DARWIN REVIEW

More than just a vulnerable pipeline: xylem physiology in the light of ion-mediated regulation of plant water transport

Andrea Nardini1,*, Sebastiano Salleo1 and Steven Jansen2

1 Dipartimento di Scienze della Vita, Università di Trieste, Via L. Giorgieri 10, Trieste, Italia
2 Institute for Systematic Botany and Ecology, Ulm University, Albert-Einstein-Allee 11, Ulm, Germany

* To whom correspondence should be addressed. E-mail: nardini@units.it

Received 11 March 2011; Revised 6 June 2011; Accepted 7 June 2011

Abstract

Major restrictions to the hydraulic conductance of xylem (K_{XYL}) in vascular plants have traditionally been attributed to anatomical constraints. More recently, changes in the cationic concentration of xylem sap have been suggested to be responsible for short-term changes in K_{XYL} based on data for 35 dicot species, and very few gymnosperms and ferns, indicating that xylem water transport may no longer be considered as an entirely passive process. Recent studies have revealed that this so-called ionic effect: (i) varies from little or no increase to >30%, (ii) is species specific, (iii) changes on a seasonal basis, (iv) depends on the cationic concentration, (v) is enhanced in embo- lized stems, and (vi) is positively correlated with vessel grouping. Furthermore, the ionic effect has been suggested to play functional roles in planta with respect to: (i) phloem-mediated control of xylem hydraulic properties, (ii) compensation of cavitation-induced loss of hydraulic conductance, with the result of optimizing light and water utilization, and (iii) differential regulation of water delivery to branches exposed to different levels of light. Pits are likely to play a key role in the ionic effect, which has largely been explained as a consequence of the poly-electrolytic nature and hydrogel properties of the pectic matrix of interconduit pit membranes, despite little evidence that pit membrane pectins remain present after cell hydrolysis. More research is needed to address the ionic effect in more species, physico-chemical properties of pit membranes, and how the ionic effect may increase xylem hydraulic conductance ‘on demand’.

Key words: ionic effect, pectin, pit membrane, xylem.

Long-distance water transport in land plants

Land plants are constantly facing a life-or-death functional dilemma. While stomata have to be open during the day in order to allow CO\textsubscript{2} to diffuse into leaf interior spaces and fuel photosynthetic processes, the parallel loss of large amounts of water cannot be avoided (Schulze et al., 2002). Hence, land plants are continually exposed to the risk of dehydration, and water lost during transpiration must be replaced promptly by water absorbed by roots and transported up to the sites of evaporation to prevent fatal desiccation.

Long-distance water transport in land plants occurs through the xylem, a very efficient network of pipelines making up a low-resistance water pathway that connects the roots to the mesophyll cells along stem, branches, twigs, petioles, and leaf veins (Tyree and Zimmermann, 2002). The xylem represents about 99% of the entire length of the water pathway from roots to leaves and makes up approximately 50% of the overall plant hydraulic resistance, the remaining 1% length and 50% resistance being located in the few millimetres of extra-vascular pathway that water has to follow when moving from the root surface to the stele and from minor veins to the evaporation sites in the mesophyll (Cruiziat et al., 2002). According to the cohesion–tension theory (Dixon and Joly, 1894; Steudle, 2001), water transport from roots to leaves through the xylem is ensured by continuous water columns ascending in response to a gradient of negative pressures (=tension) that are generated at the evaporative...
surfaces of mesophyll cell walls. Despite vigorous challenges to its basic assumptions (Canny, 1998; Zimmermann et al., 2004), the cohesion–tension theory has proved to be a robust one that can explain available experimental data on plant water relations (Comstock, 1999; Stiller and Sperry, 1999; Wei et al., 1999).

Extant land plants dominating terrestrial habitats rely on the xylem conduits for long-distance water transport (Sperry, 2003). Xylem conduits are long-shaped cells dead at maturity, with reinforced cell walls that can sustain large negative pressures (Hacke et al., 2001; Pittermann et al., 2006). The simplest xylem conduits are represented by tracheids, that appeared soon after land colonization and remained the only conducting element available to land plants for several million years (Niklas, 1985). The maximum size attained by tracheids during evolution was about 10 mm in length and up to 140 μm in diameter, and the increase in water transport capacity in tracheid-bearing plants was eventually accomplished by the progressive increase in the number of tracheids (and hence total transverse tracheid area) per cross stem area (Roth-Nebelsick et al., 2000).

An unprecedented increase in water transport efficiency of land plants was made possible by the appearance of vessels, approximately 250 million years ago (Pittermann, 2010; Fig. 1A). Vessels can reach greater lengths (more than 10 m) and, most importantly, larger diameters (up to 500 μm) than tracheids. As water flow through capillary-sized conduits is proportional to the fourth power of conduit radius, according to the Hagen–Poiseuille equation (Giordano et al., 1978), the potential hydraulic conductance of vessels is orders of magnitude higher than that of tracheids. Plant gas exchange, and hence photosynthesis and productivity, are limited by the overall plant hydraulic conductance (Tyree, 2003) as well as by root-to-shoot chemical signalling (Liang et al., 1996; Tardieu et al., 2010). It is, therefore, not surprising that emerged habitats are now dominated by vessel-bearing angiosperms which mostly contribute to terrestrial primary productivity (Brodribb, 2009).

Vessels and tracheids have finite dimensions (Beckman and Keller, 1977; Carlquist, 2001). Although some vessels can reach considerable lengths, water transport from roots to leaves rarely, if ever, relies on single conduits directly connecting root tips to mesophyll cells. Rather, water must pass through many thousands of conduits along its way. Interconduit connections for water passage are made possible by pits, small openings in the secondary cell wall putting into communication adjacent conduits along joint walls (Choat et al., 2008).

Tracheids and vessels are the key structural components of long-distance water transport, but the xylem as a whole is not just made up by dead conduits. Living vessel-associated cells, with direct access to conduit lumina, play important functional roles in loading/unloading different solutes into/from the transpiration stream (De Boer and Volkov, 2003). Living cells are also involved in the refilling of embolized conduits (Salleo et al., 2004; Nardini et al., 2011). Although much about the exact refilling mechanisms remains unclear, the hypothesis that solutes released by living cells associated with xylem conduits can influence the ascent of water in plants was postulated almost 40 years ago (Sauter et al., 1973; Braun, 1984). Moreover, xylem conduits are both physically and functionally associated with living phloem. Physical association arises as a consequence of the proximity of the two transport systems in primary vascular bundles, or is facilitated by rays in stems with secondary growth. Here, rays are continuous along xylem and phloem as ray initials in the cambium produce cells on the two opposite sides (Fahn, 1990). Functional association is demonstrated by several examples of the interchange of water and solutes between xylem and phloem reported in the literature (Van Bel, 1990; Schneider et al., 1994; Wang et al., 1997; Lampinen and Noponen, 2003; Metzner et al., 2010).

