

Could rapid diameter changes be facilitated by a variable hydraulic conductance?

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ABSTRACT

Adequate radial water transport between elastic bark tissue and xylem is crucial in trees, because it smoothens abrupt changes in xylem water potential, greatly reducing the likelihood of suffering dangerous levels of embolism. The radial hydraulic conductance involved is generally thought to be constant. Evidence collected about variable root and leaf hydraulic conductance led us to speculate that radial hydraulic conductance in stem/branches might also be variable and possibly modulated by putative aquaporins. We therefore correlated diameter changes in walnut (*Juglans regia* L.) with changes in water potential, altered by perfusion of twig samples with D-mannitol solutions having different osmotic potentials. Temperature and cycloheximide (CHX; a protein synthesis inhibitor) treatments were performed. The temperature response and diameter change inhibition found in CHX-treated twigs underpinned our hypothesis that radial hydraulic conductance is variable and likely mediated by a putative aquaporin abundance and/or activity. Our data demonstrate that radial water transport in stem/branches can take two routes in parallel: an apoplastic and a cell-to-cell route. The contribution of either route depends on the hydraulic demand and is closely linked to a boost of putative aquaporins, causing radial conductance to be variable. This variability should be considered when interpreting and modelling diameter changes.

Key-words: *Juglans regia* L. (walnut); aquaporins; composite transport model; cycloheximide; mechanistic model (HydR); radial hydraulic conductance; radial hydraulic conductivity; sap flow; stem diameter variation; water channels.

INTRODUCTION

In trees, non-stationary or dynamic flow conditions emerge from time lags that exist between water loss by leaf transpiration and water uptake by roots (e.g., Schulze *et al.* 1985; Goldstein *et al.* 1998; Steppe, Lemeur & Samson 2002). To overcome these temporal imbalances between water loss and uptake, internal water reserves are depleted daily and subsequently replenished overnight,

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causing the diameter of stem, branch and root tissues to shrink and swell accordingly (e.g. Améglio & Cruiziat 1992; Herzog, Häsler & Thum 1995; Zweifel, Item & Häsler 2000; Zweifel & Häsler 2001; Steppe & Lemeur 2004; Daudet *et al.* 2005; Steppe *et al.* 2006). These diameter changes are mainly determined by water content changes in the elastic living tissue of the bark (Zweifel *et al.* 2000; Cochard, Forestier & Améglio 2001; Steppe & Lemeur 2004; Steppe *et al.* 2006), whereas the xylem undergoes only small fluctuations (Irvine & Grace 1997; De Schepper & Steppe 2010). Water released from the bark serves as an important buffering system for smoothing abrupt changes in xylem water potential. It is suggested to prevent xylem dysfunction by cavitation events that might occur when sudden transpiration peaks exceed root water uptake (Zweifel, Item & Häsler 2001).

Water in internal storage locations in the bark is hydraulically connected to water in the xylem. Based on the small time lags in dimensional changes, Scholz *et al.* (2008) suggested that the parenchyma cells between the dead bark tissue of the periderm and the cambium in stems of Brazilian cerrado tree species are hydraulically well connected with the xylem. A tight hydraulic coupling enables water stored in the elastic storage tissues to contribute directly to the transpiration stream (Simonneau *et al.* 1993; Génard *et al.* 2001; Zweifel *et al.* 2001; Steppe & Lemeur 2004; Steppe *et al.* 2006; Sevanto, Hölttä & Holbrook 2011). The current understanding of this phenomenon is that a drop in xylem water potential creates a water potential difference ($\Delta\Psi$) between the xylem and the elastic tissues located in the bark, which induces a radial flow of water and, thus, a shrinkage in diameter (Cochard *et al.* 2001). This radial water flow (mg s^{-1}) is commonly modelled as (Génard *et al.* 2001; Steppe *et al.* 2006):

