A STRUCTURAL AND COMPOSITIONAL ANALYSIS OF INTERVESSEL PIT MEMBRANES IN THE SAPWOOD OF SOME MANGROVE WOODS

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SUMMARY

Intervessel pits are prominent wall structures involved in the water transport mechanism of land plants. The role of their intra-tree variation in the regulation of water transport, however, remains enigmatic. The hypothesis was tested that pit membrane thickness and degree of impregnation with phenolic substances increase along the stem axis with increasing tension on the water column as an adaptation to the higher risk for cavitation. Wood samples were taken at different heights from the mangrove tree \textit{Rhizophora mucronata} growing at Gazi Bay (Kenya). Additional samples were taken along the stem radius to distinguish height from age effect, and from six other mangrove species growing in the same forest. Intervessel pit membranes were studied via transmission and scanning electron microscopy and cellular UV-microspectrophotometry. The hypothesis of pit membrane thickness and composition as a static adaptation to the hydrostatic conditions during vessel differentiation could be refuted. Instead, our findings point to a more dynamic pit membrane appearance with seasonal changes in thickness and chemical composition.

\textbf{Key words:} Intervessel pit membrane, intra-tree variation, mangrove, water transport, electron microscopy, UV-microspectrophotometry.

INTRODUCTION

Following the air-seeding theory for vessel-bearing land plants (Sperry & Tyree 1988; Oertli 1993; Jarbeau \textit{et al.} 1995; Salleo \textit{et al.} 1996; Cochard 2006), it is currently believed that drought-induced cavitation, \textit{i.e.} the phase change from water to vapour, is initiated at the intervessel pits. Pit pairs between adjacent water conducting cells consist of a narrow pit aperture and pit border, which is formed by an overhanging

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secondary cell wall that is separated from the neighbouring pit border by a more or less porous pit membrane (Sperry et al. 1988; Choat et al. 2003; Jansen et al. 2009). Pit membranes are composed of remnants of the primary cell walls and the middle lamella (Schmid 1965; O’Brien 1970; Barnett 1982). Although the function of intervessel pits is radial water transport from one vessel to another, pit membranes are responsible for at least 50% of the total hydraulic resistance in a plant (Choat et al. 2008; Höllttä 2011). The limited permeability of the pit membrane for water is thought to be the result of a trade-off between hydraulic efficiency and safety. A higher pit membrane porosity would increase the hydraulic efficiency but also the vulnerability to air-seeding (Christman et al. 2009), when gas bubbles are sucked in from neighbouring air-filled vessels (Tyree et al. 1994).

Pit membranes can be described qualitatively by their porosity, thickness and chemical composition. As they are derived from the primary cell wall and the middle lamella, they are composed of cellulose microfibrils, cemented by varying proportions of hemicelluloses, pectins and lignin (Schmitz et al. 2008; Plavcová et al. 2011). It remains, however, unclear what determines the chemical nature of pit membranes and its variation between and within species. Pit membrane porosity is related to its thickness. There are indications that the porosity can change due to mechanical stresses (Hacke et al. 2001) as well as to changes in ionic composition of the xylem sap (Nardini et al. 2011). Among 26 species, Jansen et al. (2009) found a variation in pit membrane thickness from 0.07–1.18 µm that was reflected in their resistance to cavitation. Having fewer microfibrillar layers, thin pit membranes are expected to have large pores and to increase significantly in pore size when subject to stretching and flexing forces (Choat et al. 2004; Jansen et al. 2009). The variation in pit membrane thickness within species and even trees reached 59% of the mean pit membrane thickness (Schmitz et al. 2007; Schmitz et al. 2008; Jansen et al. 2009). In earlywood of the gymnosperm *Pseudotsuga menziesii*, size of pit apertures and size and number of margo pores decreased along the tree’s axis, from roots to branches (Domec et al. 2006a). In angiosperms, variation in pit membrane properties remains underexplored.