### The vulnerable pipeline

Water in the xylem remains in liquid phase while being maintained below its vapour pressure (+2.33 kPa at 20 °C), i.e. it is in a metastable state (Steudle, 2001). Under these conditions, xylem water is at risk of cavitation-induced embolism, as a consequence of the transition from the liquid to the vapour phase (Cochard, 2006). Xylem embolism is thought to arise when gaseous bubbles are aspirated into xylem conduits from adjacent gas-filled compartments through pores of interconduit pit membranes (Delzon et al., 2010), a phenomenon which becomes more and more likely as xylem pressure drops under high daily transpiration rates and/or during prolonged drought conditions leading to a substantial decrease of soil and plant water potential (Tyree and Sperry, 1989). The final outcome of this ‘air seeding’ process (Zimmermann, 1978; Cochard et al., 1992) is that conduits become filled with a gas phase. Embolism can also be induced by freeze–thaw cycles that cause gases to expand into the conduit during thawing (Sperry and Sullivan, 1992), although recent evidence suggests that cavitation events can occur during the freezing phase (Mayr et al., 2007).

Xylem embolism causes a reduction in plant hydraulic conductance (Sperry and Pockman, 1993; Nardini and Pitt, 1999) with a consequent drop in leaf water potential, stomatal closure, and the impairment of photosynthesis and productivity. Nonetheless, xylem embolism is far from being a rare event. Xylem pressures inducing 50% embolism-induced loss of conductance range between –0.2 and –14 MPa for species of wet tropical and dry tropical or Mediterranean forests, respectively (Maherali et al., 2004). These values overlap the range of minimum water potential values recorded in different habitats (Scholander et al., 1965; Lenz et al., 2006; Kursar et al., 2009). Moreover, xylem pressure thresholds triggering cavitation events are even closer to water potential values commonly experienced by plants throughout the vegetative season (Nardini and Salleo, 2000; Brodribb et al., 2003; Meinzer et al., 2009). In fact, the embolism-induced reduction of xylem hydraulic conductance (\(K_{\text{XYL}}\)) is commonly detected even in well-watered plants as...
a consequence of dynamic daily water stress conditions (Domec et al., 2007).

Day-by-day accumulation of embolism in the xylem under prolonged drought conditions can lead to the complete failure of water transport and plant death (Davis et al., 2002; Brodribb and Cochard, 2009). Plants prevent fatal drops in $K_{XYL}$ by adopting one or more of three strategies: (i) embolism avoidance, based on the stomatal control of xylem pressure; (ii) embolism reversal, based on refilling mechanism of gas-filled conduits; and (iii) the production of new xylem, based on cambial activity. Stomatal control of xylem embolism has been reported in several plant species (Jones and Sutherland, 1991; Cochard, 2002). The reversal of xylem embolism has been reported in plant species where water refilling the conduits is driven by over-atmospheric root pressures developed during the night or in spring (Sperry et al., 1987; Cobb et al., 2007). Embolized conduits can also refill while adjacent functioning conduits are under substantial negative pressure, a process that has been named ‘novel refilling’ (Tyree et al., 1999; Hacke and Sperry, 2003). In this case, embolized conduits are likely to be pressurized independently of negative pressures in neighbouring functional conduits (Brodersen et al., 2010). In every case, embolism reversal is a relatively

---

**Fig. 1.** Light (A–D) and transmission electron (E–F) microscope images of secondary xylem illustrating the major structures associated with water transport in xylem cells in angiosperms. A. Silicon resin cast of two adjacent vessels with scalariform (arrowheads) and simple (arrows) perforation plates in *Meryta tenuifolia* (Araliaceae); scale bar=200 μm. (B) Transverse section of *Umbellularia californica* (Lauraceae) stained with ruthenium red after 48 h treatment with NH$_4$OH suggesting pectins in the primary cell wall (arrowheads) and intervessel pit membranes (arrow); scale bar=50 μm. (C, D) Transverse wood sections of *Nerium oleander* (C, Oleaceae) and *Quercus ilex* (D, Fagaceae), showing radial vessel multiples and mainly solitary vessels, respectively; high vessel grouping as in *N. oleander* is suggested to be positively correlated with the magnitude of the ionic effect; scale bar=500 μm. (E) Transverse section of *Hibiscus syriacus* (Malvaceae) showing vessels (V), a perforation plate (PP), imperforate tracheary elements (*), and various types of vessel pitting; living parenchyma cells are in contact with the large vessel on the right; scale bar=20 μm. (F) Transverse section of *Lindera megaphylla* (Lauraceae) showing two pairs of bordered intervessel pits, a thick, electron dense pit membrane (arrow), pit chamber (*), pit aperture (PA), and secondary cell wall (SW), the black lines in the secondary cell wall represent preparation artefacts; scale bar=5 μm.
slow process, requiring several hours to be completed (Vesala et al., 2003; Scheenen et al., 2007; Salleo et al., 2009; Brodersen et al., 2010), so that xylem cavitation is predicted to have a negative impact on photosynthesis over the short term.

Interconduit pits: a key determinant of xylem functional properties

The structure and function of pits and their impact on xylem hydraulics and vulnerability to cavitation has received considerable attention during recent years (Choat et al., 2008). Pits in water-transporting xylem cells are always bordered, which means that they present openings in the secondary cell wall overhanging a pit chamber with a diameter that is larger than the pit aperture (Fig. 1E, F). BORDERED pits are designed to enable water flow through the common walls of connected conduits, providing a compromise between water transport efficiency, mechanical strength of the cell wall, and hydraulic safety, i.e. preventing the spread of gas bubbles and pathogens (Tyree and Sperry, 1989; Carlquist, 2001; Choat et al., 2008).

While most gymnosperms have developed pit membranes with a central thickening (torus) and a porous margo (Liese, 1965; Bauch et al., 1972), angiosperms typically have pit membranes that are homogeneous in thickness (Schmid and Machado, 1968). The inter-vessel pit membranes of angiosperms are composed of a tightly woven mesh of microfibrils with pore sizes ranging from 5 nm to 420 nm, although pores are usually <100 nm (Choat et al., 2003, 2004; Fig. 2). Water moving through the xylem encounters two principal resistances: the dense microfibril network of pit membranes and the conduit lumen. While the pit resistance to water flow is almost 60 times lower in conifer pit membranes than in those of angiosperms (Pittermann et al., 2005), the proportion of total resistance attributed to pits is rather similar, i.e. on average, 64±4% and 56±2% in conifer tracheids and eudicot vessels, respectively (Wheeler et al., 2005; Hacke et al., 2006; Pittermann et al., 2006). These findings indicate that there is scaling between pit and lumen resistance in the xylem: when lumen resistance increases (longer or narrower conduits), pit resistance also increases.

