Irradiance-induced changes in hydraulic architecture

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Abstract: The ability to acclimate to a range of light regimes is important, given that shady understory habitats can receive only a fraction of the light available at the top of the canopy. Sun and shade leaves are known to differ in their set of biochemical and morphological characteristics. In recent years, much has also been learned about the effect of shade on xylem structure and function. Several studies found that shaded plants had narrower xylem conduits than plants growing in full sun. Among the most notable responses induced by shade is a shift of xylem vulnerability to cavitation. Shaded plants are typically more vulnerable to cavitation than plants exposed to full light. This appears to coincide with the construction of weaker intervessel and intertracheid pit membranes in shade. Before entering and after exiting the xylem, water moves through living cells in roots and leaves, respectively. This nonvascular pathway can be modified by aquaporins. Rapid changes in root and leaf hydraulic conductance in response to changes in light and transpirational demand have been described. The role of aquaporins in these responses is discussed.

Key words: aquaporins, hydraulic conductivity, light level, vascular cambium, wood formation, xylem.

Introduction

Light interception and hydraulicities are two of the principal vegetative functions that must be performed by plants (Niklas 1992). Light has a profound effect on plant morphology and function (Lo Gullo et al. 2010; Walters and Reich 1999). Differences in light level can induce phenotypic plasticity in terms of biomass allocation, leaf morphology, and other plant traits (Schultz and Matthews 1993; Valladares et al. 2000). Plants growing in the understory usually experience reduced evaporative demand (Bladon et al. 2006), which is paralleled by a decreased need for water transport. In addition, carbon resources tend to be more limited in shaded plants. As a result, the hydraulic architecture of shaded plants is likely to be altered in multiple ways.

The hydraulic architecture of a tree may be described by the pattern of hydraulic conductances throughout the crown of a tree (Tyree and Zimmermann 2002). In this review, the term “hydraulic architecture” is defined rather loosely so that it also includes the nonvascular pathway for water. Before entering the xylem in roots, and after exiting the xylem conduits in leaf veins, water flows through living cells. The nonvascular pathway involves the passage of the root endodermis and the bundle sheath of leaves. Flow through these cells is modified by aquaporins, and root and leaf hydraulic conductance can change rapidly in response to environmental conditions (Heinen et al. 2009; Laur and Hacke 2013; Sakurai-Ishikawa et al. 2011).

The first part of this review considers irradiance-driven changes in xylem structure and function. The second part focuses on aquaporins in roots and leaves, and how they respond to changes in light level.

Leaf-specific conductivity

A study comparing hydraulic traits of four conifer species growing in full sun versus in a shaded understory environment found that leaf-specific xylem conductivity ($K_{L}$) was lower in understory conifers (Schoonmaker et al. 2010). The $K_{L}$ is equal to the hydraulic conductivity of a stem segment divided by the leaf area distal to the segment. It is a measure of the “sufficiency” of the segment to supply water to leaves (Tyree and Zimmermann 2002).

Lower $K_{L}$ values in plants growing in shade have also been found in other studies (Barigah et al. 2006; Plavcová et al. 2011; Schultz and Matthews 1993; Shumway et al. 1993). A large total leaf area in shade is desirable to capture more light. Moreover, in the absence of water stress, shaded plants can maintain large leaf...
areas even with smaller xylem areas and lower xylem transport efficiency (Plavcová et al. 2011; Schultz and Matthews 1993).

However, the strategy of shifting allocation from hydraulics to leaf area and light interception is not without risk. If xylem pressure decreases as a result of increased evaporative demand (e.g., via the formation of a canopy gap) or soil drought, water supply to the leaves may be insufficient, possibly resulting in stomatal closure, elevated levels of xylem embolism, and leaf loss. When stomata of shaded branches close during periods of water stress to minimize embolism levels, photosynthesis and carbon fixation are also reduced. As a result, there may not be enough carbon to sustain respiration, and shaded branches could become a carbon sink (Protz et al. 2000). Moreover, coping with an increased evaporative demand would usually require a shift of biomass allocation to roots and the production of new xylem in the medium to long term (Barigah et al. 2006), and these growth requirements seem incompatible with prolonged stomatal closure.