$$\text{Radial water flow} = L_p(\Psi_x - \Psi_b), \quad (1)$$

where L_p is the radial hydraulic conductance of the water pathway ($\text{mg MPa}^{-1} \text{s}^{-1}$) and $\Psi_x - \Psi_b$ the difference in total water potential between xylem and bark (MPa). Values of radial water flow are negative when water is withdrawn from the elastic bark tissues and positive when the bark tissues are refilled. Total water potential in the xylem equals, in this study, the osmotic potential of the perfused

solution (Π_x , see below), while total water potential in the bark is the algebraic sum of the hydrostatic pressure P_b (i.e. turgor) and the osmotic potential Π_b . The radial hydraulic conductance is commonly associated with a 'virtual' membrane that separates two coaxial cylinders representing the xylem and the bark, and is generally accepted to be constant (Génard *et al.* 2001; Steppe *et al.* 2006; De Pauw, Steppe & De Baets 2008; Steppe, De Pauw & Lemeur 2008a,b; De Schepper & Steppe 2010, 2011; De Swaef & Steppe 2010).

Recent evidence, however, shows that leaf and root hydraulic conductance can be highly variable due to an up-regulation of plasma membrane aquaporin abundance and/or activity (Javot & Maurel 2002; Tyerman, Niemietz & Bramley 2002; Cochard *et al.* 2007). Aquaporins are water channel proteins that facilitate the transport of water across cell membranes (Maurel *et al.* 2008). The possible involvement of plasma membrane aquaporins in the radial water movement of water between the bark and the xylem in stem and branches remains, however, unclear. Comparable with the composite water transport discovered in roots and leaves (e.g. Steudle 2000; Cochard *et al.* 2007), it can be expected that radial water transport in stem and branches may also follow an apoplastic (through cell walls) and/or a cell-to-cell pathway (through plasmodesmata or across cell membranes) depending on the environmental conditions (Supporting Information Fig. S1). The notion that aquaporins might contribute to modulating hydraulic conductance in roots and leaves was found from studies in which the activity of some plasma membrane aquaporins was blocked by HgCl_2 (Javot & Maurel 2002) or a physiological concentration of cycloheximide (CHX) (Cochard *et al.* 2007).

The objective of this study is to investigate the relative importance of putative plasma membrane aquaporins in modulating radial hydraulic conductance (L_p) and, thus, water movement between the elastic water storage locations in the bark and the xylem. The study focuses on walnut tree (*Juglans regia* L.) twigs. We correlate changes in twig diameter with changes in $\Delta\Psi$, $\Delta\Psi$ being altered by perfusion of twig samples with D-mannitol solutions having different osmotic potentials in order to provoke changes in Π_x . D-mannitol is used because this solute is known to be a non-permeating osmoticum (Cochard *et al.* 2001). So far, no plasma membrane transporter for mannitol has been identified in walnut, while transporters for sucrose and glucose, and their involvement in bud break and radial stem growth, have been described (Decourteix *et al.* 2006, 2008; Bonhomme *et al.* 2010). D-mannitol is therefore the preferred solute in this study because its use will exclude possible disturbing effects of active sugar uptake from the perfused xylem solution into the bark during the assessment of radial water movement. From the measured twig diameter changes, we estimate L_p with a mechanistic model. We examine whether L_p alters by applying step changes in temperature and whether radial water movement between the bark and the xylem is inhibited by a physiological concentration of CHX, a protein synthesis inhibitor.

MATERIALS AND METHODS

Plant material

Experiments were conducted on cut twigs of mature 10 m tall walnut (*J. regia* L. cv. Franquette) trees growing in an orchard at the Institut National de la Recherche Agronomique, Physique et Physiologie Intégratives de l'Arbre Fruitier et Forestier (INRA PIAF) site (Clermont-Ferrand, France). All measurements were conducted on 1-year-old twigs with a diameter of 9.4 ± 1.5 mm. At each sampling event, three randomly selected twigs of about 1 m length were harvested between 0800 and 0900 h. The twigs were immediately defoliated in the field to prevent dehydration by leaf transpiration, recut under water to minimize cavitation, enclosed in black plastic bags and brought to the laboratory, where they could rehydrate in full darkness for at least 3 h.

