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Investigating water transport through the xylem network in vascular plants

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Abstract

Our understanding of physical and physiological mechanisms depends on the development of advanced technologies and tools to prove or re-evaluate established theories, and test new hypotheses. Water flow in land plants is a fascinating phenomenon, a vital component of the water cycle, and essential for life on Earth. The cohesion-tension theory (CTT), formulated more than a century ago and based on the physical properties of water, laid the foundation for our understanding of water transport in vascular plants. Numerous experimental tools have since been developed to evaluate various aspects of the CTT, such as the existence of negative hydrostatic pressure. This review focuses on the evolution of the experimental methods used to study water transport in plants, and summarizes the different ways to investigate the diversity of the xylem network structure and sap flow dynamics in various species. As water transport is documented at different scales, from the level of single conduits to entire plants, it is critical that new results be subjected to systematic cross-validation and that findings based on different organs be integrated at the whole-plant level. We also discuss the functional trade-offs between optimizing hydraulic efficiency and maintaining the safety of the entire transport system. Furthermore, we evaluate future directions in sap flow research and highlight the importance of integrating the combined effects of various levels of hydraulic regulation.

Key words: Water flow, sap flow dynamics, hydraulic regulation, cohesion-tension theory, efficiency/safety trade-off, embolism.

Introduction

In land plants, water and minerals are taken up from the soil by the roots and transported through the xylem network to the leaves. Some trees can lift water over distances of more than 100 metres from the roots to the uppermost leaves (Ryan *et al.*, 2006). This ability has fascinated scientists through the centuries and the study of plant hydraulics remains an active topic of research open to new methods of investigation (Tyree, 2003). Independent of plant size, water movement is at the crossroads of all plant growth and development processes, from transpiration and photosynthesis to the distribution of organic and inorganic molecules throughout the plant.

When Einstein formulated the equation for the interconversion of matter and energy in 1905, it inspired decades of research and the revision of the law of conservation of energy.

In plant physiology, the cohesion-tension theory (CTT) represents a similar conceptual breakthrough; however, some aspects of the CTT still require experimental validation. The CTT originated at the end of the 19th century, when Boehm (1893) proposed an initial framework, based on the cohesion and adhesion properties of water, to explain water transport in plants. The CTT attributes the main driving force for water transport to the tension (i.e. negative hydrostatic pressure) generated at the leaf surface by evaporation. The fundamental principles of the CTT summarized by Dixon and Joly (1894) have withstood persistent challenges (Zimmermann *et al.*, 1994; Canny, 1995; Milburn, 1996; Meinzer *et al.*, 2001; Zimmermann *et al.*, 2004) and remain the most comprehensive explanation of water transport in plants (Angeles *et al.*,

2004). The CTT is based on the physical properties of water: cohesion between dipolar water molecules gives water its high tensile strength, which maintains hydraulic continuity throughout the plant vasculature. The negative pressure that causes water to move up through the xylem develops at the surface of cell walls, which act as a very fine capillary wick. Water molecules adhere to the cellulose microfibrils and other hydrophilic components of the wall (Somerville *et al.*, 2004; Oda and Hasezawa, 2006). As water evaporates from a thin film permeating through an extensive system of intercellular air spaces in the substomatal chambers of leaves, cohesive forces result in the formation of curved air/water interfaces. The surface tension at the interface induces a negative pressure and that generates the motive force that drives sap ascent in the xylem (Zimmermann, 1983). Ultimately, the surface tension generated at the air/water interfaces of cell walls is assumed to be transmitted through a continuous water column to the roots. However, this system is highly prone to cavitation owing to the metastable state of water (Tyree and Sperry, 1989; Hacke and Sperry, 2001; Meinzer *et al.*, 2001; Zimmermann *et al.*, 2004; Herbert *et al.*, 2006). Cavitation in the xylem can lead to a major reduction in hydraulic conductivity. Such a disruption in water flow poses a serious threat to photosynthetic efficiency and plant survival.

The first extensive integration of water transport and xylem structure was proposed by Zimmermann (Zimmermann *et al.*, 1971; Zimmermann, 1983) and was updated by Tyree and Zimmerman (2002). The overview presented by Holbrook and Zwieniecki (2005) is probably the most comprehensive formulation of vascular transport in plants. However, new conceptual models and experimental methods that emerged in the past decade have brought new insights. Many research groups now examine the plant-water relationship at various scales, from the level of the cellular water exchange to that of the whole-plant canopy. In that respect, although the discovery of aquaporins (Murata *et al.*, 2000) represent a significant advance in our understanding of intercellular water flow, we will restrict our review to water flow in the xylem network. Numerous tools have been developed to probe the mechanism underlying the passive transport of water in plants. During the past two decades, the concept of passive water transport has been heatedly debated in the scientific community (Zimmermann *et al.*, 1994; Canny, 1995; Milburn, 1996; Meinzer *et al.*, 2001; Zimmermann *et al.*, 2004). In this review, we highlight the major experimental tools that have provided insight into sap flow through the xylem network. From the broader perspective of the Blue Revolution (Pennisi, 2008), understanding how water is transported from the soil through the intricate plant xylem network to the atmosphere can lead to innovative ways to optimize each drop of water in applied scientific fields such as molecular biology and agronomy, and in breeding programmes that seek to improve drought-resistance in crop plants. Some industrial applications based on our understanding of microfluidics and nanofluidics have already started to emerge in the form of plant-inspired devices, such as synthetic trees (Wheeler and Stroock, 2008). In recent reviews, Pittermann (2010) presented an integrated approach of the evolution of the plant vascular system, and Lucas *et al.* (2013)

