Intervessel Pit Structure and Histochemistry of Two Mangrove Species as Revealed by Cellular UV Microspectrophotometry and Electron Microscopy: Intraspecific Variation and Functional Significance

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Abstract: Intervessel pits play a key role in trees' water transport, lying at the base of drought-induced embolism, and in the regulation of hydraulic conductivity via hydrogels bordering pit canals. Recently, their microstructure has been the focus of numerous studies, but the considerable variation, even within species and the histochemistry of pit membranes, remains largely unexplained. In the present study, intervessel pits of the outermost wood were examined for *Avicennia marina*, of dry and rainy season wood separately for *Rhizophora mucronata*. The thickness of the pit membranes was measured on transmission electron micrographs while their topochemical nature was also analyzed via cellular UV microspectrophotometry. Pit membranes of *R. mucronata* were slightly thicker in dry season wood than in rainy season wood, but their spectra showed for both seasons a lignin and a yet unidentified higher wavelength absorbing component. It was suggested to be a derivative of the deposits, regularly filling pit canals. The vestures of *A. marina* chemically resembled pit membranes rather than cell walls.

Key words: intervessel pit, pit membrane, cellular UV microspectrophotometry (UMSP), TEM, mangrove, vesture, lignin, *Rhizophora mucronata*, *Avicennia marina*, deposit

Introduction

Intervessel pits are small openings in the vessel wall enabling communication between vessels. Instead of mere holes, porous intervessel pit membranes provide a way for a regulated sap flow (Zwieniecki et al., 2001; Gascó et al., 2006; van Ieperen, 2007). They play a central but dual role in the water transport. On the one side, they are the Achilles heel of the hydraulic structure where tiny air bubbles can be sucked through, that expand under the tension in the vessel (e.g., Sperry & Tyree, 1988; Sperry & Hacke, 2004; Wheeler et al., 2005; Domec et al., 2006). On the other side, they offer a way to circumvent these expanded air bubbles or drought-induced embolisms that block the water transport (Orians et al., 2004; Ellmore et al., 2006; Hacke et al., 2006). Given their role in sap flow, the observation of a large variation in pit membrane thickness and porosity should

not be surprising. Numerous recent studies focused on the microstructure of the intervessel pits and particularly their pit membranes (Singh et al., 1999; Pesacreta et al., 2005; Sano, 2005; Schmitz et al., 2007a). A tenfold variation in pit membrane thickness and a variation of even two orders of magnitude in maximum pit membrane porosity were demonstrated across 14 hardwood species (Choat et al., 2008).

This structural variation of pit membranes can be related among others to different stages in pit development (Wardrop et al., 1963; Schmid & Machado, 1968) or to secondary deposits. Besides heartwood formation (Bonner & Thomas, 1972; Wheeler & Thomas, 1981; Wheeler, 1982; Sano & Fukuzawa, 1994; Streit & Fengel, 1994; Sano & Nakada, 1998; Koch et al., 2006), secondary deposits could be due to mechanical wounding (Morrow & Dute, 1999), biotic (Nemec, 1975; Hammerschmidt & Kuć, 1982; Street et al., 1986), or abiotic stresses (Robb et al., 1980; Robb & Busch, 1982) including seasonal changes (Yang, 1978; Wheeler, 1981; Sano et al., 1999). Under a seasonal tropical climate, trees experience an increased risk for drought-induced embolism during the dry season. In this case,

Figure 1. Transmission electron micrographs of longitudinal sections of intervessel pits of *R. mucronata* (**a–k**) and *A. marina* (l). (**a–c**) Intervessel pits with dark staining pit membranes and (**d–f**) nearly transparent pit membranes with pit annuli present (arrows). (**g, h**) Remarkably thick pit border linings at only one side of the vessel pair. (**i, j**) Membrane-like structures bordering inner and outer pit apertures, respectively (arrow heads). (**k**) Granular material lining pit borders. (**l**) Intervessel pits of *A. marina* with dark staining vestures (arrows), pit membranes of rather low electron density, and a thin layer lining pit borders. Samples shown are 3878D, 3870D, 3884D, 3872D, 3873R, 3882D, 3876D, 3885R, 3879R, 3874D, 3871R, and 3886 with D, dry season wood, and R, rainy season wood (Table 1). L_v, vessel lining; L_{pc}, pit canal lining; PC, pit canal; Pch, pit chamber; PM, pit membrane; W, intervessel wall thickness.

thicker pit membranes are less likely to develop exceptionally large pores by excessive stretching when aspirated by the pressure difference between a functional and an embolized vessel (Hacke et al., 2001, Choat et al., 2003). It is this maximum pore size that is hypothesized to determine embolism vulnerability rather than the average porosity (Wheeler et al., 2005; Hacke et al., 2006; Choat et al., 2008). Next to changes of the pit membrane itself, vestures or appendices of the vessel wall in pit canals and/or pit chambers (Fig. 1a) can impede excessive stretching by supporting the pit membrane and hence lowering the vulnerability to embolism (Zweypfenning, 1978; Jansen et al., 1998, 2003; Choat et al., 2004; Jansen et al., 2004; Sperry & Hacke, 2004).