Twig perfusion

The aim of the experiment was to perfuse the xylem of short walnut twig segments with D-mannitol solutions of different osmotic potentials at constant temperature and follow the changes in diameter. To this end, 10 cm long twig segments were cut under tap water in the laboratory. Both cut surfaces were trimmed under water using a razor blade to reopen all the vessels. The bark at the top of the twig segments (a 1 cm strip) was removed to ensure that only the xylem was exposed to the perfused solution. Parafilm was used to seal the naked xylem/bark intersection, leaving the cut surface open. Initial twig diameters were measured and further used to normalize the measured diameter changes. Next, the segments were flushed with a pressure of 100 kPa (Xyl'em, Bronkhorst, Montigny-Les-Cormeilles, France) using degassed water that was deionized and filtered through a $0.1 \mu\text{m}$ filter with an ultrapure water system (Milli Qplus 185, Millipore, Molsheim, France) for at least 1 min to dissolve air bubbles. After flushing, the twig segments were placed vertically in a climate chamber (serie KBF, Binder GmbH, Tuttlingen, Germany) to assure accurate control of the air temperature (Fig. 1). The samples were mounted in an Invar-made array holder, for which no temperature expansion correction was needed. The samples were mounted with the upper part down to mimic the natural sap stream circulation and fitted to plastic tubing (Fig. 1). The samples were first perfused with deionized water to make sure that the xylem water potential remained at 0 MPa, while a linear variable displacement transducer (LVDT; model DF2.5, Solartron Metrology, Massy, France) was mounted on each twig segment and connected to a datalogger (model DL2e, Delta-T Devices Ltd, Cambridge, UK). The changes in twig diameter (accuracy $< 1 \mu\text{m}$) were recorded every minute. Perfusion with deionized water continued for at least 2 h after sensor installation at constant air temperature in the climate chamber. The twig temperature, measured with a thermocouple attached to one of the samples, reached fairly quickly the set air temperature during this stabilization period. Xylem water potential was



Figure 1. Measurement set-up showing the climate chamber and the twig samples mounted in a linear variable displacement transducer (LVDT)-array. The twig samples were fitted to plastic tubing and connected to a reservoir filled with either deionized water or a D-mannitol solution. The inset shows a detail of the mounting of a twig segment.

close to zero with this set-up because xylem conduits were exposed to a very low and constant hydrostatic pressure. When the diameter readings stabilized, the tubing was emptied, and samples were perfused with a D-mannitol solution from a reservoir (Fig. 1).

Different solutions were obtained by diluting the appropriate quantity of D-mannitol with distilled water in order to obtain different osmotic potentials (Π_x). The Π -value of each solution was verified at the beginning of each experiment with a freezing-point osmometer (model 13DR, Roebing, Berlin, Germany). The samples were perfused with the solutions for at least 13 h. As controls, two segments per experiment were continuously perfused with deionized water. Diameter changes were barely detectable on the control segments. At the start of the experiment, the Π -values of the liquid dripping out of the twig segments was periodically measured with the osmometer. A few minutes after the reservoir solution was connected, the dripping solution was at the same Π -value as the solution in the reservoir, indicating that a step change in Π_x was realized.

Model

To determine radial hydraulic conductance (L_p) from the measured changes in twig diameter, a mechanistic model (HydR) was built using the same basic principles of the flow and storage model originally developed by Steppe *et al.* (2006). The HydR model is described in the Supporting Information in the online version of this paper. Briefly, the model uses the osmotic potential of the perfused solution (Π_x) as an input and links this directly to changes in twig diameter through radial water transport across a radial hydraulic resistance ($R_p = 1/L_p$). Water transport out of the elastic bark tissues into the xylem induces changes in bark water content (W_b) and, hence, bark turgor (P_b), causing the

twig diameter (D) to shrink accordingly (Supporting Information Fig. S1). Measurements of D were used to calibrate the model and estimate L_p .