summarized our current understanding of plant vascular biology and emphasized the major impact of the tracheophyte-based vascular system on all terrestrial organisms. Two recent international meetings (the 9th International Workshop on Sap Flow, 2013, and the Third International Conference on Plant Vascular Biology, 2013) demonstrated that sap flow is an area of prolific and inspiring research. However, there is no agreement as to which methods are best for examining sap flow or how the new results contribute to unravel sap flow dynamics in vascular plants.

In this review, we briefly retrace the scientific investigation of water transport in vascular plants, and evaluate basic concepts and theories in light of new experimental methods. We will assess our current understanding of the structure/function relationships of the xylem hydraulic architecture and provide an overview of experimental tools and methods used to unravel sap flow dynamics through the xylem network. Real-time imaging emerges as the most promising approach for integrating the xylem network structure and its multiple layers of regulation.

Linking xylem structural components and their functions

Our understanding of xylem hydraulic properties has evolved with the development of theoretical modelling and novel experimental tools to visualize the cross-sectional and three-dimensional structure of xylem. Tracheary elements (TEs) are the elementary units of xylem. After a complex process of differentiation, TEs lose their nuclei and cell contents, leaving behind a central lumen surrounded by secondary cell walls, which together form tracheids or vessels (Fukuda, 1997). The structural characteristics of tracheids in conifers and vessel elements in angiosperms have been well characterized using optical and electron microscopy. The diameter of TEs varies from a few micrometres to a few hundred micrometres. Their association in series to form long-distance pathways can attain a few millimetres up to several metres. Torus-margo or pit membranes integrated in the secondary cell wall provide various levels of subcellular resistance to water flow (Schulte and Castle, 1993; Hacke *et al.*, 2006; Sperry *et al.*, 2007). Although the structural characteristics of TEs are well established, our understanding of water flow dynamics is limited to the tissue or organ level. From a bottom-up perspective, water and minerals from the soil are absorbed through apoplastic and symplastic pathways into protoxylem vessels of the roots (Passioura, 1988). Then, long-distance transport in the stem is generally attributed to large metaxylem vessels. The vascular bundles in leaves become highly branched reducing the distance of most leaf cells to less than a few hundred micrometres from a vessel (Fig. 1). The mesophyll at the interface with air represents the highest resistance to water flow (Cochard *et al.*, 2004; Sack *et al.*, 2008).

In analogy with Ohm's law, water uptake and transport are associated with a hydraulic flow process that is controlled by resistance and hydraulic gradients (van den Honert, 1948). The overall resistance is determined by soil water potential,
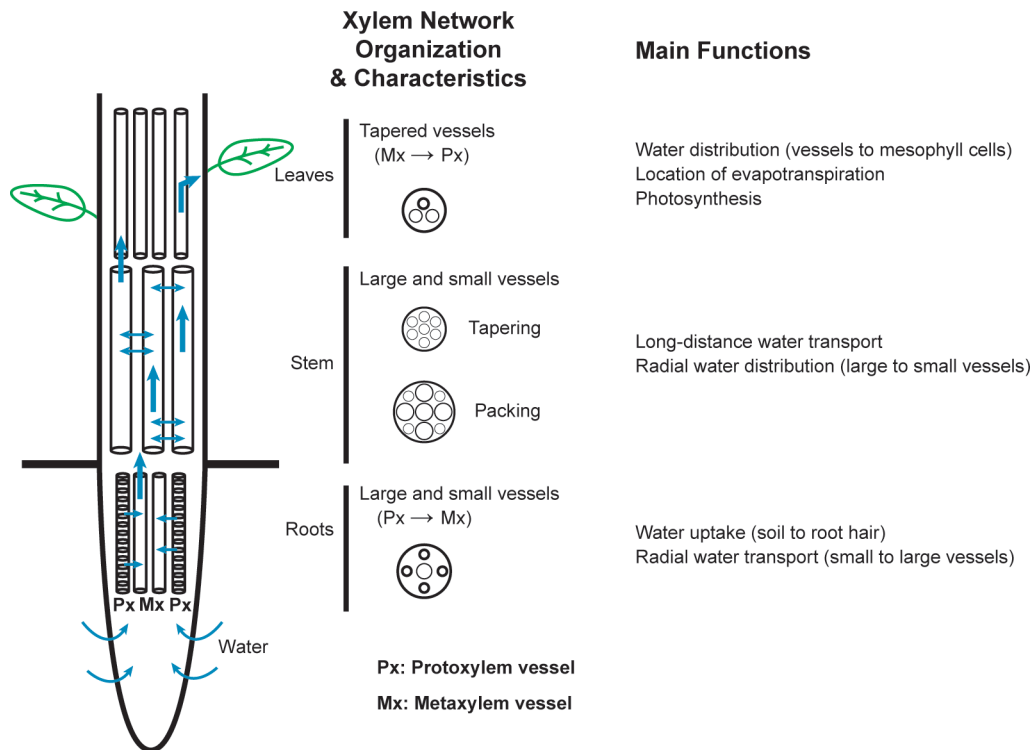
**Xylem Network
Organization
& Characteristics**