**Xylem anatomy**

The xylem of trees probably accounts for over 99% of the total length of the flow path from root tips to evaporating surfaces in the leaf. Profound changes in xylem anatomical traits have been reported in response to different light levels. Studying leaf xylem, Nardini et al. (2005) found that leaves of sun-adapted species had wider xylem conduits than species adapted to shady habitats, as well as larger vein densities. The authors concluded that sun-adapted species have developed a highly efficient conducting system to supply mesophyll cells with water. Within the crown of mature beech (*Fagus sylvatica* L.) trees, there was variation in xylem traits between branches exposed to full sun and shaded branches from the lower canopy (Lemoine et al. 2002); sun-exposed branches had wider vessels than shaded branches. In hybrid poplar stems, vessels of shaded plants were narrower but longer than those of control plants (Fig. 1; Plavcová et al. 2011). Individual vessel elements of shaded plants were also longer than in control plants. Further, shading resulted in significantly thinner secondary cell walls in fibers, and this was paralleled by lower wood densities in stems of shaded plants (Plavcová et al. 2011), possibly resulting from a reduced carbon supply.

An intriguingly complex effect of shade on xylem structure and function was found in conifers. In the tracheid-based xylem of boreal spruce and pine species, the trend for narrower tracheid lumen diameters of shaded plants corresponded with higher wood densities in stems of shaded trees (Schoonmaker et al. 2010). Tracheid length did not differ. Based on narrower tracheid diameters in shaded plants, one would expect to find decreased xylem transport capacity in understory conifers. Surprisingly, stems of shaded trees had similar xylem transport efficiencies (expressed as xylem area-specific conductivity, $K_{c,j}$) as open-grown trees with wider tracheids. Scanning electron microscopy (SEM) micrographs revealed that this was probably driven by extensive changes in pit structure (Schoonmaker et al. 2010).

Schulte (2012) recently calculated fluid flow through models of torus-margo pits based on some of the micrographs published in Schoonmaker et al. (2010). Model results indicated that the larger size of the margo pores of shaded trees (Fig. 2) had a strong effect on the flow through the margo. For the sun pit model, the calculated margo resistivity was more than twice as high as for the shade model. Only a few percent of the largest pores accounted for nearly half of the flow through the margo (Schulte 2012). Such a nonlinear relationship between pore size and flow is to be expected based on a predicted diameter to the third power relationship with flow for an isolated pore in an infinitely thin plate (Hacke et al. 2004; Schulte 2012; Vogel 2003).

**Cavitation resistance**

Among the most notable phenotypic responses induced by shade is a shift of xylem vulnerability to cavitation (Fig. 3). Shaded trees typically have more vulnerable xylem (Barigah et al. 2006; Lemoine et al. 2002; Plavcová et al. 2011; Schoonmaker et al. 2010), although there are exceptions to this general trend (Holste et al. 2006). While the mechanisms driving cavitation resistance are not completely understood, intervessel and intertracheid pits are thought to play an important role in preventing the spread of air from embolized conduits to adjacent, functional ones (Tyree and Zimmermann 2002). Available evidence indicates that pit membrane structure may be altered by shade, and that these changes are associated with changes in cavitation resistance. The following two case studies illustrate how shade may result in the construction of weaker pit membranes.

Transmission electron microscopy images revealed that intervessel pit membranes of shaded hybrid poplar plants were thinner than in control plants, which received a higher light level (Plavcová et al. 2011). The association of increased vulnerability with thinner pit membranes is consistent with a study on 26 angiosperm species, which found correlations between pit membrane thickness, membrane porosity, and vulnerability to air-seeding (Jansen et al. 2009). Thinner membranes may represent a weaker barrier between air and water-filled vessels, although the link between pit membrane thickness and porosity awaits further study. Further, differences in membrane thickness are...
likely related to the mechanical strength of the membrane and to the amount of deflection and enlargement of existing pores (Jansen et al. 2009).