The chemical nature of the intervessel pit membranes is just as variable and important for sap flow in trees as is its structure. Pit membrane porosity is not the sole determinant of the pressure that is needed between a functional and an air-filled vessel for air-seeding to occur. Also the contact angle between pit membrane and air-water interface, which is a function of the surface chemistry of the pit membrane, has an influence (Holbrook & Zwieniecki, 1999; Zwieniecki & Holbrook, 2000; Meyra et al., 2007). More hydrophobic substances, such as lignin, increase the contact angle and lower the air-seeding pressure. In addition, high lignin content has been assumed to hinder the hydrogel activity of pectins in pit membranes (Tibbits et al., 1998; Ridley et al., 2001; Boyce et al., 2004). Pectic substances can swell and shrink in reaction to changes in the ionic composition of the xylem sap, altering pit membrane porosity. Lignin would hamper this adjustment and thus the regulation of the hydraulic conductance via hydrogels as found in some species (Zwieniecki et al., 2001; Gascó et al., 2006; van Ieperen, 2007). The functional advantage of pit membranes of low lignin content is supported by the reduced lignin biosynthesis under water stress (Donaldson, 2002; Alvarez et al., 2008).

Considering the significance of the chemical nature of pit membranes for water transport in trees, variation in the histochemistry of pit membranes is not unexpected. However, a conclusive explanation could not yet be given. Non-lignified pit membranes were observed in both softwoods and hardwoods between rays and tracheary elements (Bamber, 1961; Chafe, 1974) and between fiber cells (Wardrop, 1957) using different staining methods or UV microscopy,

respectively. Rudman (1965) expected lignin-free pit membranes between vessel cells too. In confirmation, little or no lignin was observed in juvenile wood of different hardwood species after diverse staining (O'Brien, 1970; Coleman et al., 2004) or immunolocalization techniques (Chaffey et al., 1997). It remains, however, unclear whether mature wood would have given the same results. Besides, the negative staining reaction for lignin in *Salix* (O'Brien, 1970) could be due to its extremely thin and porous pit membranes (Sano, 2005). These doubts are enforced by staining as well UV microspectrophotometry of mature wood samples showing lignified pit membranes between tracheary elements in different softwoods (Bauch & Berndt, 1973; Sano & Nakada, 1998; Donaldson, 2002). Atomic force microscopy of the hardwood *Sapium sebiferum* supported this finding (Pesacreta et al., 2005), as did a backscattered electron microscopy analysis of vessel-fiber pit membranes in *Fagus sylvatica* (Fromm et al., 2003). Nevertheless, adding to the variation are the pit membranes of *Pinus* that only seemed to contain lignin in heartwood (Fengel & Wolfsgruber, 1971).