Given the large variety in pit size, shape, membrane structure, border configuration, vesture presence, pit-field arrangement, interconduit wall thickness, and interconduit pit-field fraction, pits provide an adaptive trait in the overall hydraulic efficiency of plants with the potential to drive ecological differences between species. The ‘air seeding’ hypothesis for drought-induced cavitation in xylem (Sperry and Tyree, 1988; Tyree and Zimmermann, 2002) implies that air bubbles pass through the largest pore that connects two vessels. Observations based on electron microscopy and atomic force microscopy (Fig. 2) reveal that microfibrils in angiosperm pit membranes are typically around 20 nm (Schmid and Machado, 1968; Pesacreta et al., 2005; Jansen et al., 2009). Interestingly, the 25-fold difference in pit membrane thickness, varying from 70 nm to 2.000 nm is significantly correlated with maximum pore diameter and air-seeding pressure (Jansen et al., 2009). Because greater pit membrane porosity reduces hydraulic resistance but increases vulnerability to embolism, differences in pit membrane thickness and porosity correlate with vulnerability to cavitation (Lens et al., 2011; Plavcova et al., 2011). In addition to pit membrane thickness, other micromorphological features such as vestures, warts, and pit geometry have been suggested to influence hydraulic traits (Choat et al., 2004; Jansen et al., 2004a; Meyra et al., 2007; Kohonen and Helland, 2009).

Trade-offs in hydraulic safety and efficiency of angiosperm wood are not only influenced by qualitative pit characteristics, but also by quantitative features such as the total intervessel pit membrane area per vessel (=A_P), which depends on average vessel length, vessel diameter, and spatial vessel distribution (i.e. intervessel connectivity). More specifically, A_P has been found to explain interspecific variation of vulnerability to air-seeding in various Rosaceae and across a broad range of not closely related angiosperms (Wheeler et al., 2005; Hacke et al., 2006). However, no

---

**Fig. 2.** Intervessel pit membranes of *Sapium sebiferum* (A, Euphorbiaceae) and *Laurus nobilis* (B, C, Lauraceae) imaged with an atomic force microscope using amplitude mode (A, B) and phase mode (C). (A) Intervessel pit membrane showing randomly arranged microfibrils and fractured secondary wall of the pit chamber; tip artefacts are visible near the edge of the pit membrane; scale bar=2 μm. (B) Detail of intervessel pit membrane showing cellulose microfibrils (arrow), which are 20–30 nm thick, and globules, scale bar=250 nm. (C) Phase image corresponding to amplitude image shown in (B); phase bright layer of microfibrils (arrow) and globules in combination with a phase dark layer with unknown chemical identity; scale bar=250 nm.
support for this ‘rare pit hypothesis’ has been found within the genus Acer and across conifer species (Pittermann et al., 2006; Hacke and Jansen, 2009; Lens et al., 2011). Cavitation resistance in conifers seems most closely correlated to the ratio of the torus to pit aperture diameter but does not vary systematically with margo porosity (Delzon et al., 2010; Pittermann et al., 2010).

**Ion-mediated changes of xylem hydraulic properties: the ionic effect**

Because long-distance water transport occurs through pipelines made up by the lignified walls of dead cells, it was assumed for a long time that xylem water transport properties arise as a mere consequence of the physical/geometrical features of xylem conduits. In particular, $K_{XYL}$ is generally thought to be a complex function of conduits’ diameter, length, and number per unit cross stem area (Ewers, 1985; Comstock and Sperry, 2000; Nijssse et al., 2001; Sperry et al., 2006; Poorter et al., 2010; McCulloh et al., 2010). Hence, the xylem has been classically envisioned as an ‘inert’ transport system, whose hydraulic properties can change only as a consequence of diurnal or seasonal embolism/refilling cycles (Melcher et al., 2001; Domec et al., 2007), the collapse of conduits under high tension (Cochard et al., 2004; Brodribb and Holbrook, 2005), or as the result of cambial activity and the production of new xylem conduits (Oosterhuis and Wulkschleger, 1987; Cochard et al., 1997).

In contrast to this paradigm, data gathered over the last ten years suggest that plants are potentially capable of modulating xylem hydraulics over the short term by regulating xylem sap ionic content. Zimmermann (1978) appears to be the first to report that $K_{XYL}$ measurements are influenced by the composition of the fluid perfused through the stem sample and to provide a tentative interpretation of this phenomenon. It is worth reporting the exact words by MH Zimmermann to appreciate the value of his intuition in the light of discoveries to come several years later.

‘...Distilled water was replaced by tap water in the middle of a flow-rate measurement [...] The result was surprising: flow rate instantaneously increased to above the initial rate with distilled water and remained constant [...] We now think that the phenomenon might be based upon swelling or shrinking of the vessel-to-vessel pit membranes...’

It was only more than 20 years later that the impact of the composition of perfused fluids on $K_{XYL}$ measurements received renewed interest by Van Ieperen et al. (2000), who reported 5–8% increase in $K_{XYL}$ of Dendranthema stems perfused with different salt solutions compared with distilled water. Interestingly, the enhancement of $K_{XYL}$ was observed only when salt solutions were used, while isosmotic carbohydrate solutions did not induce any change in $K_{XYL}$ with respect to values measured using distilled water. This finding suggested that purely osmotic effects were not involved in the phenomenon. As a consequence, the $K_{XYL}$ dependence on the ionic concentration of xylem sap is referred to as the ‘ionic effect’.

Zwieniecki et al. (2001) provided evidence for very rapid variations of $K_{XYL}$ upon alternative perfusion of Nicotiana tabacum, Laurus nobilis, and Fraxinus americana stems with deionized water and diluted salt solutions, and also showed that $K_{XYL}$ enhancement was a function of the salt concentration in the fluid, the response being initiated and saturated at KCl concentrations ranging between 5 mM and 20–50 mM, respectively. In some cases, $K_{XYL}$ increased by about 150% when measured with KCl solutions compared with deionized water. The range of concentrations effective on $K_{XYL}$ fits well with the physiological range of potassium content reported for xylem sap of different plant species (Herdel et al., 2001; Malone et al., 2002; Siebrecht et al., 2003; Goedger et al., 2005). Zwieniecki et al. (2001) confirmed that the presence of ionic solutes was necessary for the enhancement of $K_{XYL}$, while non-ionic solutes like sucrose or ethanol had no effect on xylem hydraulic properties. Based on such observations and on the dynamics of $K_{XYL}$ enhancement, Zwieniecki and co-workers re-proposed the interpretation originally suggested by Zimmermann (1978), namely that $K_{XYL}$ changes upon modifications of xylem sap ionic content was due to dynamic swelling/shrinking of pit membranes and, in particular, of the pectic matrix that is supposed to be a major component of these structures (see below).