The HydR model was implemented, simulated and calibrated using the plant modelling software PhytoSim (Phyto-IT BVBA, Mariakerke, Belgium; further details are given in the online Supporting Information). HydR is available in the built-in plant model library of PhytoSim, including simulation and calibration examples.

Treatments

Changes in twig diameter were evaluated at 1, 10, 20 and 30 °C using solutions of D-mannitol with a Π -value of -1.0 MPa. To account for the change in L_p (estimated from the measured twig diameter changes using HydR) caused by the temperature dependence of the viscosity of water, L_p was standardized at 20 °C as follows:

$$L_{p20^\circ\text{C}} = L_{pT} \times \eta_T / \eta_{20^\circ\text{C}} \quad (2)$$

where $L_{p20^\circ\text{C}}$ is the temperature-corrected radial hydraulic conductance, L_{pT} is the actual radial hydraulic conductance at temperature T , and η_T and $\eta_{20^\circ\text{C}}$ is the dynamic water viscosity at T and 20 °C, respectively. No standardization of L_p was needed for the viscosity effects of the perfused solution because the radial water flow from the elastic bark tissues into the xylem was D-mannitol free. To determine the temperature dependence of the diameter change response, six to eight samples were used for each temperature. Each sample yielded its unique L_p value through model calibration, from which the mean and standard deviation (SD) were calculated.

To link possible plasma membrane aquaporins to variable L_p and diameter changes, and, hence, changes in the protein-mediated cell-to-cell pathway, a series of cut twig segments were treated with a 100 μM CHX (an inhibitor of protein biosynthesis) solution. CHX experiments were performed at 20 °C. The experimental samples were first perfused with deionized water containing 100 μM CHX for at least 2 h to ensure that aquaporin abundance and/or activity was already affected before the samples were perfused with D-mannitol solutions having different osmotic potentials and 100 μM CHX. Solutions with Π -values of -0.5 MPa, -1.0 MPa and -2.0 MPa were evaluated and compared with control D-mannitol solutions (without CHX). The change in contribution of the cell-to-cell pathway to the overall radial water transport was estimated by subtracting L_p in the presence of CHX from total L_p (in the absence of CHX) divided by total L_p . To determine the effect of CHX on diameter changes, six to eight samples were used for each osmotic solution. Through model calibration, a unique value was found for L_p for each sample, from which the mean and SD were calculated.

Statistical analysis

The statistical significance of the difference in average L_p for the temperature and CHX treatments was determined

using the two-sample *t*-test for comparison of means with equal variances (Rosner 2000).

RESULTS

Temperature effect on diameter changes in cut walnut twigs

To understand the temperature effect on radial water movement between the bark and the xylem, twig samples were perfused with D-mannitol having an average Π_x of -1.05 ± 0.05 MPa (Fig. 2). Despite the same $\Delta\Psi$ created between the xylem and the elastic tissues in the bark, the response curves were different and dependent on temperature. The diameter showed the steepest decrease at 30 °C ($2.47 \pm 0.31\%$ of the initial twig diameter or a maximum shrinkage of 208 ± 40 μm). This was followed by the decrease obtained at 20 °C ($1.85 \pm 0.45\%$ or 154 ± 59 μm) and 10 °C ($0.95 \pm 0.21\%$ or 79 ± 21 μm), while at 1 °C the shrinkage was barely detectable ($0.42 \pm 0.14\%$ or 36 ± 13 μm) (Fig. 2).

L_p derived with HyDR from the twig diameter changes showed an important dependence on temperature (Fig. 3). When L_p was corrected for changes in water viscosity with temperature (Eqn 2), an important dependence with temperature remained (Fig. 3). Average corrected L_p at 30 °C was significantly different from that at 1 and 10 °C ($P < 0.01$). Corrected L_p increased with temperature (T) across the 1–30 °C temperature range according to the following equation ($R^2 = 1.000$):

$$L_p = 9.95 \cdot 10^{-6} \cdot T^2 - 5.51 \cdot 10^{-5} \cdot T + 1.33 \cdot 10^{-2} \quad (3)$$

