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

Nele Schmitz[1,2,*], **Gerald Koch**[3], **Hans Beeckman**[2], **Nico Koedam**[1], **Elisabeth M.R. Robert**[1,2] and **Uwe Schmitt**[3]

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 *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.

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 *et al*. 1995; Salleo *et al*. 1996; Cochard 2006), it is currently believed that drought-induced cavitation, *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

1) Vrije Universiteit Brussel (VUB), Laboratory for Plant Biology and Nature Management (APNA), Pleinlaan 2, 1050 Brussels, Belgium.
2) Royal Museum for Central Africa (RMCA), Laboratory for Wood Biology and Xylarium, Leuvensesteenweg 13, 3080 Tervuren, Belgium.
3) Johann Heinrich von Thünen-Institut (vTI), Federal Research Institute for Rural Areas, Institute for Wood Technology and Wood Biology, Leuschnerstrasse 91, 21031 Hamburg, Germany.
*) Author for correspondence [E-mail: nschmitz@vub.ac.be].

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; Hölttä 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 $5 \times 5 \times 8$ mm from equally spaced positions along the medium stem radius from bark to pith, as no growth rings could be distinguished.

Table 1. Site description of the species studied.

Site		Species	Range of soil water sailinity (‰)		Inund. freq.[‡] (days month[-1])	Radial increment[†] (mm yr^{-1})
			Dry season	Rainy season		
1	landward	H*	40–90	10–57	4	–
1	landward	C	40–90	10–57	5	0.28 ± 0.09
1	landward	L	40–90	10–57	5	0.81 ± 0.08
2	riverside	R	7–31	0–11	10	–
2	riverside	X*	7–31	0–11	12	2.31 ± 1.09
3	landward	R	52–76	10–51	17	–
4	seaward	B*	34–46	21–35	27	0.2 ± 0.12
4	seaward	R	34–46	21–35	30	–
4	seaward	S	34–46	21–35	30	2.62 ± 2.11

B, *Bruguiera gymnorrhiza*; C, *Ceriops tagal*; H, *Heritiera littoralis*; L, *Lumnitzera racemosa*;
R, *Rhizophora mucronata*; S, *Sonneratia alba*; X, *Xylocarpus granatum*.
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).

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 $2 \times 2 \times 3$ 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 μm^2 and a photometrical resolution of 4096 grey scales converted into 14 basic colours (Koch & Kleist 2001).

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.

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 $2 \times 2 \times 3$ 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 $2 \times 2 \times 3$ mm wood sample.
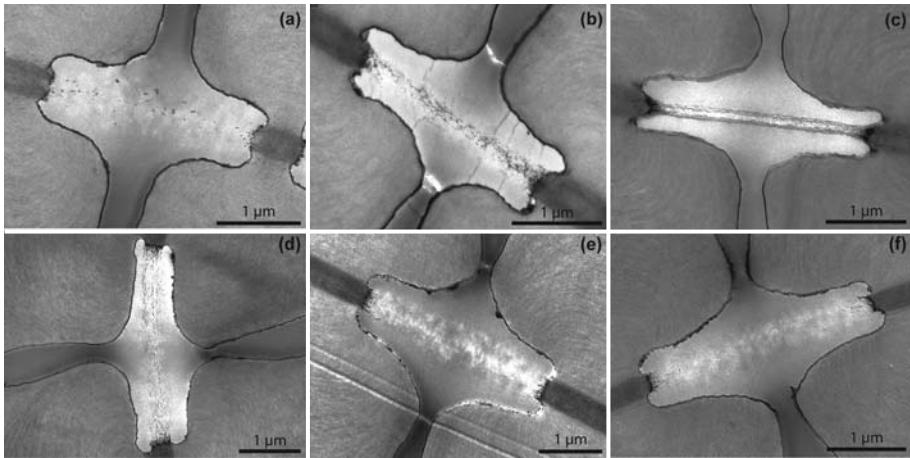
Figure 2. Transmission electron micrographs (potassium permanganate staining) of intervessel pit membranes from the sapwood of *Rhizophora mucronata* trees along the stem axis from root to canopy. – a & b: Two different positions in a single vessel of the root. – c: Aerial root showing thinner pit membrane in a vessel with tyloses. – d: Stem sample just above the highest aerial root. – e: Stem sample at 130 cm above ground. – f: stem sample from within the canopy.