Pectins are major components of the middle lamella and primary wall, accounting for about 30% of wall macromolecules (Ridley et al., 2001; Willats et al., 2001; Kaczkowski, 2003). Pectins are known to be among the ‘most complex macro-structures in nature’ (Vincken et al., 2003) and consist of complex polysaccharides rich in galacturonic acid (GalA). GalA can be assembled into two structural types forming the backbone of three polysaccharide domains: homogalacturonan (HGA), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II). The most abundant components of primary wall matrix are HGA and RG-I. Although the RG-II fraction is generally small, it can have a major impact on cell wall porosity (Matsunaga et al., 2004). RG-I is based on a backbone of rhamnose and GalA residues with lateral arabinan or galactan chains. HGA is a linear homopolymer of GalA, which is highly methyl-esterified when synthesized and can be de-esterified later by apoplastic pectin methyl-esterases. Acidic HGA can link Ca$^{2+}$ thus forming ‘egg box’ junction zones that increase cell wall stiffness and can impact on cell wall porosity (Tibbits et al., 1998). However, some of the carboxyls do not link calcium and the pectic matrix is hence characterized by the presence of negative charges exposed. As a consequence, pectins behave like anionic polyelectrolytes that can reversibly switch from a gel status when fully hydrated to an amorphous solid status when dehydrated (Ryden et al., 2000). Pectin hydration depends on the equilibrium between neutral carboxylic residues and negative charges (Dähnert and Huster, 1999), and cations can interfere with this equilibrium and cause some shrinking of pectins (Ryden et al., 2000; Willats et al., 2001).
The hydrogel properties of pectins are currently thought to be the major cause of the ionic effect. Cation-mediated volume changes of pit membrane pectins would modify the diameter of nanometer-sized pores of pits and thus change their hydraulic conductance, which would influence the overall $K_{XYL}$ (Fig. 3). The dynamics of pectins swelling/shrinking as a function of ion concentration as measured in synthetic polyampholytic hydrogels (English et al., 1996) is consistent with observed changes of $K_{XYL}$ in response to the modification of xylem sap salinity in two mangroves (López-Portillo et al., 2005). Moreover, the ionic effect is larger in species where acidic pectins of the pit membranes are more abundant (Gortan et al., 2011), which is in agreement with predictions based on the hydrogel hypothesis. On the other hand, targeted modification of the degree of neutrality of tobacco homogalacturonan did not impact on the ion-mediated enhancement of $K_{XYL}$ (Nardini et al., 2007a). Hence, clear-cut evidence of cation-mediated reversible changes of pit membrane volume changes is still missing.

Cochard et al. (2010) have recently reported contrasting effects of ionic solutions on $K_{XYL}$ of different species. In some cases, $K_{XYL}$ actually decreased when measured with KCl solutions compared with deionized water, leading the authors to conclude that the current interpretation of the ionic effect, being based on swelling/shrinking dynamics of a porous pectic matrix, is probably too simplistic to account for all the observations reported in the literature. Alternatively, Cochard et al. (2010) proposed that, in some species, pit membranes would behave like a gelled non-porous structure. In this case, an increase in gel water content under low ionic concentrations of xylem sap would increase the water permeability of the pit membrane (Tibbits et al., 1998). By contrast, high ionic concentration would be expected to dehydrate the gel and decrease its water permeability and, hence, $K_{XYL}$.

Clearly, more information about the structure and composition of interconduit pit membranes is needed in order to confirm or discard some of the hypotheses advanced to explain the ionic effect. Although the role of intervessel pits in the ionic effect is suggested by several lines of evidence (Gascò et al., 2006; Aasamaa and Sober, 2010; Jansen et al., 2011), data supporting the original explanation of the ionic effect as provided by Zimmermann (1978) and later re-launched by Zwieniecki et al. (2001) are still scarce and controversial. The putative mechanism of the ionic effect, based on changes in pectin volume, still awaits convincing proof. In this sense, atomic force microscopy (AFM) could provide a necessary tool to test the validity of the current paradigm (Kirby et al., 1996; Santos and Castanho, 2004; Fig. 2) and to investigate the chemical identity of the interstitial phase bright coating of pit membranes, which is suggested to correspond to the soft, non-crystalline nature of both pectin and polyphenolic
materials (Pesacreta et al., 2005; Fig. 2C). Until such direct evidence is obtained, alternative interpretations of the biophysical bases of the ionic effect, like electro-viscous effects in pit membranes microchannels (Ren et al., 2001), should not be discarded.

After the new surge of interest into the ionic effect, several studies have appeared in the literature reporting ion-mediated increases of $K_{XYL}$. The magnitude of the $K_{XYL}$ increase tested up to now for different species is reported in Table 1. Although Table 1 reports only results gathered in angiosperms, the ionic effect has also been reported to occur in some ferns and gymnosperms (Boyce et al., 2004; Domec et al., 2007). The ion-mediated $K_{XYL}$ increase ranged from less than 2% to almost 60% when low concentration solutions (or deionized water) perfused through fully hydrated stems, were enriched with 10–170 mM KCl (or NaCl). In most studies KCl solutions were used to test the magnitude of the ionic effect. The choice of potassium as a reference cation to induce $K_{XYL}$ enhancement is justified by the fact that K+ is one of the most common ions found in the xylem sap, representing about 50% of the inorganic solutes (Herdel et al., 2001). Hence, any eventual ion-mediated regulation of $K_{XYL}$ in planta is likely to be accomplished by modulation of the xylem sap [K⁺]. As already reported above, early studies have shown that the ionic effect is concentration-dependent and is generally saturated at 20–50 mM KCl. However, in various studies, different KCl concentrations have been used to test the occurrence and magnitude of the ionic effect, so that data across species are not easily comparable.

One further important source of variation in the magnitude of the ionic effect is related to the length of samples