Also, the intra-species variation in pit membrane electron density as viewed with TEM (Schmitz et al. 2008), determined by both pit membrane structure and chemical composition remains unclear. So far, the only explanation that could be given to these variations in electron density of the pit membranes is the deposition/hydrolysis of phenolic and/or pectic substances. Although there is little or no evidence on the chemical nature of pit membranes, pectic substances would explain the ionic effect on the hydraulic conductivity observed in several angiosperm tree species (Zwieniecki et al. 2001; Gortan et al. 2011; Nardini et al. 2011; Plavcová & Hacke 2011) and the seasonal changes of this effect (Gascó et al. 2007; Aasamaa & Sober 2010). Lignin, as a hydrophobic substance, is thought to hinder these interactions between hydrophilic pectin and ions in the xylem sap (Boyce et al. 2004) and would make pit membranes less prone to excessive stretching upon air-seeding and thus to increase of the pit membrane’s porosity (Hacke et al. 2001; Choat et al. 2003; Choat et al. 2004).

Unlike the clearly established close relationship between pit membrane thickness and cavitation vulnerability (Jansen et al. 2009; Lens et al. 2011; Plavcová et al. 2011), the
function of intra-species variability of intervessel pit membranes in mature sapwood vessels remains hardly understood (Choat & Pittermann 2009). Pit membranes of the mangrove *Rhizophora mucronata* formed during the dry season were thicker than those formed during the rainy season (Schmitz et al. 2008). Although seasonality seems to affect pit membrane thickness, our earlier work could not fully explain the range of variation observed. Therefore, this study aimed to test the hypothesis that the intra-tree variation in pit membrane structure and composition is related to the increasing tension on the water column from root to canopy. This hypothesis was recently supported by a carbon cost-gain model predicting less porous pit membranes near the apex (Höllttä 2011). There is currently no reliable method to measure the water potential at different heights along an unbranched stem. Since a small increase in tension can lead to a relatively large loss in hydraulic conductance (Sperry et al. 1988; Ewers et al. 2004), it is suggested that pit membrane thickness is optimized for the water potential experienced. Especially in mangroves, tensions in the xylem can be high due to the regular inundation with saline sea water and the frequently high temperatures (Suarez & Sobrado 2000; Aziz & Khan 2001; Melcher et al. 2001; Sobrado 2004). Since the hydrostatic gradient due to height coincides with a trend in the age of the cambium, variation of intervessel pit membranes was also studied from pith to bark at 130 cm height to disentangle both effects. For the interpretation of the data and in order to widen our understanding of pit membrane chemistry, a comparison was made with six Kenyan mangrove species.

**MATERIALS AND METHODS**

**Sample collection**

Wood samples were collected from seven mangrove species (*Bruguiera gymnorrhiza*, *Ceriops tagal*, *Heritiera littoralis*, *Lumnitzera racemosa*, *Rhizophora mucronata*, *Sonneratia alba*, *Xylocarpus granatum*) in the forest of Gazi Bay, Kenya in February 2009 (Table 1). Wedges of the outermost sapwood were taken at a height between 63 cm and 158 cm from the ground (average: 97.5 cm) to avoid interference with proproots or buttresses, wounds and bark irregularities. To investigate the intra-tree variation in intervessel pit anatomy, five discs per tree were collected. We compared two *R. mucronata* trees growing at sites of contrasting environmental conditions (soil water salinity: 10–76‰ vs 0–31‰, inundation frequency: 17 vs 10 days month⁻¹) and within each tree we compared discs collected from 1) a root just below the surface, 2) a proproot, 3–5) the stem between the last proproot and the top of the tree (4.5–5 m). An additional stem disc was taken above the last proproot to evaluate the radial variation from bark to pith.

**Sample preparation and analysis**

Immediately after cutting, samples were put in plastic bags with some distilled water. In the lab (max. 9 hours later) the bark was removed and blocks were cut of the outermost wood in the direction of the medium stem radius. For the intra-tree investigation, five blocks were cut of about 5 × 5 × 8 mm from equally spaced positions along the medium stem radius from bark to pith, as no growth rings could be distinguished.
Samples were frozen for later analysis with a scanning electron microscope (SEM) or stored in 70% ethanol for analysis with a transmission electron microscope (TEM) and UV-microspectrophotometer (UMSP). Additional images were used of *R. mucronata* intervessel pits from material previously collected at the same study site (Schmitz et al. 2007; Schmitz et al. 2008).