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.

Site*:	Stem axis				Stem radius	
	Low salinity	High salinity	Pooled			High salinity
root	–‡	0.30 ± 0.08 a	0.30 ± 0.08 a	pith		0.45 ± 0.04 ab
proproot	0.48 ± 0.03 ab†	0.22 ± 0.04 a	0.48 ± 0.03 b			0.46 ± 0.04 a
above proproot	0.51 ± 0.13 a	0.45 ± 0.05 b	0.48 ± 009 b	↓		0.42 ± 0.03 a
DBH	0.38 ± 0.04 b	0.56 ± 0.06 c	0.49 ± 0.11 b			0.49 ± 0.07 ab
canopy	0.44 ± 0.03 ab	0.45 ± 0.06 bd	0.45 ± 0.05 b	bark		0.53 ± 0.03 b

*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* (Domec *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).
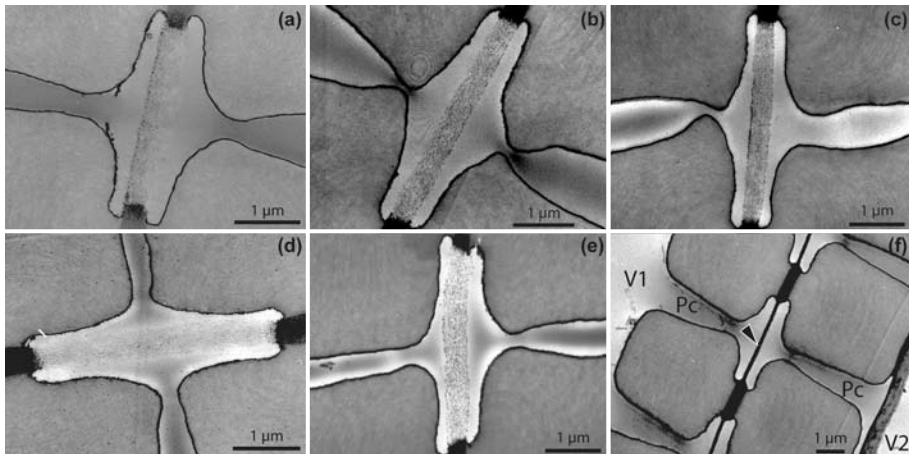

Figure 3. a–f: Transmission electron micrographs (potassium permanganate staining) of intervessel pit membranes along the stem radius (4.5 cm) of a *Rhizophora mucronata* tree from bark to pith. – e & f: Pit membranes from the same wood sample but from different vessels. In f a tylosis wall can be seen at the right of the picture. V1-2, two neighbouring vessels; Pc = pit canal; arrowhead points to the pit membrane.