Main Functions

Tapered vessels
(Mx → Px)

Leaves

Water distribution (vessels to mesophyll cells)
Location of evapotranspiration
Photosynthesis

Large and small vessels

Tapering

Stem

Packing

Long-distance water transport
Radial water distribution (large to small vessels)

Large and small vessels
(Px → Mx)

Roots

Water uptake (soil to root hair)
Radial water transport (small to large vessels)

Px Mx Px

Water

Px: Protoxylem vessel

Mx: Metaxylem vessel

Fig. 1. Main characteristics of the xylem network. Organization and characteristics of the xylem network: water flow throughout the plants depends on characteristics of the xylem in different organs. Water absorbed by the roots moves radially from small protoxylem vessels, which have high hydraulic resistance, to larger metaxylem vessels, with reduced hydraulic resistance. In the stem, the number and organization of vessels vary along the height of the plant height. Packing and tapering functions can be used to characterize each level of organization. In the leaves, water travels through small xylem vessels. During transpiration, negative hydrostatic pressure is generated at the interface between mesophyll cells and air.

conducting vessels, transpiration rate, plant height, and gravity. In this physical conceptualization of the soil-plant-atmosphere continuum, the tension of the driving force of sap ascent continuously decreases in the direction of flow, and the pressure gradient is proportional to the evaporative flux density from the leaves (Tyree, 1997). The xylem provides a low-resistance pathway for long-distance water movement by minimizing the pressure gradients required to transport water from the soil to the leaves (Jeje and Zimmermann, 1979). In its most simplified representation, the xylem is often modelled as an assemblage of 'unit pipes' (Shinozaki et al., 1964) and water flow is generally approximated with the Hagen-Poiseuille equation (Dimond, 1966; Schulte et al., 1989; Lewis and Boose, 1995). The pipe model has contributed to the estimation of canopy-level parameters by incorporating variations in vessel size and number at the tissue and organ levels, and was also used to understand tree growth, resource allocation, and plant biomechanics (Niklas et al., 2006). However, the functionalities of the xylem network integrate different structural organization at the tissue and organ levels that cannot be supported by this simplified model. Hydraulic resistance is highly variable depending on the species and organ. Unravelling how water is collected from all the vessels in the roots, passes through the stem, and is distributed in the leaves requires an integrated functional approach at the whole-plant level (Sperry, 2003; Loepfe et al., 2007; Page et al., 2011).

At the tissue level, the hydraulic conductivity per unit of cross-sectional area generally defines efficiency. The

constraints on the maximum diameter, length, and number of xylem vessels for a given cross-sectional area limit efficiency: this is related to a species-dependent limit on conduit frequency. For instance, vessel lumens in angiosperms occupy less than 10% of the cross-sectional wood area at the midpoint of their diameter range, whereas tracheid lumens in conifers can occupy over 40% (Sperry et al., 2008). Such variation is due to the lower investment in mechanical strength in angiosperms, which rely on wood fibres, whereas conifer tracheids provide both transport and support functions. Conduit diameter and frequency are not the only factors determining efficiency of water flow, because the conductivity of conduits of a given diameter can also vary. In angiosperms, simple or schalariform perforation plates and conduit end walls create differences in actual conductivity compared with the theoretical maximum set by the Hagen-Poiseuille equation. Lumen and end-wall resistance is relatively constant and flow resistance through pits does not increase with cavitation safety. Pit membrane porosity does not seem to be related to cavitation pressure (Hacke et al., 2006). Despite difference in size, the end wall resistance at a given diameter seems to be relatively similar between conifers and angiosperms. The presence of specialized structures, such as the torus-margo in conifers, greatly reduces the resistance of inter-tracheid water flow (Pittermann et al., 2005).

In summary, the most important structural features of the xylem at the cellular level are conduit diameter, length, wall features (i.e. annular, spiral, or reticulate thickening or pits),

and the presence or absence of end walls (simple or scalariform). At the tissue level, inter-conduit pitting (determined by the density and size of torus-margo or pit membrane) and the number of conduits define the connectivity of the xylem network.