For SEM analysis (Hitachi S520, accelerating voltage 15 kV), wood samples were gradually dehydrated in a 30–100% ethanol series, split tangentially and gold-coated with a sputter coater (Agar Scientific Ltd., USA). For TEM analysis (Philips CM 12, accelerating voltage of 60 kV), wood samples were trimmed into cubes of 2 × 2 × 3 mm. After dehydration in an acetone series (30–100%), samples were infiltrated with Spurr’s epoxy resin through a series of propylene oxide/resin mixture, embedded and polymerized at 70 °C. Longitudinal sections of 100 nm thickness were cut with an ultramicrotome (Ultracut E, Reichert-Jung) equipped with a holder for glass/diamond knife and stained with 1% (w/v) potassium permanganate. Photographs were taken from three to six positions along one or two intervessel walls. For UMSP analysis, unstained sections of 1 μm thickness were transferred to quartz slides, immersed in a drop of non-UV absorbing glycerine and covered with a quartz cover slip. UMSP scanning profiles were made along the same intervessel walls as where the TEM images were taken. Scanning profiles were made with a Zeiss UMSP 80 at a constant wavelength of 278 nm (representing the absorbance maximum of lignin) using an ultrafluar 100:1 objective. Data were recorded and processed with the software programme APAMOS (Zeiss). The scan programme digitizes rectangular tissue portions with a local geometrical resolution of 0.25 μm² and a photometrical resolution of 4096 grey scales converted into 14 basic colours (Koch & Kleist 2001).

Table 1. Site description of the species studied.

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Range of soil water salinity (%e)</th>
<th>Inund. freq‡</th>
<th>Radial increment †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry season</td>
<td>Rainy season</td>
<td>(days month⁻¹)</td>
</tr>
<tr>
<td>1 landward</td>
<td>H*</td>
<td>40–90</td>
<td>10–57</td>
<td>4</td>
</tr>
<tr>
<td>1 landward</td>
<td>C</td>
<td>40–90</td>
<td>10–57</td>
<td>5</td>
</tr>
<tr>
<td>1 landward</td>
<td>L</td>
<td>40–90</td>
<td>10–57</td>
<td>5</td>
</tr>
<tr>
<td>2 riverside</td>
<td>R</td>
<td>7–31</td>
<td>0–11</td>
<td>10</td>
</tr>
<tr>
<td>2 riverside</td>
<td>X*</td>
<td>7–31</td>
<td>0–11</td>
<td>12</td>
</tr>
<tr>
<td>3 landward</td>
<td>R</td>
<td>52–76</td>
<td>10–51</td>
<td>17</td>
</tr>
<tr>
<td>4 seaward</td>
<td>B*</td>
<td>34–46</td>
<td>21–35</td>
<td>27</td>
</tr>
<tr>
<td>4 seaward</td>
<td>R</td>
<td>34–46</td>
<td>21–35</td>
<td>30</td>
</tr>
<tr>
<td>4 seaward</td>
<td>S</td>
<td>34–46</td>
<td>21–35</td>
<td>30</td>
</tr>
</tbody>
</table>


Soil water for salinity measurements was collected at about 25 cm depth.

* For this specific site no salinity data are available for both seasons and several years so that data of the nearest site are given.

‡ Inundation frequency based on the tide tables of 2009.

† Values are means ± standard deviation. Data from Robert et al. (2010).
**Measurements and statistics**

Thicknesses of the pit membranes were measured with the image analysis software AnalySIS 3.2 (Soft Imaging System GmbH, Münster, Germany) near the centre of the pit membranes on the TEM images. Statistical analyses were performed in STATISTICA (StatSoft, Inc. 2006, data analysis software system, version 7.1, www.statsoft.com). One-way ANOVA and post-hoc tests (unequal n HSD test) were carried out to test for differences in pit membrane thickness with position along the stem axis or stem radius.

![Image of electron micrographs showing variability in inter-vessel pit membrane thickness and electron density within the sapwood of *Rhizophora mucronata* trees.](image-url)

**Figure 1.** Transmission electron micrographs (potassium permanganate staining) showing the variability in inter-vessel pit membrane thickness and electron density within the sapwood of *Rhizophora mucronata* trees. – a & b: Pit membranes from the same 2×2×3 mm wood sample, collected in the rainy season. – c: Collection in the dry season. – d–f: Collection in the short rainy season with e & f from the same 2×2×3 mm wood sample.
To investigate the chemical content of the pit membranes, point measurements (diameter 1 µm) were carried out with a Zeiss UMSP 80, using an ultrafluar 100:1 objective, covering a wavelength range from 240 nm to 700 nm in 1 nm steps using the programme LAMWIN (Zeiss). To estimate the lignin content of the pit membranes the median was taken of 9–11 spectra of pit membranes per species at 278 nm. Lignin concentration of vessel walls was measured as the mean absorbance of the intervessel wall within the field of view, with 9–12 fields analysed per species.