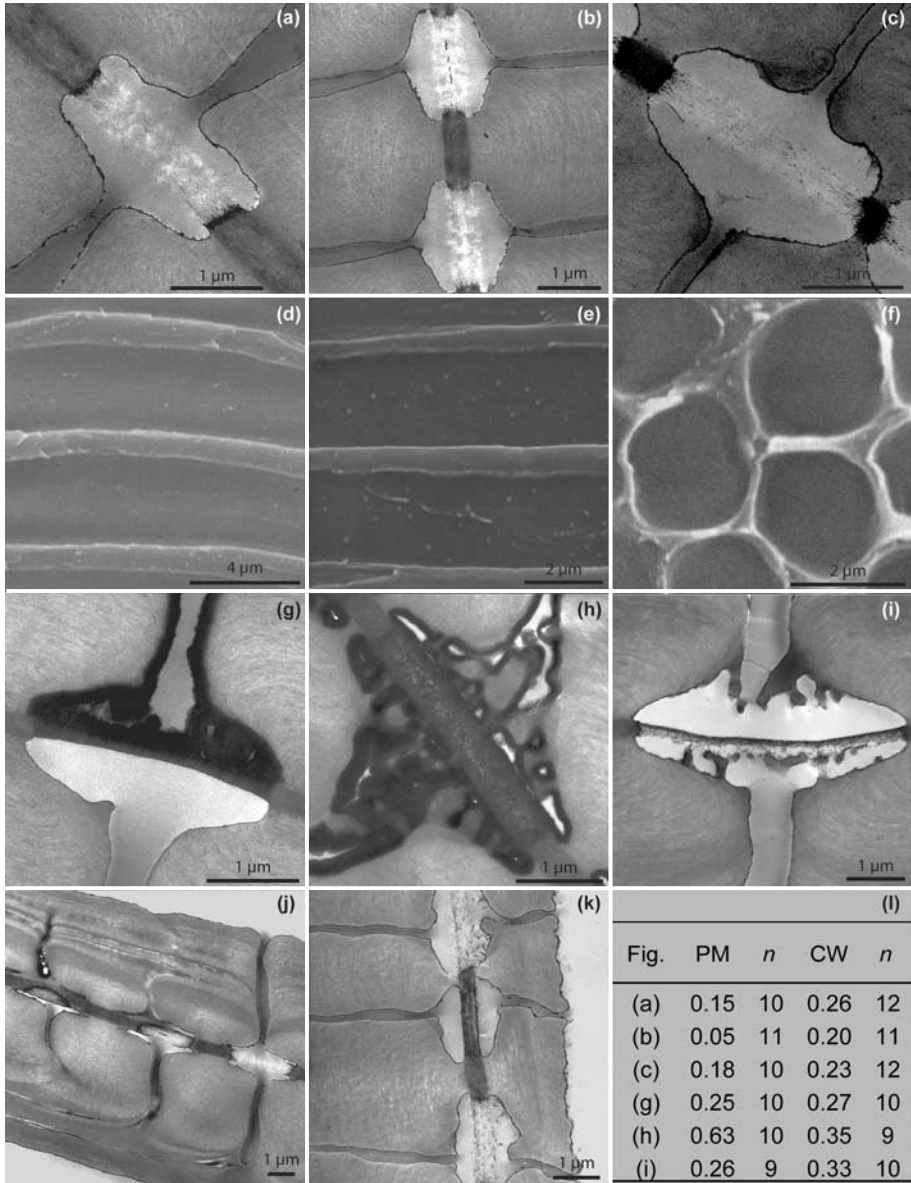| Fig.	PM	n	CW	n
(a)	0.15	10	0.26	12
(b)	0.05	11	0.20	11
(c)	0.18	10	0.23	12
(g)	0.25	10	0.27	10
(h)	0.63	10	0.35	9
(i)	0.26	9	0.33	10