Functionality of the xylem network: bottleneck for efficiency or smart design for safety?

The two main functions of the xylem hydraulic network in vascular plants are (i) to supply water and minerals to all tissues and (ii) to provide mechanical support. In living organisms, similar functions are generally carried out by similar structures. A large diversity in xylem hydraulic architecture exists between organs and among species, and the initial structures are even modified during growth and development. Among seed plants, coniferous, diffuse-porous, and ring-porous trees have radically different xylem anatomy (McCulloh *et al.*, 2010). Within angiosperms, the vascular bundles of dicot and monocot plants have distinct organizations that vary in different organs. These differences in organization are ultimately due to differences in conduit tapering and packing of similar elementary structures. However, the functional consequences of these distinct organizations are not well understood at either the conduit or the whole-organism level. The integration of organ-level variation in xylem architecture at the whole-plant level is essential for unravelling the mechanisms that maintain the integrity of water transport from roots to leaves. The elementary elements of the system (i.e. tracheids or vessel elements) are organized to withstand strong physical constraints and simultaneously achieve efficient water transport with minimal resistance, while protecting against cavitation (Tyree and Sperry, 1989). To integrate structural characteristics into functional roles, it requires determining how the dynamic hydraulic properties at the cellular level are incorporated into tissue and organ levels. For example, the hydraulic efficiency per conduit diameter and length is higher for conifer tracheids than for angiosperm vessel elements; however, the wider diameter and greater length of angiosperm vessels provide greater conductivity per xylem area.

In terms of the biophysical mechanisms underlying these processes, the major challenge is to understand how the trade-off between efficiency and safety is achieved at different levels of organization. The hydraulic regulation attributed to the xylem is generally considered to depend on the specific organization in each organ; however, the respective contributions are difficult to integrate into the entire network (Fig. 1). Unravelling the relationship between the structural complexity of hydraulic architecture and efficiency/safety functions remains one of the main issues in understanding plant-water relations. In leaves, direct pressure-drop measurements confirmed that mesophyll cells are the major component of hydraulic resistance, even though the vascular system accounts for the longest distance (Cochard *et al.*, 2004). From the soil to the atmosphere, the relationship between hydraulic resistance and stomatal conductance is a key component

(Cruiziat *et al.*, 2002) as environmental factors can influence the efficiency of water absorption and uptake. When transpiration is high, maintaining the continuity of flow in individual vessels is seriously challenged owing to cavitation risks (Cochard, 2006). However, cavitation can be reduced by the hydraulic capacitance of the xylem and the water storage capacity of each organ, and the network organization can also provide alternative pathways to avoid disruption of water flow (Tyree and Ewers, 1991; Sperry *et al.*, 2008; Höltta *et al.*, 2009). Ultimately, water transport and gas exchange in the leaves have a major physiological effect on the photosynthetic capacity of the plant (Tyree and Ewers, 1991).

The structural model of the hydraulic transport system proposed by West, Brown, and Enquist (WBE model; 1999) has been widely used to explain the maintenance of a constant flow rate along the entire flow path. In this model, it is assumed that plants minimize the effect of hydraulic resistance imposed by increasing height and total path-length conductance by tapering the xylem conduits. Plant size is related to the geometry of the branching architecture and metabolism. Based on the fact that all living organisms contain a transport system for aqueous materials, the plant vascular system should minimize the hydrodynamic resistance of nutrient transport, while maximizing the exchange surface with the environment (Petit and Anfodillo, 2009). The ideas that (i) all plants adopt a universal architecture of the xylem transport system, and (ii) hydraulic efficiency is independent of plant height are very attractive. Although a wide range of plants seemed to comply with these assumptions (West *et al.*, 1999), numerous studies challenged the validity of a universal rule given the diversity of vascular plants (Dodds *et al.*, 2001; Coomes, 2006; Apol *et al.*, 2008), and hydraulic constraints seem to increase with plant height (Koch *et al.*, 2004). Despite controversies, the WBE model highlights the value of architectural modelling in simplifying plant diversity and stimulated prolific empirical research. Now, complementary models of the vascular system not only include a more realistic view of the hydraulic architecture (Savage *et al.*, 2010), but also incorporate physiological considerations (von Allmen *et al.*, 2012).