This response was not attributable to a change in water viscosity and suggests that other potential mechanisms,

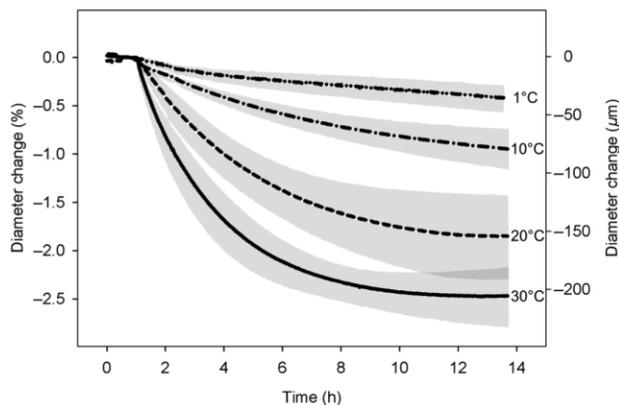


Figure 2. Temperature dependence of diameter change of walnut twig segments perfused (at time = 1 h) with D-mannitol solutions, having an average Π_x -value of -1.05 ± 0.05 MPa. Diameter changes normalized with the initial twig diameter are shown on the left-hand y-axis, while the absolute diameter shrinkage is shown on the right-hand y-axis. Error bands indicate ± 1 standard deviation (SD).

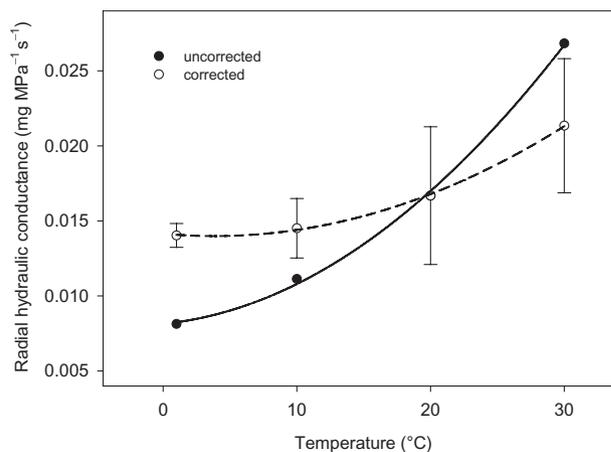


Figure 3. Temperature response of radial hydraulic conductance (L_p) for walnut twig segments perfused with D-mannitol solutions, having an average Π_x -value of -1.05 ± 0.05 MPa. Uncorrected L_p values were directly derived with HyDR from the diameter changes of the individual twig samples constituting Figure 2. These values were averaged per temperature treatment and corrected [\pm standard deviation (SD)] to account for the temperature dependence of water viscosity.

such as aquaporin activity and/or abundance, might play a role in possible modification of the radial hydraulic conductance.

Water moves between the bark and the xylem via two distinct water pathways

To investigate the potential role of putative aquaporins in modifying L_p , twig samples were perfused with D-mannitol solutions, either enriched with 100 μM CHX or not (Fig. 4). CHX has been reported to inhibit protein biosynthesis, depending on the ribosomes' affinity to bind CHX (MacDonald & Ellis 1969; Ellis & MacDonald 1970; Voicu & Zwiazek 2004; Cochard *et al.* 2007). CHX is expected to decrease L_p if the protein-mediated cell-to-cell pathway is important for the hydraulic coupling between the bark and the xylem. The response curves obtained at 20 °C with decreasing Π_x were markedly different because greater $\Delta\Psi$ between the xylem and the elastic tissues in the bark were created. The diameter changes were zero when samples were perfused with deionized water (Fig. 4a). This finding indicates that no masking effects on the L_p estimates occurred of the expected increase in xylem hydraulic resistance because of the use of deionized water and swollen pectins in the inter-vessel pit membranes (Zwieniecki, Melcher & Holbrook 2001). While barely detectable (19 ± 1 μm) when perfused with a Π_x of -0.52 ± 0.00 MPa, the exponential decrease in twig diameter became more pronounced when more negative Π_x were used (-1.07 ± 0.00 MPa and -2.33 ± 0.01 MPa), reaching a maximum shrinkage of 176 ± 59 μm and 375 ± 117 μm , respectively (Fig. 4a).