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
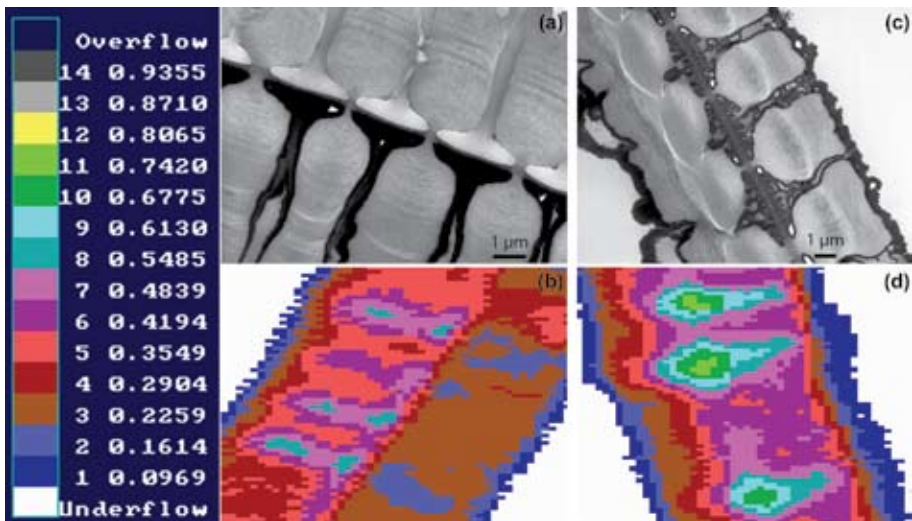

Figure 5. Transmission electron micrographs (potassium permanganate staining) showing deposits in intervessel pit canals (a & c) that increase the pit membrane lignin content as seen by UV scanning profiles at 278 nm (b & d). – a, b: Deposits in pit canals at one side of the intervessel pits in *Heritiera 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. 4 l).

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.

REFERENCES

Aasamaa, K. & A. Sober. 2010. Sensitivity of stem and petiole hydraulic conductance of deciduous trees to xylem sap ion concentration. Biol. Plantarum 54: 299–307.

Alder, N.N., J.S. Sperry & W.T. Pockman. 1996. Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. Oecologia 105: 293–301.

Aziz, I. & M.A. Khan. 2001. Effect of seawater on the growth, ion content and water potential of *Rhizophora mucronata* Lam. J. Plant Res. 114: 369–373.

Barnett, J.R. 1982. Plasmodesmata and pit development in secondary xylem elements. Planta 155: 251–260.

Benayoun, J., A.M. Catesson & Y. Czaninski. 1981. A cytochemical study of differentiation and breakdown of vessel end walls. Ann. Bot.-London 47: 687–698.

Bonsen, K.J.M. & L.J. Kučera. 1990. Vessel occlusions in plants - morphological, functional and evolutionary aspects. IAWA Bull. n.s. 11: 393–399.

Boyce, C.K., M.A. Zwieniecki, G.D. Cody, C. Jacobsen, S. Wirick & A.H. Knoll. 2004. Evolution of xylem lignification and hydrogel transport regulation. Proc. Natl. Acad. Sci. USA 101: 17555–17558.

Choat, B., M. Ball, J. Luly & J. Holtum. 2003. Pit membrane porosity and water stress-induced cavitation in four co-existing dry rainforest tree species. Plant Physiol. 131: 41–48.

Choat, B., A.R. Cobb & S. Jansen. 2008. Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function xylem. New Phytol. 177: 608–625.

Choat, B., S. Jansen, M.A. Zwieniecki, E. Smets & M. Holbrook. 2004. Changes in pit membrane porosity due to deflection and stretching: the role of vestured pits. J. Exp. Bot. 55: 1569–1575.

Choat, B., E.C. Lahr, P. Melcher, M.A. Zwieniecki & N.M. Holbrook. 2005. The spatial pattern of air seeding thresholds in mature sugar maple trees. Plant Cell Environ. 28: 1082–1089.

Choat, B. & J. Pittermann. 2009. New insights into bordered pit structure and cavitation resistance in angiosperms and conifers. New Phytol. 182: 557–560.

Christman, M.A., J.S. Sperry & F.R. Adler. 2009. Testing the 'rare pit' hypothesis for xylem cavitation resistance in three species of *Acer*. New Phytol. 182: 664–674.

Cochard, H. 2006. Cavitation in trees. C. R. Phys. 7: 1018–1026.

Cochard, H., S. Herbette, E. Hernández, T. Hölttä & M. Mencuccini. 2010. The effects of sap ionic composition on xylem vulnerability to cavitation. J. Exp. Bot. 61: 275–285.