Although the plant xylem is non-living tissue, there is an extraordinary degree of coordination between the hydraulic capacity and photosynthetic assimilation because both of these pathways intersect at stomata during the exchange of water and CO_2 at the leaf surface (Brodribb, 2009). The rate of transpiration and gas exchange via stomata are limited by the xylem hydraulic system. Packing and taper functions are the backbone of a robust framework for modelling network transport (Sperry *et al.*, 2008; McCulloh *et al.*, 2010). Strength and storage requirements set a packing limit and influence the conducting capacity (Zanne *et al.*, 2010). Theoretically, a small number of wide conduits are more efficient than a large number of narrow ones. This is reflected by the more efficient networks of ring-porous trees compared with conifers (McCulloh *et al.*, 2010). Without tapering of the xylem conduits, branches would have the highest conductivity in a tree. In other words, tapering counterbalances the decline in conductance due to increasing path length, but maintaining similar conductivity

requires an increase in the number of xylem vessels per unit cross-sectional area as conduits become narrower. The organization of the xylem network thus defines the functional trade-off between efficiency and safety in each organ.

Building on these concepts, Höltta *et al.* (2011) proposed a carbon-cost gain model, which calculates the xylem structure that maximizes carbon-use efficiency while simultaneously accounting for intervessel pit structures that increase flow resistance. As the water potential is lower at the plant apex, fewer pores in the pits near the apex would also restrict the spreading of embolisms. An optimal hydraulic structure would have conduits that decrease in size from the base to the apex (defining tapering function). In parallel, the vulnerability to cavitation can be reduced by increasing conduit number (defining the packing function). Indeed, whole-plant carbon-use efficiency demands that conduit size decreases and conduit number increases simultaneously (Lancashire and Ennos, 2002; Choat *et al.*, 2003; Höltta *et al.*, 2009).

What is the appropriate approach to investigate the regulation of sap flow dynamics?

The theoretical and conceptual bases of water transport and xylem hydraulic architecture have been examined by various experimental methods (Fig. 2). Technical reliability of new methodology is of prime importance in investigating the processes of water transport. Moreover, subsequent results are rarely cross-validated with those obtained using other methods. A difficulty in making proper comparisons is that the measurement techniques do not address the same level of the xylem network. For instance, the technical limitations of new methods in measuring internal pressure or vulnerability to cavitation have sometimes resulted in a misunderstanding of the elementary processes and have given erroneous interpretations. The invasive methods using excised tissues do not change the internal xylem structure, but water flow generated artificially in isolated leaves, stems or roots does not accurately reflect water flow in intact plants.

Three categories of methods are currently available for investigating xylem sap flow: (i) continuous measurement of sap flow velocity (to confirm the relationship between transpiration and water uptake); (ii) internal pressure measurement (to confirm that negative hydrostatic pressure is the main driving force of sap flow); and (iii) visualization of sap flow through the xylem. Experimental data obtained using these different methods were frequently not in agreement, because the scale of the xylem architecture examined (from the whole-plant network to individual vessels) generally differed. Futhermore, sap flow dynamics were not always measured with the same hydraulic parameters. Therefore, it is crucial to understand the advantages and limitations of different techniques to compare the characteristics of sap flow across different species.

Continuous sap-flow monitoring has been most commonly used to measure water flux through the stems and branches of trees, but the resolution is not sufficient for determining leaf-level responses to environmental changes. Flow monitoring techniques using tracers and histological sections enabled the identification of the water-conducting vessels of the xylem network and provided a snapshot of how they function under different environmental conditions. The injection of different dyes (e.g. fuchsin or safranin) is the most common method used to visualize water-conducting pathways at the tissue level in conifers (Harris, 1961; Kozlowski and Winget, 1963; de Faÿ *et al.*, 2000), dicotyledonous trees (Kramer and Kozlowski, 1960; Ellmore and Ewers, 1986), and herbaceous plants (Hargrave *et al.*, 1994; Tang and Boyer, 2002). Recently, a number of concerns have been raised in interpreting the results of dye injection (Umebayashi *et al.*, 2007). First, the type of dye and the method used for sample preparation greatly affect the distribution and diffusion of the dye through the xylem. Second, the diversity in plant size, and vessel size and organization do not generally allow extrapolation of the results obtained for a stem, root, or leaf sections to other organs. Third, it is difficult to compare the results of studies conducted at the whole-plant level under various environmental conditions with those obtained from the isolated tissues. Using improved preparation methods, stabilized dye can
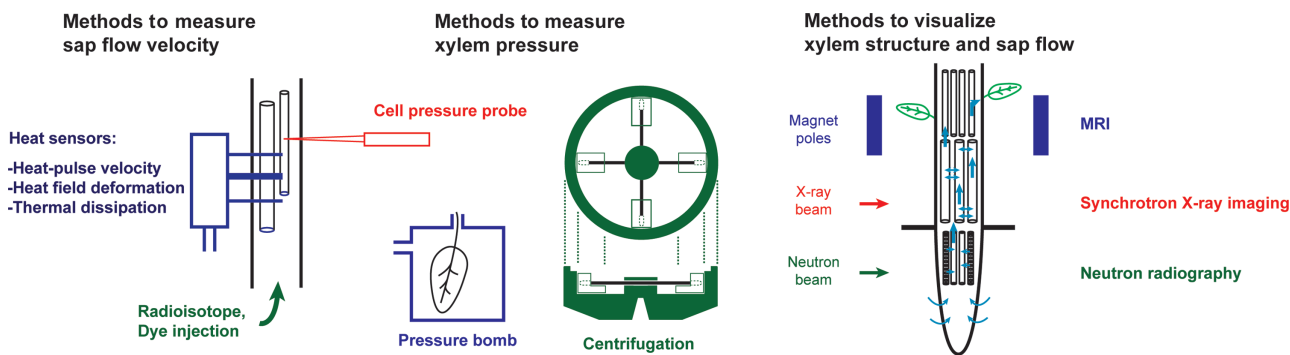