<table>
<thead>
<tr>
<th>Species</th>
<th>$\Delta K_{XYL}$ (%)</th>
<th>Concentration range, solute type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus ilex L. (Fagaceae)</td>
<td>1.9</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Betula alba L. (Betulaceae)</td>
<td>3.0</td>
<td>0–50 mM, KCl</td>
<td>Cochard et al. (2010)</td>
</tr>
<tr>
<td>Fagus sylvatica L. (Fagaceae)</td>
<td>3.5</td>
<td>0–50 mM, KCl</td>
<td>Cochard et al. (2010)</td>
</tr>
<tr>
<td>Platanus orientalis L. (Platanaceae)</td>
<td>4.7</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Salix caprea L. (Salicaceae)</td>
<td>5.0</td>
<td>0–30 mM, KCl</td>
<td>Aasamaa and Sober (2010)</td>
</tr>
<tr>
<td>Litssea sericea (Wallich ex Nees) Hooker fil. (Lauraceae)</td>
<td>6.7</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Dendranthema grandiforum Tzelev cv Cona (Asteraceae)</td>
<td>7.0</td>
<td>0–10 mM, KCl</td>
<td>Van Ieperen et al. (2000)</td>
</tr>
<tr>
<td>Robinia pseudoacacia L. (Fabaceae)</td>
<td>7.1</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Phillyrea latifolia L. (Oleaceae)</td>
<td>8.4</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Lindera megaphylla Hemsl. (Lauraceae)</td>
<td>9.7</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Padus avium Mill. (Rosaceae)</td>
<td>10.0</td>
<td>0–30 mM, KCl</td>
<td>Aasamaa and Sober (2010)</td>
</tr>
<tr>
<td>Populus tremula L. (Salicaceae)</td>
<td>11.1</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Arbutus unedo L. (Ericaceae)</td>
<td>11.7</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Tilia cordata Mill. (Malvaceae)</td>
<td>12.0</td>
<td>0–30 mM, KCl</td>
<td>Aasamaa and Sober (2010)</td>
</tr>
<tr>
<td>Quercus robur L. (Fagaceae)</td>
<td>13.0</td>
<td>0–30 mM, KCl</td>
<td>Aasamaa and Sober (2010)</td>
</tr>
<tr>
<td>Helianthus annuus L. (Asteraceae)</td>
<td>14.7</td>
<td>0.05–25 mM, KCl</td>
<td>Nardini et al. (unpublished data)</td>
</tr>
<tr>
<td>Acer platanoides L. (Sapindaceae)</td>
<td>15.0</td>
<td>0–30 mM, KCl</td>
<td>Aasamaa and Sober (2010)</td>
</tr>
<tr>
<td>Viburnum tinus L. (Adoxaceae)</td>
<td>15.5</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Nicotiana tabacum L. (Solanaceae)</td>
<td>16.1</td>
<td>0–25 mM, KCl</td>
<td>Nardini et al. (2007a)</td>
</tr>
<tr>
<td>Ceratonia silqua L. (Fabaceae)</td>
<td>16.9</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Prunus laurocerasus L. (Rosaceae)</td>
<td>17.1</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Neolitsea sericea (Blume) Koidz (Lauraceae)</td>
<td>18.0</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Phytolacca dioica L. (Phytolaccaceae)</td>
<td>19.3</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Prunus avium L. (Rosaceae)</td>
<td>20.0</td>
<td>0–50 mM, KCl</td>
<td>Cochard et al. (2010)</td>
</tr>
<tr>
<td>Lycopersicum esculentum (L.) Karsten ex Farw. (Solanaceae)</td>
<td>20.0</td>
<td>0–20 mM, KCl</td>
<td>Zwieniecki et al. (2003)</td>
</tr>
<tr>
<td>Laurus nobilis L. (Lauraceae)</td>
<td>21.5</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Acer campestre L. (Sapindaceae)</td>
<td>22.3</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Umbellularia californica (Hook &amp; Arn.) Nutt. (Lauraceae)</td>
<td>24.2</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Persea americana Mill. (Lauraceae)</td>
<td>27.3</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Citrus aurantium L. (Rutaceae)</td>
<td>28.0</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Olea europaea L. (Oleaceae)</td>
<td>31.5</td>
<td>0.05–25 mM, KCl</td>
<td>Jansen et al. (2011)</td>
</tr>
<tr>
<td>Nerium oleander L. (Apocynaceae)</td>
<td>32.0</td>
<td>0–170 mM, NaCl</td>
<td>López-Portillo et al. (2005)</td>
</tr>
<tr>
<td>Conocarpus erectus L. (Combretaceae)</td>
<td>32.0</td>
<td>0–170 mM, NaCl</td>
<td>López-Portillo et al. (2005)</td>
</tr>
<tr>
<td>Avicennia germinans L. (Acanthaceae)</td>
<td>34.0</td>
<td>0–170 mM, NaCl</td>
<td>López-Portillo et al. (2005)</td>
</tr>
<tr>
<td>Tilia platyphyllos Scop. (Malvaceae)</td>
<td>58.0</td>
<td>0–50 mM, KCl</td>
<td>Cochard et al. (2010)</td>
</tr>
</tbody>
</table>
used for hydraulic measurements. Gascò et al. (2006) have shown that the ionic effect is proportional to the sample length. In fact, short samples have a higher probability to have some (or even most) vessels cut open at both ends. In this case, water would flow through xylem conduits without crossing interconduit pits and the ionic effect would be expected to be very small. Very moderate $K_{XYL}$ enhancement was reported for 3-cm-long laurel stems, whilst the effect increased for longer samples and became saturated for samples 12-cm-long. Measurements of vessel length distribution revealed that more than 80% of xylem conduits were intact in 12-cm-long laurel stems. Hence, the choice of sample length for stems of different species should be preceded by measurements of vessel length distribution or maximum vessel length (Gascò et al., 2007), but this information is not always available in the papers listed in Table 1.

The third important source of variation in the magnitude of the ionic effect as reported in different studies is related to the reference fluid used to measure values of $K_{XYL}$. In several studies (Zimmermann, 1978; Van Ieperen et al., 2000; Zwieniecki et al., 2001; Boyce et al., 2004; Aasamaa and Sober, 2010), $K_{XYL}$ enhancement induced by salt solutions was calculated relative to values measured during perfusion with deionized water. In fact, deionized water is a very artificial medium and is by no means representative of xylem sap. Xylem sap always contains at least small amounts of different ions including potassium, calcium, and magnesium among the principal cations (Siebrecht et al., 2003; Googder et al., 2005). Van Ieperen and Van Gelder (2006) have reported suppression of ion-mediated flow changes in Chrysanthemum sp. and Prunus laurocerasus L. whenever solutions containing even small amounts of calcium (1 mM) were used as a reference fluid instead of deionized water. Hence, the authors concluded that complete removal of cations from the xylem fluid as during perfusion with deionized water, would be responsible for about 95% of the measured ion-mediated $K_{XYL}$ changes. They interpreted this result as evidence that the small amount of cations usually present in the xylem sap would be sufficient to maximize the electrostatic screening of the de-esterified groups in the pectic matrix of pit membranes and, hence, maximize pit membrane porosity and $K_{XYL}$. They further suggested that a high $K^+/Ca^{2+}$ ratio in the solution flowing through the xylem conduits would cause $Ca^{2+}$ loss from the pectic matrix, therefore modifying the degree of cross-linking of the pectins and causing reduced rigidity and larger swelling of the pectic matrix. Hence, injecting $K^+$ solutions after deionized water would strongly but artefactually amplify the ionic effect (Van Ieperen, 2007) thus raising doubts about the actual possibility that the ionic effect can occur to a significant extent in planta.

Nardini et al. (2007b) and Cochard et al. (2010) later showed that the suppression of ion-mediated $K_{XYL}$ changes, when $Ca^{2+}$ is added to the reference solution, is not a general phenomenon. Different species as well as different physiological states were reported to be important factors causing different responses to ionic solutes in terms of magnitude of the $K_{XYL}$ increase as well as eventual modulation/suppression of the phenomenon by $Ca^{2+}$. This might suggest that differences in the chemical nature of the pectins at the pit membrane level, due to inter- and intra-specific variation, make the ion-mediated regulation of xylem hydraulics a largely variable and partially unpredictable phenomenon. In any case, low-concentration poly-ionic solutions containing at least 0.5 mM $Ca^{2+}$ should be preferred over deionized water as reference fluids during hydraulic measurements aimed at evaluating the ionic effect in different plant species in future studies.

Despite the limitations listed above, Table 1 suggests that the ionic effect is variable across species. Other studies have shown that the ionic effect can be variable even within single species, for example, on a seasonal time scale (Gascò et al., 2007; Trifilo et al., 2008). Recent studies have highlighted some of the anatomical and biochemical bases of this variability.