Domec, J.C., B. Lachenbruch & F.C. Meinzer. 2006a. Bordered pit structure and function determine spatial patterns of air-seeding thresholds in xylem of Douglas-fir (*Pseudotsuga menziesii*; Pinaceae) trees. Amer. J. Bot. 93: 1588–1600.

Domec, J.C., F.G. Scholz, S.J. Bucci, F.C. Meinzer, G. Goldstein & R. Villalobos-Vega. 2006b. Diurnal and seasonal variation in root xylem embolism in neotropical savanna woody species: impact on stomatal control of plant water status. Plant Cell Environ. 29: 26–35.

Donaldson, L. 1992. Lignin distribution during latewood formation in *Pinus radiata* D.Don. IAWA Bull. n.s. 13: 381–387.

Dute, R.R., A.E. Rushing & J.D. Freeman. 1992. Survey of intervessel pit membrane structure in *Daphne* species. IAWA Bull. n.s. 13: 113–123.

Ewers, F.W., J. Lopez–Portillo, G. Angeles & J.B. Fisher. 2004. Hydraulic conductivity and embolism in the mangrove tree *Laguncularia racemosa*. Tree Physiol. 23: 1057–1062.

Gascó, A., E. Gortan, S. Salleo & A. Nardini. 2008. Changes of pH of solutions during perfusion through stem segments: further evidence for hydrogel regulation of xylem hydraulic properties? Biol. Plantarum 52: 502–506.

Gascó, A., S. Salleo, E. Gortan & A. Nardini. 2007. Seasonal changes in the ion-mediated increase of xylem hydraulic conductivity in stems of three evergreens: any functional role? Physiologia Plantarum 129: 597–606.

Gortan, E., A. Nardini, S. Salleo & S. Jansen. 2011. Pit membrane chemistry influences the magnitude of ion-mediated enhancement of xylem hydraulic conductance in four Lauraceae species. Tree Physiol. 31: 48–58.

Hacke, U.G., V. Stiller, J.S. Sperry, J. Pittermann & K.A. McCulloh. 2001. Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. Plant Physiol. 125: 779–786.

Hargrave, K.R., K.J. Kolb, F.W. Ewers & S.D. Davis. 1994. Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). New Phytol. 126: 695–705.

Hölttä, T. 2011. A carbon cost–gain model explains the observed patterns of xylem safety and efficiency. Plant Cell Environ. 34: 1819–1834.

Jansen, S., B. Choat & A. Pletsers. 2009. Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. Amer. J. Bot. 96: 409–419.

Jarbeau, J.A., F.W. Ewers & S.D. Davis. 1995. The mechanism of water-stress-induced embolism in two species of chaparral shrubs. Plant Cell Environ. 18: 189–196.

Koch, G. & G. Kleist. 2001. Application of scanning UV microspectrophotometry to localise lignins and phenolic extractives in plant cell walls. Holzforschung 55: 563–567.

Koch, G., H.G. Richter & U. Schmitt. 2006. Topochemical investigation on phenolic deposits in the vessels of afzelia (*Afzelia* spp.) and merbau (*Intsia* spp.) heartwood. Holzforschung 60: 583–588.

Lens, F., J.S. Sperry, M.A. Christman, B. Choat, D. Rabaey & S. Jansen. 2011. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. New Phytol. 190: 709–723.

Lopez-Portillo, J., F.W. Ewers & G. Angeles. 2005. Sap salinity effects on xylem conductivity in two mangrove species. Plant Cell Environ. 28: 1285–1292.

Melcher, P.J., G. Goldstein, F.C. Meinzer, D.E. Yount, T.J. Jones, N.M. Holbrook & C.X. Huang. 2001. Water relations of coastal and estuarine *Rhizophora mangle*: xylem pressure potential and dynamics of embolism formation and repair. Oecologia 126: 182–192.