Fig. 2. Methods and instruments used to analyse sap flow in plants. A. Schematic representation of different methods used to measure sap flow velocity. In heat-based methods, heat sensors (heat pulse velocity, heat field deformation, or thermal dissipation) are installed radially into a segment. In radioisotope or dye methods, tracers are injected into the xylem or uptaken from a cut segment. B. Methods used to measure negative pressure in the xylem. The observation scale and measurement target (i.e. cell, tissue, or organ) differ between indirect (i.e. pressure bomb, centrifugation) or direct (i.e. cell pressure probe) methods. C. Simultaneous visualization of xylem structure and sap flow using magnetic resonance, neutron or synchrotron X-ray imaging methods. The temporal and spatial resolutions vary for each imaging method.

enable the identification of water-conducting vessels in trees at the cellular level (Sano *et al.*, 2005); however, it remains technically challenging to visualize sap flow at the subcellular level (Geitmann, 2006). Dye injection is a relatively easy technique, but it gives misleading interpretations about the functional water-conducting pathways if the procedures are not well defined and standardized (Umebayashi *et al.*, 2007).

More accurate modelling of leaf and plant-level responses to abiotic stresses is essential to predict the canopy response to future climate change. In forest ecosystems, water fluxes in trees can be monitored at the stem or leaf level (Fig. 2A). Heat-balance and heat-pulse methods estimate whole-plant water flow using heat-based sensors (Smith and Allen, 1996). In both cases, probes inserted into the stem of a tree generate heat that is used as a tracer. The heat-balance method calculates the mass flow of sap in the stem from the amount of heat taken up by the moving sap stream. In the heat-pulse method short pulses of heat are applied, and the mass flow of sap is determined from the velocity of the heat pulses moving along the stem (Cohen *et al.*, 1981; Burgess *et al.*, 2001). The thermal dissipation method, which is based on the propagation of heat pulses and was initially developed by Huber (1932) and refined by Vieweg and Ziegler (1960), is also widely used to estimate sap flow rates. The direction of volume flow is derived from the asymmetry of thermal dissipation; however, reliable estimates of the sap-conducting surface area and size are essential to compare the deduced sap flow rates with the actual sap flow rates (Green *et al.*, 2003). One of the major limitations of theses techniques is that the inserted probes disrupt the sap stream, which alters the thermal homogeneity of the sapwood. Recently, mathematical corrections of sap velocity include effects due to heat-convection (Vandegehuchte and Steppe, 2012*b*) or natural temperature gradients (Lubczynski *et al.*, 2012). In ecophysiological studies, technically improved probes are now available for continuous sap flow measurements in trees (Burgess *et al.*, 2001). A sophisticated four-needle, heat-pulse sap flow probe even permits measurement of non-empirical sap flux density and water content (Vandegehuchte and Steppe, 2012*a*).

Measurements of sap flow alone do not provide sufficient spatial resolution to evaluate the variations in xylem water transport properties. Spatial variations in xylem structure and hydraulic properties have to be compared with the actual patterns of *in vivo* water flow dynamics. Measuring the sap flow (i.e. the velocity and amount of water transported through the xylem) and pressure (i.e. the driving force responsible for the transport) are technically and conceptually challenging. A reliable interpretation of instrumentation outputs requires an integrated understanding of both the structural complexity and technical limits of each measurement method. In particular, the velocity or pressure measurements should be evaluated with respect to the hydraulic architecture of the xylem network. Tension measured using pressure bombs and xylem pressure probes were only in accordance for non-transpiring leaves and differed considerably for transpiring leaves (Melcher *et al.*, 1998). The deviations were later attributed to technical limitations, as the range of sensitivity of the initially developed pressure probes was below 0.8 MPa, and insertion of the glass

tip of the probe frequently disrupted a vessel under tension (Wei *et al.*, 1999*a*,*b*; Wei *et al.*, 2001) (Fig. 2B). Pressure probes can now be used to measure negative pressures; however, theoretical values of up to –10 MPa cannot be verified. The existence of negative hydrostatic pressure is no longer a question. Meanwhile, how this pressure is transmitted through the xylem network requires a better understanding of the relationship between changes in pressure and network architecture.