Table 2. Variation in pit membrane thickness (means ± standard deviation in µm) along the stem axis of two Rhizophora mucronata trees growing at relatively low and high soil water salinity (see Table 1, site 2: 0–31‰ vs site 3: 10–76‰) and of both trees with pooled data.

<table>
<thead>
<tr>
<th>Site*</th>
<th>Low salinity</th>
<th>High salinity</th>
<th>Pooled</th>
<th>High salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>root</td>
<td>0.30 ± 0.08 a</td>
<td>0.30 ± 0.08 a</td>
<td>pith 0.45 ± 0.04 ab</td>
<td></td>
</tr>
<tr>
<td>proproot</td>
<td>0.48 ± 0.03 ab†</td>
<td>0.22 ± 0.04 a</td>
<td>0.48 ± 0.03 b</td>
<td></td>
</tr>
<tr>
<td>above proproot</td>
<td>0.51 ± 0.13 a</td>
<td>0.45 ± 0.05 b</td>
<td>0.48 ± 0.09 b</td>
<td></td>
</tr>
<tr>
<td>DBH</td>
<td>0.38 ± 0.04 b</td>
<td>0.56 ± 0.06 c</td>
<td>0.49 ± 0.11 b</td>
<td></td>
</tr>
<tr>
<td>canopy</td>
<td>0.44 ± 0.03 ab</td>
<td>0.45 ± 0.06 bd</td>
<td>0.45 ± 0.05 b</td>
<td></td>
</tr>
</tbody>
</table>

* The number of pit membranes measured per position in the tree and per study site varied between 5 and 12.
† No root data are available for site 2.
Different letters indicate significant differences, p-values < 0.01, as given by unequal n HSD-tests.

To investigate the chemical content of the pit membranes, point measurements (diameter 1 µm) were carried out with a Zeiss UMSP 80, using an ultrafluar 100:1 objective, covering a wavelength range from 240 nm to 700 nm in 1 nm steps using the programme LAMWIN (Zeiss). To estimate the lignin content of the pit membranes the median was taken of 9–11 spectra of pit membranes per species at 278 nm. Lignin concentration of vessel walls was measured as the mean absorbance of the intervessel wall within the field of view, with 9–12 fields analysed per species.
RESULTS AND DISCUSSION

Structural analyses with TEM revealed that pit membrane thickness in the sapwood varied between positions along the stem for both study sites (ANOVA: F = 45.54 / 5.15, p < 0.0001 / < 0.01, df = 3/4 for site 2/3 respectively), whereby differences were small without a trend along the trees' axis (Fig. 2, Table 2). Only pit membranes in the root were clearly thinner than in the stem (Table 2) but simultaneously varied in thickness within a single vessel (Fig. 2a, b). As the tension on the water column in vessels is smaller in the roots, thinner (i.e. more porous) pit membranes would allow a more efficient water flow. Besides, thinner pit membranes in the roots agree with their higher vulnerability to embolism, as observed in two Acer species (Alder et al. 1996; Choat et al. 2005) and the gymnosperm Pseudotsuga menziesii (Doméic et al. 2006a).

With increasing height along a stem the age of the secondary meristem decreases. Although the age of the cambium is known to affect the anatomy of several wood anatomical traits (Rao et al. 1997; Sun & Suzuki 2001), no effect was found on pit membrane thickness (Fig. 3). Pit membranes in the proproot of trees of site 3 were twice as thin as those in trees of site 2 (Fig. 2c, Table 2). Interestingly, all extraordinarily thin pit membranes occurred in vessels with tyloses. The pit membranes near the pith were on average 2.7 times thicker in vessels without tyloses than in vessels with tyloses (Fig. 3e, f). Despite exclusion of these thinner pit membranes from calculations and pooling the data of both sites, no positive trend was found in pit membrane thickness along the stem axis (Fig. 2, ANOVA: F = 10.15, p < 0.0001, df = 4). Similarly, pit membrane thickness differed only slightly along the stem radius (ANOVA: F = 8.86, p < 0.0001, df = 4) without an obvious trend from bark to pith (Table 2, Fig. 3).