Meyra, A.G., V.A. Kuz & G.J. Zarragoicoechea. 2007. Geometrical and physicochemical considerations of the pit membrane in relation to air seeding: the pit membrane as a capillary valve. Tree Physiol. 27: 1401–1405.

Nardini, A., A. Gascó, F. Cervone & S. Salleo. 2007a. Reduced content of homogalacturonan does not alter the ion-mediated increase in xylem hydraulic conductivity in tobacco. Plant Physiol. 143: 1975–1981.

Nardini, A., A. Gascó, P. Trifilò, M.A. Lo Gullo & S. Salleo. 2007b. Ion-mediated enhancement of xylem hydraulic conductivity is not always suppressed by the presence of Ca2+ in the sap. J. Exp. Bot. 58: 2609–2615.

Nardini, A., F. Grego, P. Trifilò & S. Salleo. 2010. Changes of xylem sap ionic content and stem hydraulics in response to irradiance in *Laurus nobilis*. Tree Physiol. 30: 628–635.

Nardini, A., S. Salleo & S. Jansen. 2011. More than just a vulnerable pipeline: xylem physiology in the light of ion-mediated regulation of plant water transport. J. Exp. Bot. 62: 4701–4718.

O'Brien, T.P. 1970. Further observations on hydrolysis of the cell wall in the xylem. Protoplasma 69: 1–14.

Oertli, J.J. 1993. Effect of cavitation on the status of water in plants. In: M. Borghetti, J. Grace & A. Raschi (eds.), Water transport in plants under climatic stress: 27–40. Cambridge University Press, Cambridge.

Plavcová, L. & U.G. Hacke. 2011. Heterogeneous distribution of pectin epitopes and calcium in different pit types of four angiosperm species. New Phytol. 192: 885–897.

Plavcová, L., U.G. Hacke & J.S. Sperry. 2011. Linking irradiance-induced changes in pit membrane ultrastructure with xylem vulnerability to cavitation. Plant Cell Envir. 34: 501–513.

Rao, R.V., D.P. Aebischer & M.P. Denne. 1997. Latewood density in relation to wood fibre diameter, wall thickness, and fibre and vessel percentages in *Quercus robur* L. IAWA J. 18: 127–138.

Rioux, D., M. Nicole, M. Simard & G.B. Ouellette. 1998. Immunocytochemical evidence that secretion of pectin occurs during gel (gum) and tylosis formation in trees. Phytopathology 88: 494–505.

Robert, E.M.R., N. Schmitz, J.A. Okello, I. Boeren, H. Beeckman & N. Koedam. 2010. Mangrove growth rings: fact or fiction? Trees-Struct. Funct. 25: 49–58.

Salleo, S., M.A. Lo Gullo, D. De Paoli & M. Zippo. 1996. Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. New Phytol. 132: 47–56.

Sano, Y. 2005. Inter- and intraspecific structural variations among intervascular pit membranes as revealed by field-emission scanning electron microscopy. Amer. J. Bot. 92: 1077–1084.

Sano, Y. & K. Fukuzawa. 1994. Structural variations and secondary changes in pit membranes in *Fraxinus mandschurica* var. *japonica*. IAWA J. 15: 283–291.

Sano, Y. & R. Nakada. 1998. Time course of the secondary deposition of incrusting materials on bordered pit membranes in *Cryptomeria japonica*. IAWA J. 19: 285–299.

Schmid, R. 1965. The fine structure of pits in hardwoods. In: W.A. Côté, Jr. (ed.), Cellular ultra-structure of woody plants: 291–304. Syracuse University Press, New York.

Schmid, R. & R.D. Machado. 1968. Pit membranes in hardwoods – fine structure and development. Protoplasma 66: 185–204.