A lack of consistency between results obtained using tracer dyes and probes called into question which velocity component each method measures. Flow velocities obtained from heat-pulse or particle-type tracers, such as radioisotopes, probably differ owing to the way in which axial and radial flow components are measured. Vessels involved in the flow and the total lumen area are generally not known and it is technically difficult to insert the glass tip of a pressure probe into a vessel without causing cavitation (Heine and Farr, 1973; Dye *et al.*, 1992). In a tropical forest canopy, axial long-distance flow and transport of radial water were affected by the internal water-exchange capacity and the transpiration stream (James *et al.*, 2003). An inverse relationship between the internal water-exchange capacity and the specific hydraulic conductivity confirmed a trade-off between transport efficiency and water storage. By combining the thermal-dissipation technique with infrared gas analysis, sap flow and transpiration could be measured simultaneously (Ziegler *et al.*, 2009).

Since the formulation of the CTT, multiple instruments and techniques have been developed to measure the negative pressure in xylem vessels. Inspired by Renner's (1911) technique using a potometer attached to an excised leafy twig, Scholander *et al.* (1965) developed the pressure bomb technique. It rapidly became a reference tool to measure negative hydrostatic pressures in excised leaves. Despite initial disagreement between the results obtained from the pressure bomb, *in situ* psychrometry (Turner *et al.*, 1984), and the root pressurization method (Passioura and Munns, 1984; Passioura, 1988), the high negative value given by the pressure bomb was considered to be the decisive proof supporting the CTT. Later on, cell pressure probes developed by Balling and Zimmermann (1990) gave access to *in vivo* measurements of pressure in individual xylem vessels (Pockman *et al.*, 1995). Measurements of xylem pressures, leaf balancing pressures, transpiration rates, and leaf hydraulic properties are now possible; however, the reasons behind the large variations in pressure obtained using different techniques need further investigation. Better integration of the hydraulic regulation at each level of organization of the xylem network should thus be the next step (Fig. 2B). How is water from individual vessels in the roots transmitted to a network of vessels in the stem? How is long-distance water transport redistributed to vessels in the leaves? How is each level of hydraulic regulation coordinated at the whole-plant level?

Visualization of *in vivo* water flow dynamics using magnetic resonance imaging (MRI) and synchrotron X-ray imaging provided the first tools for examining flow regulation and a specific level of structural organization. In particular, it is now possible to visualize the functionality of individual

xylem vessels under different environmental conditions. Nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) is the least invasive method to investigate sap flow, and provide spatially and temporally resolved information on sap flow at the level of membrane, cell-to-cell, and long-distance transport (Witsuba *et al.*, 2000; Scheenen *et al.*, 2007). Relative differences in flow volume in different vascular bundles suggested that each vascular bundle is under different tension. Also, root pressure can be estimated non-destructively by taking continuous measurement of sap flow and variations in root segments of different stem diameter and integrating this information with a mechanistic flow and storage model (De Swaef *et al.*, 2013) (Fig. 2C).

Numerous studies examine sap flow as a combination of flow velocity and pressure measurements under different environmental conditions (Witsuba *et al.*, 2000). A wide range of devices are available to measure pressure and flow at different scales: mobile MRI systems for outdoor tree measurements (Kimura *et al.*, 2011), patch-clamp pressure-probes to monitor leaf water status non-invasively and record variations in turgor pressure gradients in leaves (Zimmermann *et al.*, 2008), and even simultaneous dendrometer and leaf patch-clamp pressure-probe measurements for the effects of microclimate and soil moisture on diurnal variations in leaf turgor pressure and water in stems (Ehrenberger *et al.*, 2012). Now, measurements of sap flow velocity, xylem pressure at the level of individual vessels and *in-vivo* real-time visualization are required to completely unravel the dynamics of sap flow regulation in the xylem network.

Toward real-time imaging of flow dynamics in the xylem network

Real-time imaging methods, such as synchrotron X-ray imaging, have recently revealed that radial flow of water can occur during refilling of dehydrated xylem vessels in monocot leaves (Kim and Lee, 2010) and in the roots of *Arabidopsis* plants (Lee *et al.*, 2013b). A major challenge for plants under high evaporative demand or low soil water availability is to resist cavitation and/or recover from the reduction in water transport (Hacke and Sperry, 2001; Lens *et al.*, 2013). Embolism of xylem vessels reduces hydraulic conductivity, and the percentage loss of conductivity (PLC) is used to estimate cavitation and embolism repair (Zwieniecki and Holbrook, 1998). For a long time, the experimental research was focused on trying to identify how frequently embolism occurred and how it could be repaired, especially in trees. The refilling of embolized vessels is not explained by thermodynamic laws (Holbrook and Zwieniecki, 1999). However, the latest comparison of different methods used to measure the PLC showed that embolism repair is largely due to technical artefacts (Wheeler *et al.*, 2013). The ability to limit embolism occurrence is a major component of hydraulic safety and the frequent cavitation reported in earlier studies was due to erroneous interpretations. In particular, inappropriate dehydration methods to generate vulnerability curves led to an overestimation of the vulnerability to cavitation (Cochard *et al.*, 2013). Nonetheless,