Figure 3. a–f: Transmission electron micrographs (potassium permanganate staining) of intervessel pit membranes along the stem radius (4.5 cm) of a Rhizophora mucronata tree from bark to pith. – e & f: Pit membranes from the same wood sample but from different vessels. In f a tylosis wall can be seen at the right of the picture. V1-2, two neighbouring vessels; Pc = pit canal; arrowhead points to the pit membrane.
Figure 4. Transmission electron micrographs (potassium permanganate staining) of intervessel pit membranes of Kenyan mangrove species showing that low electron density is not related to high porosity, as seen on scanning electron micrographs (a–c vs. d–f), but to a lower lignin content as estimated by the absorbance of UV light at a wavelength of 278 nm (a–c, g–i vs. l). – j & k: Electron density of intervessel pit membranes varied within fully differentiated sapwood vessels of *Rhizophora mucronata* (j) and *Xylocarpus granatum* (k). – l: Lignin concentration of cell walls (CW) followed the pit membrane (PM) lignin content across species. – a, d: *Bruguiera gymnorrhiza*; b, e: *Ceriops tagal*; c, f: *Xylocarpus granatum*; g: *Heritiera littoralis*; h: *Lumnitzera racemosa*; i: *Sonneratia alba.*
In contrast to what was assumed earlier (Schmitz et al. 2008), the occurrence of faint (electron transparent) pit membranes was not related to the cambial age (Fig. 3). Pit membranes often showed lower electron density than the surrounding vessel wall with its dark compound middle lamella portion (Fig. 2 & 3). Low electron density suggests lower contents of lignin and/or other aromatic compounds (e.g. Donaldson 1992; Schmitt & Melcher 2004) as supported by the lower UV absorbance at 280 nm (the absorbance maximum of lignin) for the faint than for the dark pit membranes (Fig. 4 l (a)–(c) vs (g)–(i)). When considering all seven species investigated, the flimsy looking, faint pit membranes were not remarkably more porous than the dark pit membranes when observed with SEM in surface view (Fig. 4d, f). SEM could not resolve the apparently tiny pores, neither in the electron dense (Fig. 4g–i) nor in the nearly electron transparent (Fig. 4a–c) pit membranes. At the species level, lignin content of the pit membranes was roughly reflected in the lignin content of the vessel walls (Fig. 4 l, PM vs CW; r = 0.85, p = 0.03, n = 6).

According to our results, a wide variation of intervessel pit membrane thickness and chemistry, also within an individual tree, was found. The observations of the present study support a dynamic nature of pit membrane structure and composition in the sapwood. Extremely faint pit membranes were observed by TEM in all samples collected in the dry season (Fig. 1c, 2 & 3). Samples collected in the rainy season showed darker pit membranes (Schmitz et al. 2007) (Fig. 1a, b), while samples taken in the period in between, during the short and less heavy rainy season, displayed a mix of dark and light pit membranes (Schmitz et al. 2008) (Fig. 1d–f). Pit membranes are generally thought of as static structures once they are fully developed (e.g. Schmid & Machado 1968; Benayoun et al. 1981). In fully differentiated vessels, modifications of pit membranes have in most cases been related to the formation of heartwood, when vessels lose their conductive capacity permanently. At that time, pit membranes get incrusted with deposits assumed to be rich in phenolics (Wheeler & Thomas 1981; Sano & Fukuzawa 1994). In contrast to heartwood deposits, there have been only few observations of deposits in the pit membranes of the sapwood (Thomas 1976; Sano & Nakada 1998; Sano 2005). Wheeler (1981) reported deposits in Fraxinus americana during winter that disappeared from late spring onwards and re-appeared in autumn. It was suggested that the deposits could help prevent water loss during the stressful winter months in a temperate region. Altogether we hypothesize that the observed differences in electron density of the pit membranes may be caused by seasonal deposition or degradation of thus far chemically unidentified substances. One possible substance is pectin and especially the subgroup homogalacturonans. Pectic homogalacturonans are thought to be involved in the regulation of the water transport via their hydrogel character, at least in some species (Lopez-Portillo et al. 2005; Van Ieperen 2007; Gascó et al. 2008; Trifilò et al. 2008; Nardini et al. 2011). The degree of swelling and shrinking depends on the chemical composition of the pit membranes and the composition and concentration of ions as well as enzymes in the xylem sap modifying the amount of charged residues of the pectins in the pit membranes. These factors can change pit membrane flexibility and porosity and affect hydraulic conductance (Nardini et al. 2007a, b; Cochard et al. 2010; Gortan et al. 2011). Deposition of a pectin-rich compound in the dry season, in times of
water stress, would agree with a variation in the ion sensitivity of the xylem hydraulic conductance, with a maximum in late summer in six temperate deciduous tree species (Aasamaa & Sober 2010), and higher values in sunlit than in shaded laurel branches (Nardini et al. 2010). The process was assumed to be a strategy for conservative water use and an adaptation to varying environmental conditions. In addition, the apparent mismatch between increased hydraulic conductance but not cavitation vulnerability, in response to changing ion concentrations in the xylem sap (Cochard et al. 2010), could be explained by a thin pectin-rich deposit, which would not necessarily change the porosity of the pit membrane that mainly depends on the number of superposed microfibrillar layers. Next to ionic effects of the deposits, refilling of embolized vessels (Melcher et al. 2001) might be facilitated by a changing amount of lignin or lignin-like substances as the hydrophobic nature of lignin affects the wetting angle at the water-gas interface (Zwieniecki & Holbrook 2000; Meyra et al. 2007).