As outlined above, shade also had a profound effect on the structure of conifer pits (Fig. 2). Based on SEM imaging of a large number of pits, Schoonmaker et al. (2010) concluded that margo strands were narrower in understory conifers than in open-grown plants. This, and the fact that there were fewer margo strands in pits of understory conifers, suggests that these strands would be more likely to tear and prevent the torus from sealing properly. In addition, for a given aperture size, understory black spruce (Picea mariana (Mill.) Britton, Sterns & Poggenb.) trees had smaller tori than their open-grown counterparts. This may mean that the torus of shaded plants would be more easily dislodged or that it may not completely cover the pit aperture, allowing air entry to adjacent tracheids at less negative xylem pressure than in open-grown plants (Domec et al. 2008; Hacke et al. 2004). Overall, understory conifers appeared to invest less carbon into torus-margo pits by producing pit membranes with a more porous but fragile structure, corresponding with relatively efficient transport but increased xylem vulnerability.

It may also be interesting to test if and how shade affects cell wall chemistry. If lignification patterns were altered, then this may also have an effect on water transport and vulnerability to cavitation (Voelker et al. 2011).

Cambial activity and gene expression

Irradiance-driven changes in xylem structure and function are the result of developmental adjustments. In woody plants, these adjustments are established as a result of altered activity of the vascular cambium. As vessel elements and fibers mature, their development is sensitive to environmental conditions. In poplar, the type of xylem that is produced by the cambium is known to differ in response to different levels of nutrition, water, and light (Arend and Fromm 2007; Fichot et al. 2010; Fromm 2010; Hacke et al. 2010; Harvey and van den Driessche 1997, 1999; Pitre et al. 2007; Plavcová and Hacke 2012). In recent years, we have improved our understanding of the changes in gene expression that accompany some of these developmental changes (Janz et al. 2012; Pitre et al. 2010). For instance, Plavcová et al. (2013) reported that three genes encoding aquaporins of the tonoplast intrinsic protein (TIP) class were up-regulated in the cambial region of poplar plants receiving high levels of nitrogen. These plants exhibited enhanced radial growth, wider vessels and fibers, as well as thinner fiber walls than plants that only received adequate levels of nitrogen. Water uptake is essential to drive cell expansion, and it has been hypothesized that aquaporins play a role in xylogenesis by facilitating the flow of water into the zone of expanding cells (Domec et al. 2010; Hacke et al. 2010). Following this hypothesis, the up-regulation of the three TIPs may be linked with the wider vessels found in high-nitrogen plants (Plavcová et al. 2013).

Work with the Arabidopsis model system and Populus trichocarpa Torr. & A. Gray ex Hook. showed that expression of many regulatory and structural genes related to xylem differentiation is under...
Fig. 4. Effect of a sudden change in transpirational demand on aquaporin transcript amounts in poplar roots. Cumulative aquaporin transcript amounts in roots. Individual genes are labeled with different colours in Web version only. One subset of plants was grown at adequate light level in the growth chamber (“Light control”). Other subsets of plants were grown in shade (“Shade”) or in a humidified box at ~95% relative humidity (“High RH”). Shaded plants were exposed to a ~4-fold increase in light level. Gene expression was measured 4 h (“Light increase, 4 h”) and 28 h (“Light increase, 28 h”) after the increase in light level. Plants growing at high humidity were removed from their humidified box and were exposed to a ~4-fold increase in vapor pressure deficit while light levels remained adequate. Gene expression was measured 4 h and 28 h after the decrease in relative humidity. From Laur and Hacke (2013).

Aquaporin-mediated changes in root and leaf hydraulic conductance

In addition to the developmental plasticity described above, plants are able to respond to dynamic changes in light availability by changes in leaf and root hydraulic conductance (Almeida-Rodriguez et al. 2011; Guyot et al. 2012; McElrone et al. 2007; Sakurai-Ishikawa et al. 2011). Water transport through roots and leaves involves flow through living cells and is therefore potentially modified by aquaporins.

When light levels and canopy water demand increase, dynamic changes in root and leaf hydraulic conductance may be required to restore the water balance at the whole plant level. Diurnal cycling of fine root hydraulic conductance has been observed in many species (Clarkson et al. 2000; Henzler et al. 1999; Sakurai-Ishikawa et al. 2011). However, root hydraulic conductance is not just governed by diurnal cycles, but is also impacted by environmental factors, including light. Canopy shading resulted in a strong decrease in root hydraulic conductance in Sideroxylon lanuginosum Michx. (McElrone et al. 2007). Conversely, the hydraulic conductance of fine roots of hybrid poplar increased significantly in response to an increase in irradiance (Almeida-Rodriguez et al. 2011).