Anatomical and biochemical evidence for the ionic effect

The phylogenetic variation in the magnitude of the ionic effect has been explained by differences in lignification patterns of vascular cell walls (Boyce et al., 2004). These authors suggested that a weak primary wall lignification corresponds to high concentrations of middle-lamella pectins. Given the hydrogel properties attributed to pectins, variation in the ionic effect was attributed to volume changes of middle-lamella pectins, but no evidence for the distribution of pectins in pit membranes was provided (Boyce et al., 2004).

Recently, Jansen et al. (2011) have reported a positive correlation between the magnitude of the ionic effect and spatial vessel distribution (Fig. 1C, D). Across a wide range of 20 angiosperm species covering 13 families, the ionic effect was found to show a significant correlation with the following features: (i) intervessel contact fraction (=portion of the vessel wall in contact with adjacent vessels based on transverse sections), (ii) intervessel pit fraction (=mean fraction of the total vessel wall area occupied by intervessel pits), (iii) intervessel contact length fraction (=ratio of average contact length between adjacent vessels to mean vessel length), (iv) vessel grouping parameters (vessel grouping index, solitary vessel index, and vessel multiple index), and (v) intervessel wall thickness. Although these findings provide further evidence that qualitative features related to pits and the three-dimensional vessel network play a central role in the ionic effect, only a weak correlation ($r=0.42$, $P=0.06$) was found between ionic effect and total intervessel pit membrane area per vessel ($A_P$). This last finding suggests that chemical characteristics of pit membranes could play an additional role in the ionic effect response. It should also be emphasized that quantitative data on vessels, especially vessel length distributions and the amount of intervessel connectivity are time-consuming.
features to quantify using light microscopy, and have therefore not been studied extensively across angiosperms (Zimmermann and Tomlinson, 1967; Huggett and Tomlinson, 2010; Brodersen et al., 2011).

In addition, variation in vessel diameter, vessel length, and vessel wall area showed no correlation with the ionic effect (Jansen et al., 2011). It is unclear whether the relatively large variation in pit membrane area resistance has an impact on the ionic effect response. Furthermore, no significant correlation was found between the ionic effect and pit membrane thickness, although a positive correlation existed with the intervessel wall thickness. Since the thickness of the primary cell wall and middle lamella is associated with the thickness of the secondary cell wall (Jansen et al., 2009), it can be suggested that higher amounts of pectins are available in species with thick secondary cell walls.

One of the most important questions related to the hydrogel hypothesis of the ionic effect is whether or not pectins remain present in fully mature pit membranes of angiosperms. While various scientists have confirmed considerable amounts of pectins in tori of conifer sapwood (Bauch and Berndt, 1973; Thomas, 1975; Tschernitz and Sachs, 1975; Hafrén et al., 2000; Putoczki et al., 2008), this issue is not without controversy for angiosperm pit membranes. Although more work on the ionic effect of conifers is needed (Boyce et al., 2004; Coillard et al., 2010), the relatively low (and even negative, see Coillard et al., 2010) ionic effect measured in conifers could be explained by the fact that water flow through conifer tracheids occurs via the porous margo of pit membranes, which shows an average radius of $r \approx 800$ nm (Petty and Preston, 1969). As a result, $K_{XYL}$ would not be affected by the presence of pectins in the impermeable torus of conifers.

There is general agreement that hydrolysis of the angiosperm pit membrane increases its permeability. During the final developmental stages of vessel elements and tracheids, hydrolytic enzymes are traditionally thought to remove all non-cellulosic pit membrane components in interconduit pit membranes, resulting in mats of cellulose microfibrils (O’Brien and Thimann, 1967; O’Brien, 1970; Butterfield and Meylan, 1982; Butterfield, 1995). Pit membranes connecting tracheary elements with parenchyma cells or fibres are only hydrolysed on the tracheary side up to the middle lamella. Pit membranes between parenchyma cells are not hydrolysed (Schmid and Machado, 1968; Meylan and Butterfield, 1982).

No hemicelluloses have been detected in intervessel pit membranes of Fagus sylvatica (Jayme and Azzola, 1965) and Citrus sylvatica (Alves et al., 2009). Immunolabelling with JIM5 and JIM7 monoclonal antibodies revealed no pectic homogalacturonans in pit membranes of Populus trichocarpa×deltoideae (Plavcova et al., 2011). Several studies, however, have provided evidence that methylated pectins remain present in mature intervessel pit membranes, based on the hydroxylamine-ferric chloride reaction, in a total of nine angiosperm species covering six non-related families (Catesson et al., 1979; Czaniński, 1979; Catesson, 1983; Gortan et al., 2011). Acidic pectins in angiosperms have only been reported in torus-bearing pit membranes of Ulmus campestris and more recently in four Lauraceae species as based on staining with ruthenium red (Czaninski, 1979; Gortan et al., 2011) (Fig. 1B). Based on various staining solutions (TBO, safranin/alcian blue, and acriflavine), the absence of lignin and the potential presence of pectins associated with tori has also been suggested for Ulmus and Zelkova (Jansen et al., 2004b; Dute et al., 2004; Coleman et al., 2004). Outside angiosperms, both acidic and methylated pectins have been reported in pit membranes of a Polypodium sp. fern (Liberman-Maxe, 1982).

Chemical properties of intervessel pit membranes were suggested to play a role in the magnitude of the ionic effect within four Lauraceae species (Gortan et al., 2011). In particular, two Lauraceae species (Laurus nobilis and Umbellularia californica) with a relatively high (c. 20%) ionic effect were found to show a similar proportion of acidic versus methylsterified pit membrane pectins, whereas methylsterified pectins showed a much higher relative abundance than acidic pectins in two species (Lindera megaphylla and Listea sericea) with a low (c. 10%) ionic effect (Gortan et al., 2011). The authors also speculated that the activity of cell-wall modifying enzymes such as pectin methylesterases (PMEs) and pectin acetylsterases (PAEs) can induce temporal changes in the sensitivity of $K_{XYL}$ to changes in sap ionic content (Gascó et al., 2007; Trifilò et al., 2008). Interestingly, the classical reaction of ruthenium red with pectins is typical but not completely specific (Bonner, 1936; Luft, 1971). Therefore, it is clear that the study of a wider range of angiosperm species and families is required and that immunolabelling techniques using a broad range of antibodies are needed to provide clear evidence for the presence of pectins in angiosperm pit membranes. In addition to further research on the chemistry of pit membranes, seasonal variation of pit membrane ultrastructure requires special attention in order to evaluate seasonal variation of the ionic effect.