It remains to be tested whether additional species with faint pit membranes (Fig. 4a–c) have darker pit membranes when sampled in the rainy season than in the dry season, as was found for R. mucronata. All samples in this study were collected in the dry season. The darker pit membranes observed in three species of this study could be exceptional cases as the pits were either vestured (Fig. 4h, i) or the pit canals filled with deposits (Fig. 4g, h, 5a, c). These deposits, which contained higher amounts of phenolic constituents than the surrounding vessel wall (Fig. 5b, d), may have infiltrated pit membranes (Dute et al. 1992). Gum-filled vessels are common to the heartwood, but are less frequent in sapwood vessels of the mangrove species studied. Deposition of phenolic substances to block vessels in a permanent way is a common reaction to

Figure 5. Transmission electron micrographs (potassium permanganate staining) showing deposits in intervessel pit canals (a & c) that increase the pit membrane lignin content as seen by UV scanning profiles at 278 nm (b & d). – a, b: Deposits in pit canals at one side of the intervessel pits in Heritiera littoralis. – c, d: Deposits lining vessel lumen and pit canals with vestures in Lumnitzera racemosa.
avoid gas embolism (Bonsen & Kučera 1990; Rioux et al. 1998). Embolisms can be triggered by water stress (Hargrave et al. 1994; Ewers et al. 2004) or by wounding (Schmitt et al. 1997; Soukup & Votrubová 2005). The deposits hinder pathogens from spreading in the tree. The deposition takes place by secretion of phenolic substances from parenchyma cells through pits into vessels (Bonsen & Kučera 1990; Schmitt et al. 1997; Koch et al. 2006).

The trigger for, and the mechanism underlying the obviously seasonal deposition/removal of pit membrane incrusting substances remains to be elucidated. Nevertheless, our results suggest that changes in pit membrane composition, which are unrelated to heartwood deposits, take place in fully differentiated sapwood vessels. Irregularities in this process could then explain variations in pit membrane electron density within a conduit (Fig. 4j, k), as observed previously in Cryptomeria japonica (Sano & Nakada 1998) and Daphne (Dute et al. 1992). Such post-differentiation events are in agreement with the dissimilar lignin content of vessel walls and pit membranes (Fig. 4l).

It can be concluded that characteristics of intervessel pit membranes are not influenced by changing conditions along the tree axis, but we found indications they are influenced by environmental conditions. The ability to dynamically change pit membranes in mature sapwood, would add an extra level to the capacity of plants to regulate sap flow in response to the ambient environment. It would help to explain the dual and contrasting role of pit membranes as gateways for the circumvention of embolisms. Further studies are needed to obtain a better understanding of how trees balance safety and efficiency of their water transport system.

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