the ability of plants to refill embolized vessels during transpiration cannot be neglected and the biophysical mechanisms that enable plants to do so remain to be elucidated (Zwieniecki and Holbrook, 2009). Synchrotron X-ray computed tomography is an extremely promising method to visualize and quantify refilling dynamics (Brodersen *et al.*, 2010; Brodersen *et al.*, 2013). In grapevines, water influx in the embolized vessels has been attributed to adjacent vessels or the surrounding living tissue. These advances in imaging techniques provide sufficient spatial and temporal resolution to visualize axial, radial, and reverse flow (Lee *et al.*, 2013a; Lens *et al.*, 2013). Although such methods cannot be used on trees due to limitations in sample size and field of view, the experimental results obtained from model plants can be integrated into a broader framework to understand the hydraulic regulation of active water flow. If refilling under tension is indeed a physical process, we need to re-evaluate the reality of this phenomenon and identify the source of the driving force that draws water into embolized vessels, localize the origin of this water, and determine how embolized and functional vessels are hydraulically compartmentalized (Holbrook and Zwieniecki, 1999). Real-time, high-resolution imaging methods are ideal for visualizing dynamic processes such as embolism repair (Brodersen *et al.*, 2010; Brodersen *et al.*, 2013). Although these methods can only be used in some small model plants, the visualization of flow dynamics in the xylem network opens new insights in understanding the hydraulic efficiency/safety trade-offs (Kim and Lee, 2010; Brodersen and McElrone, 2013). Ultimately, the different structural and functional components, such as sugar metabolism, capacitive effect (Höltta *et al.*, 2009), the presence of bordered pit membranes (Zwieniecki and Holbrook, 2009), venation architecture, and leaf size (Scoffoni *et al.*, 2011) must be incorporated in a functional model to fully comprehend the hydraulic regulation at the entire plant level.

Future directions

A multitude of tools and methods are now available to study water transport from the level of individual xylem vessels to the whole plant. It is crucial to consolidate our current knowledge in order to guide future research on plant water transport in the most relevant directions. Whereas plant physiologists are the ones who better understand the complexity of this transport system, they need support from physicists to validate the results obtained with new methods. Molecular biologists should also play a key role in incorporating the role of aquaporins in regulating plant water transport, especially in the roots and leaves. Ecologists, agronomists, and breeders can benefit tremendously by including the basic processes of water transport in their modelling and selection approaches. Currently, it is difficult to attribute structural characteristics of the xylem network to specific functions related to efficiency or safety. Developing new tools and methods that connect flow and structure at different scales is probably the most promising approach for gaining new insight into hydraulic regulation along the transpiration stream. Using a combination of structural and functional methods, it is now possible to distinguish between water-conducting and

non-functional vessels. However, given the diversity of plant hydraulic architecture and dimensions, the same methods cannot be applied to all plants.

Advanced high-resolution imaging methods such as MRI, synchrotron X-ray imaging, and neutron-based imaging, now allows the analysis of flow dynamics at the organ level, as reported for rice leaves, grapevine stems, or *Arabidopsis* roots (Kim and Lee, 2010; Brodersen, 2013; Lee *et al.*, 2013a; Warren *et al.*, 2013). The next major step will be to reconstitute a realistic 3D map of the hydraulic network of the whole organism starting with small model plants, such as *Arabidopsis*. At the subcellular level, the combination of scanning electron microscopy (nano-scale) and macroscopic techniques will enable investigations of the relationship between cell wall characteristics and the xylem network (McCully *et al.*, 2009; Zehbe *et al.*, 2010; Page *et al.*, 2011). Atomic force microscopy will provide information about the surface chemistry of xylem cell walls. Confocal microscopy of leaves can provide insight into the relationship between leaf water dynamics and transpiration (Botha *et al.*, 2008; Fitzgibbon *et al.*, 2010; Wuyts *et al.*, 2010). On the other hand, portable devices such as portable MRI are being developed to measure sap flow under real-field conditions. Infrared imaging techniques can provide a detailed map of surface temperatures and promote insight into water distribution, evaporation, ice formation, and sap flow. The development of enhanced computing power will also give rise to more realistic models and simulations of sap flow.

Transport of water and minerals is at the centre of all metabolic processes in plants, yet many variables and parameters related to this transport are unknown. In a broader perspective, a functional framework of the xylem network that integrates water flow dynamics at various levels of organizations can lead the development of bio-inspired technologies based on sap flow in plants. For decades, research on water transport in plants has hinged on a reference theory. To move forward, the research should now focus on unravelling how water transport through the xylem network is regulated using ingenious combinations of advanced techniques that probe the structure-function relationships of this fascinating transport system.

Acknowledgements

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