**Functional roles of the ionic effect in planta**

**Diurnal and/or seasonal changes of $K_{XYL}$**

Xylem sap ionic content is known to change dynamically on both a daily and a seasonal basis. As an example, Siebrecht et al. (2003) reported daily fluctuations in the concentration of three cations ($K^+$, $Mg^{2+}$, $Ca^{2+}$) in the xylem sap of poplar. Similarly, concentrations of the major cations including potassium have been reported to change on a seasonal basis in the xylem sap of different plants (Glawac et al., 1990; Bundt et al., 1997). Interestingly, in some cases, the increase in xylem sap $[K^+]$ was observed during the summer time, when transpirational demand is higher due to high air temperature, and long-distance water transport in plants is put at risk by drought-induced hydraulic dysfunction (Trifilò et al., 2008). Zwieniecki et al. (2004) were the first to report a possible link between xylem sap potassium concentration and xylem hydraulic conductance in planta.
When a ring of bark was removed from maple stems, thus interrupting phloem transport, xylem sap potassium concentration was found to decrease in girdled stems. Stem hydraulic conductance decreased as well and xylem hydraulic efficiency returned to pre-girdling values only when KCl was added to the solution perfused through the stem during hydraulic measurements. These results were interpreted as evidence for a role of phloem-to-xylem potassium recirculation in the maintenance of adequate levels of xylem hydraulic conductance via the ionic effect. Evidence for potassium recirculation between phloem and xylem was later provided by Metzner et al. (2010), but other studies (Nardini et al., 2010) failed to detect significant changes in xylem sap [K+] upon girdling. Nonetheless, the above data raise the hypothesis that xylem hydraulic properties can also change on a diurnal and/or seasonal basis as a function of changes in xylem sap cation concentration. In this view, the peaks in potassium concentration recorded during the warmest hours of the day as well as during the warmest months of the year would be consistent with the functional advantage of up-regulation of xylem (and plant) hydraulic properties during periods of high transpirational demand, when a reduction of frictional resistances along the xylem would allow higher rates of water transport to the foliage, thus preventing water potential drop and desiccation.

Further support for a role of xylem sap potassium concentration in optimizing $K_{XYL}$ came from measurements on two mangrove species, where native xylem sap osmolarity was found to be close to the optimum ionic concentration for flow enhancement as recorded during the perfusion of stem samples with different ionic solutions (López-Portillo et al., 2005). The hypothesis that xylem sap ionic composition is also regulated by plants in order to optimize $K_{XYL}$ in response to fluctuations in environmental parameters certainly deserves further experimental tests in the years to come. Recent findings also suggest that up-regulation of $K_{XYL}$ would be favoured by adequate nutritional status, which would permit plants to increase xylem sap potassium concentration to levels sufficient to enhance plant hydraulic conductance significantly (Oddo et al., 2011).

Compensation for embolism-induced loss of $K_{XYL}$

Drought-induced xylem embolism can seriously impair the water transport capacity of plants, leading to leaf water potential drop, stomatal closure, reduction of photosynthesis, and eventual dieback up to plant death (Davis et al., 2002). Mechanisms of embolism reversal based on water refilling of gas-filled xylem conduits, have received great attention in recent years. While measuring changes in xylem sap osmolarity and ionic concentration after induction of embolism in laurel stems, Tyree et al. (1999) reported an increase in xylem sap potassium concentration in embolized stems. The concentration of this and other ions were, overall, too low to account for significant osmotic gradients involved in the refilling process (Nardini et al., 2011), so that the functional role of such transitory peaks in xylem sap [K+] remained unexplained.

Significant increases in the magnitude of the ionic effect as related to the amount of embolism in the stems of different plant species have been reported (Gascó et al., 2006; Nardini et al., 2007b; Trifilò et al., 2008). As an example, $K_{XYL}$ increased by about 10% when 25 mM KCl solution was added to the xylem sap of fully functional stems of Ceratonia siliqua, but the $K_{XYL}$ increase was greatly enhanced in embolized stems, so that, in samples suffering a 70% loss of hydraulic conductance, the residual $K_{XYL}$ almost doubled in response to the experimental treatment with the KCl solution (Trifilò et al., 2008). Similar observations have been reported for other woody plants like Laurus nobilis, Platanus orientalis, and Prunus laurocerasus (Nardini et al., 2007b).

The enhancement of the ionic effect in embolized stems is thought to arise as a consequence of the higher number of interconduit pits that water has to cross to bypass gas-filled conduits along its way through the xylem. Moreover, xylem sap [K+] has been reported to increase during dry and warm periods, like the summer months in the Mediterranean area (Trifilò et al., 2008). These two concurrent factors have been hypothesized to co-operate to enhance the hydraulic conductance of still functioning xylem in partially embolized stems (Gascó et al., 2006).

An important consequence of the enhancement of the ionic effect in embolized stems is that the $K_{XYL}$ loss suffered under drought-stress conditions might be partially compensated by the up-regulation of residual hydraulic conductance of still-functioning conduits (Fig. 4). This effect might be amplified by the up-regulation of xylem sap ionic content in response to increasing embolism rates. In accordance with this view, both the ionic effect and xylem sap [K+] were found to increase during the summer drought in three Mediterranean woody species (Trifilò et al., 2008). As an example, in Ceratonia siliqua, xylem sap [K+] increased from 2.7 mM in April to 14.2 mM in July, in accordance with increased amounts of embolism in the xylem. A $K_{XYL}$ loss of about 40% was measured in July with a low [K+] solution. However, the enhancement of $K_{XYL}$ as induced by 15 mM KCl solutions changed from about +15% for fully hydrated stems to about +50–75% in stems with 50% $K_{XYL}$ loss. Hence, the [K+] recorded in xylem sap in July would cause an increase in residual xylem hydraulic conductance by about 30%. In other words, the $K_{XYL}$ loss suffered by C. siliqua in summer would be significantly buffered by the increased xylem sap [K+].

The above hypothesis has received further support from data gathered on Laurus nobilis plants growing in the field (Trifilò et al., 2011). Both xylem embolism rates and xylem sap [K+] were found to increase during the summer in drought-stressed plants compared with values recorded in control (well-watered) plants. Increased potassium concentrations were sufficient to induce significant up-regulation of residual $K_{XYL}$ so that the actual loss of water transport capacity was consistently reduced in drought-stressed plants, as revealed by constant values of stem hydraulic conductance measured in planta using an evaporative flux method.
Finally, changes in water volume flow of functional vessels after the induction of embolism in cucumber stems were observed by Scheenen et al. (2007) and interpreted as a possible consequence of changes in hydraulic resistances of vessel walls, which would be consistent with the embolism-compensation scenario detailed above.

Altogether, these findings suggest that the reduction of plant hydraulic conductance, as caused by xylem embolism, can be alleviated by increased hydraulic conductance of still functioning conduits through a synchronous increase in the ionic concentration of the xylem sap. This phenomenon might represent an important functional consequence of the sensitivity of \( K_{XYL} \) to changes in xylem sap composition, possibly mediated by phloem-to-xylem ion recirculation (Zwieniecki et al., 2004), and certainly deserves further studies aimed at verifying its occurrence in a larger number of species.

**Regulation of water delivery to different branches**

Land plants are sessile organisms and cannot move rapidly towards resources essential for their life like light, water, and nutrients. Indeterminate growth of plants can be envisioned as a functional substitute for motility, in that it allows plants to explore the surrounding environment with modular structures repeated both in space and time to exploit the available resources (Peñuelas and Munné-Bosch, 2010).