In rice, root-specific aquaporins, such as OsPIP2;5, were strongly induced by transpirational demand (Sakurai-Ishikawa et al. 2011). Protein levels of root-specific aquaporins peaked 6 h after light initiation, while mRNA levels peaked 2 h after light initiation. Changes in fine root hydraulic conductance may occur even before a peak in protein levels is observed. This could be due to gating and the assembly of PIP1-PIP2 heterotetramers in conjunction with their trafficking to the plasma membrane (Maurel et al. 2008; Zelazny et al. 2007).

Immunolabeling showed that OsPIP2;5 accumulated in the endodermis and adjacent cortex cells, as well as in cells of the vascular cylinder. Sakurai-Ishikawa et al. (2011) suggested that the rapid induction of root aquaporin gene expression by transpirational demand may increase cell-to-cell water movement to sustain a high transpiration rate during the light period.

In another study, hybrid poplar plants were subjected to a sudden increase in evaporative demand, either by increasing the light level or by reducing the relative humidity (Laur and Hacke 2013). Both treatments led to an increase in root water flow and an increase in the transcript abundance of aquaporin genes in roots (Fig. 4). Interestingly, root water flow increased more rapidly in plants experiencing a decrease in relative humidity than in shaded plants experiencing a sudden increase in light level. The relatively slow response of shaded plants to increased light may be due to the stressful growing conditions that these plants experienced. Shade-grown plants were probably energy starved and may have lacked the resources that are necessary for a rapid expression and activation of aquaporins. Regardless of the reasons for the slower response of shaded plants, transcript levels of three PIP1 aquaporin genes closely matched trends in root water flow.

Immunolabeling revealed that PIP1 protein was present in the epidermis and cortex cells, as well as in the endodermis and in vascular tissue (Laur and Hacke 2013). In situ hybridization experiments suggest that individual aquaporin genes may be expressed in different parts of the root cross section. While one gene, PIP2;10, was highly expressed in the vascular cylinder (Fig. 5), others showed higher expression in the cortex (Almeida-Rodriguez et al. 2011). More in situ hybridization studies in poplar roots would be
useful to determine the tissue distribution of expression of key aquaporin genes (such as those identified by Laur and Hacke 2013). The observed responses by roots to an increase in transpiration demand seem to involve shoot-to-root signaling. Molecular signaling in the phloem would provide hours-scale travel times from shoot to root for most small plants (Turnbull and Lopez-Cobolo 2013), but it may not be fast enough in large trees and plants with extensive and deep root systems. In addition, both electric and hydraulic signals may be involved as they have the capacity of rapidly spreading through the plant body (Grams et al. 2007).

Leaf hydraulic conductance is also impacted by light, although the leaf response to light appears to be highly variable across species. Some (but not all) species showed light enhancement of leaf hydraulic conductance within 30 min, according to measurements made with a high pressure flowmeter (HPFM) (Tyrre et al. 2005; Voici et al. 2008). Rockwell et al. (2011) cautioned that the exact interactions between stomata and the HPFM remain enigmatic and that the concept of aquaporin-mediated, light-induced changes of bulk leaf tissue conductivity requires further examination. However, light enhancement of leaf hydraulic conductance was also found when the evaporative flux method was used (Guyot et al. 2012). Recent research discusses the complex role of aquaporins in regulating leaf hydraulic conductance in response to changes in light level (Prado and Maurel 2013).

Many avenues for future research present themselves. For example, it would be interesting to investigate why species differ in how leaf hydraulic conductance responds to changes in irradiance. New research showed that bundle sheath cells of Arabidopsis play a key role in controlling leaf hydraulic conductance in response to stress signals (Shatil-Cohen et al. 2011); it could be tested if bundle sheath cells also play an important role in leaf responses to changes in light level. On the xylem level, it would be useful to learn more about the development of intervessel pit membranes, and how it is affected by genes and environment. Finally, changes in irradiance may offer an opportunity to study signaling between aboveground plant organs and roots.


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