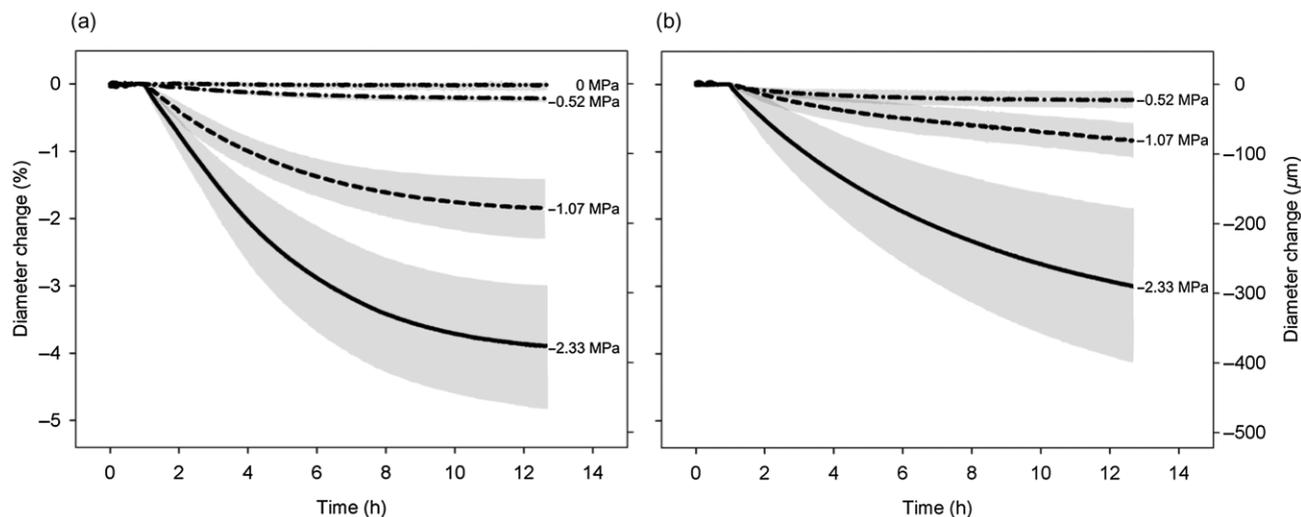


Figure 4. Dependence of diameter change of walnut twig segments on perfusion (at time = 1 h) with D-mannitol solutions, having different osmotic potentials: (a) in the absence of cycloheximide (CHX), and (b) in the presence of CHX. Treatments are performed at 20 °C. Diameter changes normalized with the initial twig diameter are shown on the left-hand y-axis, while the absolute diameter shrinkage is shown on the right-hand y-axis. Error bands indicate ± 1 standard deviation (SD).

A 100 μM CHX perfusion had an inhibiting effect on twig diameter changes when applied in combination with a Π_x of -1.07 and -2.33 MPa, while no effect could be detected at Π_x of -0.52 MPa (Fig. 4b). Maximum diameter shrinkage was 55 and 23% less when 100 μM CHX was added to the solutions with a Π_x of -1.07 and -2.33 MPa, respectively (Fig. 4).

L_p derived from the untreated CHX twigs showed a slight decreasing trend from 0.018 to 0.015 $\text{mg MPa}^{-1} \text{s}^{-1}$ with decreasing Π_x , although the effect was not significant ($P > 0.05$) (Fig. 5). While L_p was unaffected by the CHX treatment at Π_x of -0.52 MPa, a significant inhibiting effect was observed at Π_x of -1.07 MPa ($P < 0.01$) and -2.33 MPa ($0.01 < P < 0.05$), respectively (Fig. 5). This important inhibiting effect on L_p in CHX-treated twigs suggests potential modifications in putative aquaporin abundance and/or activity. The corresponding decrease in contribution of the cell-to-cell pathway to the overall radial water transport was 1, 43 and 38% for Π_x -values of -0.52 , -1.07 and -2.33 MPa, respectively. Hence, the cell-to-cell route appeared to be significantly involved in radial water transport at intermediary and higher $\Delta\Psi$ only.

