dimensions and optimal conduit tapering). Consequently, the reduction in conduit tapering would determine a domino effect that would progressively reduce the hydraulic efficiency of the whole xylem network. Once the maximum basal conduit width is reached and the plateau begins to form, the most conductive pathway would become less efficient, so increasing the whole tree hydraulic resistance. The elongation rate would hence decrease and, as the apical shoot would simply be shorter so that its resistance would be lower than before, the rate of loss of conductivity of sapwood would decrease, hence increasing the amount of conductive xylem per unit leaf, a phenomenon commonly observed in tall trees (e.g., McDowell et al. 2002a) that can only decelerate the progressive decline of tree growth.

In conclusion, this work evidenced the close connection between the hydraulic limitations to tree growth and the two most important features of xylem anatomy, the dimension of apical conduits and degree of tapering, which was demonstrated as the most effective mechanism of compensation for the increase in hydraulic resistance with increasing path length (i.e., tree height).

Moreover, our data not only supported the hypothesis that departure from the optimal degree of conduit tapering is among the most important determinants setting the limit to tree height, but may also induce structural modifications that can themselves serve as mitigation factors for the effect of hydraulic constraints, such as the decrease in leaf area to sapwood area ratio.

The last hope and recommendation is to further investigate xylem anatomy in different species, to evaluate how potential modifications induced by limiting environmental factors on conduit dimensions and degree of tapering would affect tree growth and to test the hypothesis of intra-ring sectoriality as the cause of heartwood formation.

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