Schmitt, U. & E. Melcher. 2004. Section staining with potassium permanganate for transmission electron microscopy: a useful tool for lignin localisation. In: U. Schmitt *et al*. (eds.), Wood fibre cell walls: methods to study their formation, structure and properties: 105–118. Swedish University of Agricultural Sciences, Uppsala.

Schmitt, U., H.G. Richter & C. Muche. 1997. TEM study of wound-induced vessel occlusions in European ash (*Fraxinus excelsior* L.). IAWA J. 18: 401–404.

Schmitz, N., S. Jansen, A. Verheyden, J.G. Kairo, H. Beeckman & N. Koedam. 2007. Comparative anatomy of intervessel pits in two mangrove species growing along a natural salinity gradient in Gazi Bay, Kenya. Ann. Bot.-London 100: 271–281.

Schmitz, N., G. Koch, U. Schmitt, N. Koedam & H. Beeckman. 2008. Intervessel pit structure and histochemistry of two mangrove species as revealed by cellular UV microspectrophotometry and electron microscopy: intraspecific variation and functional significance. Microsc. and Microanal. 14: 387–397.

Sobrado, M.A. 2004. Influence of external salinity on the osmolality of xylem sap, leaf tissue and leaf gland secretion of the mangrove *Laguncularia racemosa* (L.) Gaertn. Trees-Struct. Funct. 18: 422–427.

Soukup, A. & O. Votrubová. 2005. Wound-induced vascular occlusions in tissues of the reed *Phragmites australis*: their development and chemical nature. New Phytol. 167: 415–424.

Sperry, J.S. & M.T. Tyree. 1988. Mechanism of water stress-induced xylem embolism. Plant Physiol. 88: 581–587.

Sperry, J.S., M.T. Tyree & J.R. Donnelly. 1988. Vulnerability of xylem to embolism in a mangrove *vs*. an inland species of Rhizophoraceae. Physiol. Plantarum 74: 276–283.

Suarez, N. & M.A. Sobrado. 2000. Adjustments in leaf water relations of mangrove (*Avicennia germinans*) seedlings grown in a salinity gradient. Tree Physiol. 20: 277–282.

Sun, Q. & M. Suzuki. 2001. Quantitative character variations of cambial derivatives in mangroves and their functional significance. Trees-Struct. Funct. 15: 249–261.

Thomas, R.J. 1976. Anatomical features affecting liquid penetrability in three hardwood species. Wood Fiber Sci. 7: 256–263.

Trifilò, P., M.A. Lo Gullo, S. Salleo, K. Callea & A. Nardini. 2008. Xylem embolism alleviated by ion-mediated increase in hydraulic conductivity of functional xylem: insights from field measurements. Tree Physiol. 28: 1505–1512.

Tyree, M.T., S.D. Davis & H. Cochard. 1994. Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? IAWA J. 15: 335–360.

Van Ieperen, W. 2007. Ion-mediated changes of xylem hydraulic resistance in planta: fact or fiction? Trends Plant Sci. 12: 137–142.

Wheeler, E.A. 1981. Intervascular pitting in *Fraxinus americana* L. IAWA Bull. n.s. 2: 169–174.

Wheeler, E.A. & R.J. Thomas. 1981. Ultrastructural characteristics of mature wood of south ern red oak (*Quercus falcata* Michx.) and white oak (*Quercus alba* L.). Wood Fiber Sci. 13: 169–181.

Woodruff, D.R., K.A. McCulloh, J.M. Warren, F. Meinzer & B. Lachenbruch. 2007. Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. Plant Cell Environ. 30: 559–569.

Zwieniecki, M.A. & N.M. Holbrook. 2000. Bordered pit structure and vessel wall surface properties. Implications for embolism repair. Plant Physiol. 123: 1015–1020.

Zwieniecki, M.A., P.J. Melcher & N.M. Holbrook. 2001. Hydrogel control of xylem hydraulic resistance in plants. Science 291: 1059–1062.