DISCUSSION

Often the diameter changes measured with LVDT-sensors are regarded as being solely driven by the gradient in water potential between the xylem and the bark, while the radial hydraulic conductance involved is usually thought to be constant (Steudle 1998; Génard *et al.* 2001; Steppe *et al.* 2006, 2008a,b; De Pauw *et al.* 2008; De Swaef & Steppe 2010; De Schepper & Steppe 2010, 2011). Recently, Sevanto *et al.* (2011) emphasized that knowledge on the hydraulic coupling is far from complete. Evidence collected during this study demonstrates that the assumption of constant L_p

has to be modified. We found that L_p in walnut twigs is variable and can respond to temperature. The temperature effect on twig diameter changes could not be explained by changes in water viscosity, but rather reflected a possible modification in L_p (Fig. 3). An increase in environmental temperature boosted L_p (Eqn 3), enhancing diameter shrinkage despite the constant driving force applied between the xylem and the bark tissue (Fig. 2). This additional water was coming from the elastic tissue in the bark and not from a release by cavitation in the xylem conduits.

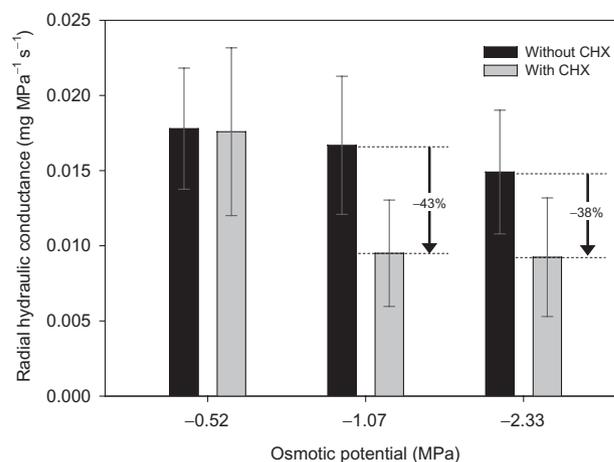


Figure 5. Inhibitory effect of cycloheximide (CHX) on average radial hydraulic conductance (L_p) [\pm standard deviation (SD)] of walnut twig segments perfused with D-mannitol solutions having different osmotic potentials. L_p values are derived with HyDR from the diameter changes of the individual twig samples constituting Figure 4. The decrease in contribution of the cell-to-cell route to the overall radial water transport (black arrow) is estimated by subtracting L_p in the presence of CHX from total L_p (in the absence of CHX) divided by total L_p .

Based on the 1:1 relationship found between the mass and volume variations in walnut segments during the first stage of dehydration, Cochard *et al.* (2001) indeed demonstrated that the water lost by dehydration originated from the elastic bark tissue as long as the diameter change was smaller than 400 μm . This 400 μm threshold was never exceeded in our study. When the diameter change was higher than 400 μm , Cochard *et al.* (2001) showed that the mass change was higher than the volume change, suggesting that water was lost by cavitation (Tyree *et al.* 1993).

In other studies on roots and leaves, temperature treatment has been reported to alter hydraulic conductance values, and this effect has been attributed to a change in abundance and/or activity of aquaporins (or water channel proteins) (e.g., Aroca *et al.* 2005 for roots; Cochard *et al.* 2007 for leaves). The clear temperature effect observed in our study therefore suggests the possible existence of a protein-mediated water transport pathway in walnut twigs.