In some cases, the amount/concentration of essential resources changes over relatively short time scales and cannot be matched by shoot and/or root production. As an example, light availability changes by orders of magnitude during the day. Nutrient availability can also change over short time intervals because the mass flow of water in the soil can transport inorganic ions rapidly and over relatively large distances (Robinson, 2001). Exploitation of such short-term changes in resource availability might be accomplished by plants through ‘functional motility’ i.e. ‘a complex of interactions enabling efficient utilization of a dynamic environment by the transfer of physiological activities among parallel organs’ (Gorska et al., 2008).

Light is one of the most variable resources among those required by plants to achieve sufficient photosynthetic rates. Mutual shading by different leaves/branches as well as daily changes in the incident light angle continuously modify the quantity/quality of light available for each leaf/branch. Both light available for photosynthesis and evaporative demand are higher in illuminated versus shaded plant sectors. Stomatal control partially keeps track of changes in light availability so that stomata are maintained relatively closed in shaded branches to prevent uncontrolled water loss from leaves whose photosynthetic rates are limited by low irradiance. On the other hand, stomata are opened in illuminated branches to lower diffusive resistance to CO₂. In turn, maximum stomatal conductance is limited by leaf
water potential, which has to be maintained above critical values like the turgor loss point (Brodrrib et al., 2003) or the cavitation threshold (Nardini and Salleo, 2000). Because water potential drop during transpiration is caused by frictional resistances to water flow in both vascular and non-vascular pathways (Sperry, 2000), plant hydraulic conductance poses a limit to maximum leaf gas exchange rates (Tyree, 2003).

On the basis of the above, any mechanism that could transiently up-regulate the hydraulic conductance of illuminated plant sectors, would be expected to buffer the water potential drop and maximize stomatal opening and photosynthesis. Both root and leaf hydraulic conductance have been reported to increase upon plant illumination (Henzler et al., 1999; Lo Gullo et al., 2003), as a likely consequence of the activation of aquaporins (Cochar et al., 2007; Kim and Steudle, 2007), although this view has recently been challenged (Lee et al., 2009; Rockwell et al., 2011).

Experimental results also suggest that stem, as well as petiole, hydraulic conductance can increase upon illumination (Tsuda and Tyree, 2000; Bucci et al., 2003). Nardini et al. (2010) have reported a significant increase (about 60%) of $K_{XYL}$ in fully illuminated *Laurus nobilis* branches compared with shaded ones when measured for this variable at the same time of day. Enhancement of $K_{XYL}$ in planta was accompanied by the up-regulation of xylem sap [K+] that was about 3 mM in shaded branches and 12 mM in illuminated ones. Such a change in xylem sap ionic content also proved to induce an increase of $K_{XYL}$ in excised stems that were artificially perfused with different ionic solutions, thus suggesting that up-regulation of $K_{XYL}$ in illuminated branches arose as a consequence of the ionic effect.

The above findings were confirmed by Sellin et al. (2010), who reported substantial differences in stem hydraulic conductance depending on the incident light in branches of *Betula pendula* Roth, both in planta and under laboratory conditions. Differences in stem hydraulics were closely related to changes in xylem sap [K+] that was found to be significantly higher in branches sampled from the upper, sun-exposed portion of the crown compared with that measured in the lower, shade-exposed sectors.

Hence, the ionic effect emerges as a potential mechanism allowing $K_{XYL}$ of illuminated branches to increase and thus to divert the water flow toward sun-exposed foliage (Fig. 5), preventing leaf dehydration and favouring stomatal aperture and photosynthesis. Such a mechanism would provide plants with a large potential for fine regulation of water flow toward canopy sectors exposed to different environmental conditions with the result of optimizing both light and water utilization.

**The xylem: just more than a vulnerable pipeline**

The recent discovery of the ionic effect as a potential mechanism involved in the fine regulation of $K_{XYL}$, together with the elucidation of cellular processes and events involved in embolism repair, is progressively changing our view of the xylem from a purely physical, inert, and vulnerable pipeline, to a more complex system designed to regulate water delivery to the foliage differentially over different temporal and spatial scales. Recent findings suggest that $K_{XYL}$ regulation and maintenance is a vital process, requiring metabolic activity of axial and radial xylem parenchyma and/or phloem.

Although the ionic effect has been recorded in a substantial number of angiosperms as well as in some gymnosperms, the underlying mechanism(s) remains elusive. Detailed knowledge of pit membrane biochemical composition and macromolecular structure under different chemical environments is needed in order to test the current paradigm predicting ion-mediated volume changes of the pectic matrix of interconduit pits. Moreover, careful analysis of daily and seasonal changes of xylem sap composition and $K_{XYL}$ sensitivity to cations are required in order to confirm the occurrence of the ionic effect in planta and its actual importance for optimization of $K_{XYL}$.

The elucidation of the metabolic requirements for xylem functioning opens some questions about the appropriateness of the experimental techniques used so far for measuring xylem hydraulic features. Current protocols are largely based on the perfusion of water or dilute salt solutions under positive pressure (either sub- or over-atmospheric) through excised stem segments. However, as $K_{XYL}$ is a function of sap ionic concentration, laboratory measurements might not provide an adequate picture of the actual values of xylem hydraulic efficiency in planta. Moreover, hydraulic measurements are destructive and generally performed under physical conditions substantially different from those experienced by the xylem in planta.
This often translates into difficulties in the interpretation of the meaning of hydraulic parameters measured using such techniques (Espino and Schenk, 2011; Beikircher et al., 2010). In recent years, development and implementation of non-invasive imaging techniques based on nuclear magnetic resonance (Holbrook et al., 2001; Windt et al., 2006; Van As, 2007; Kaufmann et al., 2009) or synchrotron X-ray sources (Westneat et al., 2008; Kim and Lee, 2010) has allowed in vivo visualization of water flow in xylem conduits, as well as quantification of hydraulic parameters. Future combination of these powerful techniques with concurrent measurements of sap ion concentration in planta will probably contribute to a more complete understanding of the physiological and functional meaning of the ionic effect.

The recent elucidation of some of the anatomical features at the base of inter-specific variability of the ionic effect opens new interpretations of the functional meaning of some neglected xylem characteristics like vessel grouping, intervessel connectivity, and pitting. In particular, a careful analysis of evolutionary trends in vessel grouping as related to adaptation to drought-prone habitats (Carlquist, 2009) would provide interesting insights into the functional advantage of xylem architectural traits favouring the ion mediated up-regulation of $K_{XYL}$. Similarly, the possible relationships between pit membrane biochemistry and its dynamic changes based on enzymatic activity and the magnitude of the ionic effect, might open new scenarios depicting new important functional roles of cell wall components for xylem and, hence, plant physiology.

References


Braun HJ. 1984. The significance of the accessory tissues of the hydrosystem for osmotic water shifting as the second principle of water ascent, with some thoughts concerning the evolution of trees. *International Association of Wood Anatomists Bulletin* 5, 275–294.


Malone M, Herron M, Morales MA. 2002. Continuous measurement of macronutrient ions in the transpiration stream of
intact plants using the meadow spittlebug coupled with ion chromatography. Plant Physiology 130, 1436–1442.