These findings lead us to speculate that water on its way from the bark into the xylem can take two routes in parallel: an apoplastic route through cell walls and a cell-to-cell route across one or more cell membranes, involving aquaporins (Supporting Information Fig. S1). Such composite water transport has been previously observed in roots (e.g. Steudle 2000) and leaves (e.g. Cochard *et al.* 2007), but has never been reported for radial water transport between the bark and the xylem in twigs and stems. Aquaporins were initially identified in walnut xylem tissue as being involved in winter embolism recovery by mediating water transport between xylem parenchyma cells and cavitated vessels (Sakr *et al.* 2003). It can be expected that these water channels also play a key role in modulating L_p between the xylem and the bark.

Indirect evidence to underpin this hypothesis came from the change in L_p when CHX was applied and Π_x was lower than -0.5 MPa (Fig. 5). Water potential gradients greater than 0.5 MPa are also commonly encountered in actively transpiring trees in the field. These findings are, hence, indicative of the possible involvement of plasma membrane aquaporins whenever radial water transport between the bark and the xylem is relatively high and quantitatively relevant to accommodate the temporal imbalance between leaf water loss and root water uptake. This leads us to speculate that any environmental condition leading to higher transpiration or sap flow rates (and consequently higher $\Delta\Psi$ between bark and xylem) may lead to higher L_p values by boosting aquaporin abundance and/or activity. The implication of L_p being positively related to transpiration is potentially very important because such a dependence provides relatively stable water potential gradients, greatly decreasing the likelihood of suffering dangerous levels of xylem embolism (Martinez-Vilalta *et al.* 2007). High presence of plasma membrane aquaporins at times of high hydraulic demands has also been previously demonstrated in roots and leaves (e.g. Javot & Maurel 2002; Kaldenhoff *et al.* 2008; Heinen, Ye & Chaumont 2009). At higher Π_x values (-0.5 MPa), no modulating effect on L_p in walnut was found, suggesting that water transport through the cell-to-cell pathway did not require any protein biosynthesis

(Fig. 5). Such lower water potential gradients are typically encountered in trees in the field in the evening when emptied water storage compartments are being refilled. This leads us to hypothesize that radial water flow from the xylem into the bark during refilling at low water potential gradients does not rely on a boost in aquaporin abundance/activity, but depends primarily on the apoplastic route.

Although no direct measurements of aquaporin abundance/activity were performed in this study, there is agreement in the literature that CHX can act at the aquaporin transcript level by enhancing its instability and/or at the protein level by decreasing the turnover of aquaporins and/or that of components essential to aquaporin activity (Cochard *et al.* 2007 and references therein). The extent of diameter change and L_p inhibition observed in walnut twigs treated with CHX suggested that the contribution of the cell-to-cell pathway to the overall radial water transport might be greater than originally thought. The apoplastic route appeared to dominate at low $\Delta\Psi$, while the cell-to-cell route was clearly activated at intermediary and higher $\Delta\Psi$ (Fig. 5).

In conclusion, our data support the fact that the radial water pathway between the bark and the xylem tissue might be closely linked to plasma membrane aquaporins and that environmental stimuli that increase transpiration (temperature, vapour pressure deficit, radiation) can lead to activation of the cell-to-cell route and, consequently, to higher L_p values. This conclusive evidence of a variable radial hydraulic conductance in twigs (and consequently stems) adds to many recent studies showing variable hydraulic conductance of other plant parts (i.e. roots and leaves). This variability should, hence, be considered when interpreting LVDT data and should be taken into account in the modelling of stem and branch diameter changes.

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SUPPORTING INFORMATION

Additional Supporting information may be found in the online version of this article:

Figure S1. Schematic of the HydR model and detailed illustration of radial water flow between the bark and the xylem in twigs. The apoplastic route refers to the

flow through the cell walls around the protoplasts. The cell-to-cell route includes the symplastic pathway through plasmodesmata and the transcellular pathway across cell membranes. Because symplastic and transcellular pathways cannot be separated for water, they are grouped into the cell-to-cell route. Symbols: Ψ_x = osmotic potential of the perfused solution; Ψ_b = total bark water potential; W_b = bark water content; and D = twig diameter.

Table S1. Symbol, unit, default value and description of the model parameters and variables.

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