In this study, intervessel pit structure and chemistry of two Kenyan mangrove species will be examined. *Rhizophora mucronata* is restricted to the seaward side of the forest and areas influenced by fresh river water. *Avicennia marina* has a much wider distribution both globally and locally, where it occurs in a disjunct pattern at both seaward and landward side of the forest (Dahdouh-Guebas et al., 2004). The extreme mangrove environment concerning stresses imposed on the water transport makes them appropriate study species to assess the ecological plasticity of intervessel pit properties. Recently, a bimodal distribution of the pit membrane thickness was reported in *R. mucronata* but not in *A. marina* (Schmitz et al., 2007a) corresponding to their periodic (Verheyden et al., 2004) and patchy wood formation (Schmitz et al., 2008), respectively. In addition, the electron density of the pit membranes using transmission electron microscopy (TEM) was considerably higher in *R. mucronata* compared to *A. marina* suggesting a different chemical composition (Schmitz et al., 2007a). The objectives of the study were approached via a combination of TEM and cellular UV microspectrophotometry (UMSP). The latter technique has proven its high value to visualize and quantify the chemical composition of cell wall structures and contents (Frankenstein et al., 2006; Koch et al., 2006). UV scans of semithin wood sections at a fixed wavelength,
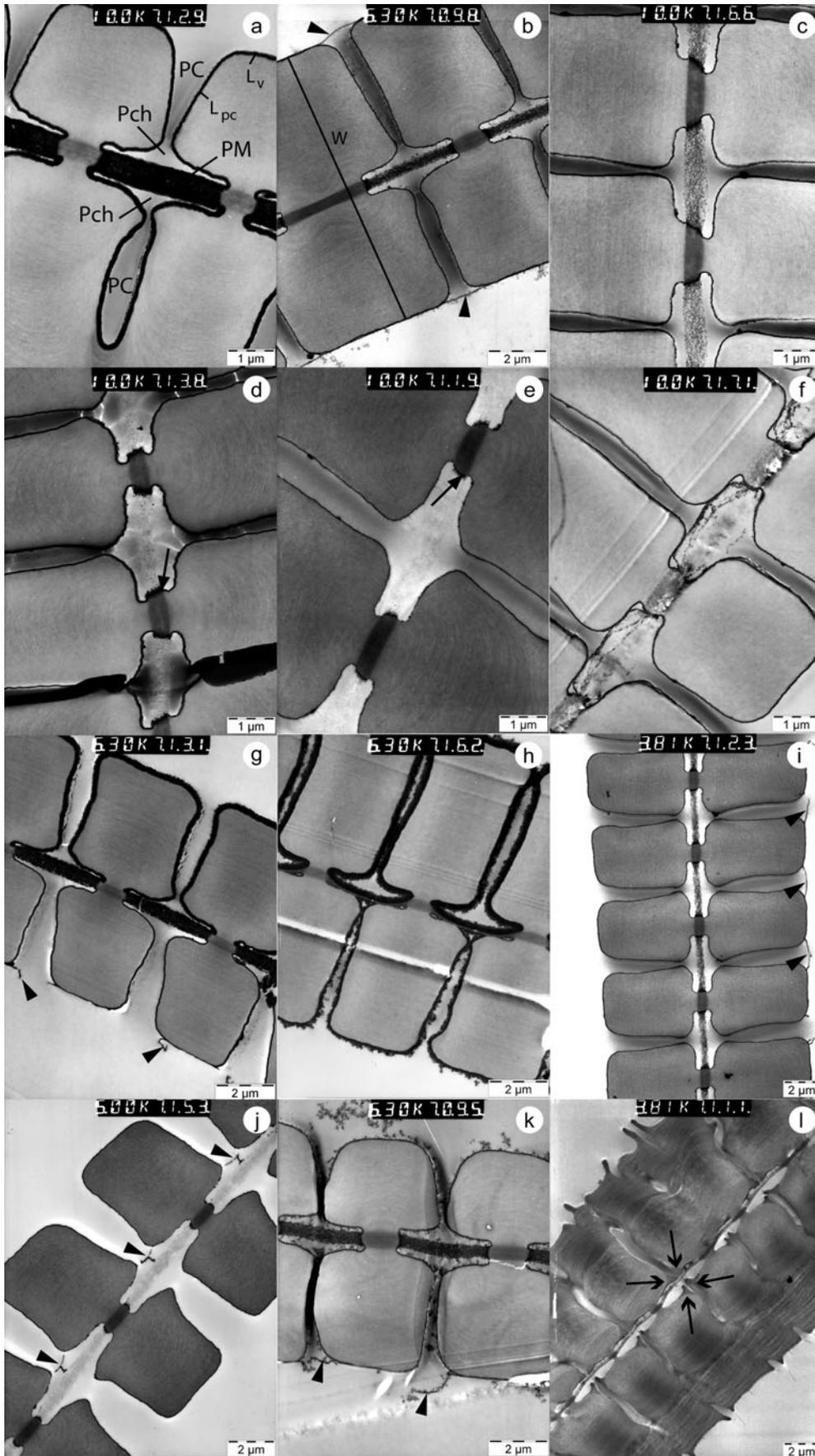
Table 1.   Environmental and Tree Characteristics of the Eight *R. mucronata* Trees and Two *A. marina* Trees Sampled; Consecutive Sample Numbers for *R. mucronata* Are from One Annual Ring.

Species/location	Sample number		C_{130}[a] (cm)	Tree height (m)	Soil water salinity (‰)		Inundation class[b]
	Dry season	Rainy season			Minimum	Maximum	
R. mucronata							
Site 1	1878, -80	1879, -81	55 ± 12	8 ± 1	21	46	1
Site 2	3870, -72	3871, -73	32 ± 7	7 ± 2	0	11	3
Site 3	3874, -76	3875, -77	51 ± 12	9 ± 0	30	33	2
Site 4	3882, -84	3881, -85	36 ± 9	6 ± 1	26	40	2
A. marina							
Site 5	3886–87[c]		25 ± 4	7 ± 1	5	68	3

[a]Stem circumference at 130 cm height.
[b]Inundation classes 1 to 3 correspond to an area being inundated by respectively 100–76%, 75–51%, and 50–26% of the high tides (Tomlinson, 1994).
[c]No distinction could be made between dry and rainy season wood (Schmitz et al., 2007*b*).

chosen at the absorbance maximum of the interested compound, inform about its absorbance intensity that is related to its concentration. UV spectra over a range of wavelengths, but at a selected position of the section, inform about the chemical composition of the spot though without identifying observed absorbance peaks (Koch & Grünwald, 2004). First, the hypothesis will be tested that the dimorphism in the thickness of the intervessel pit membranes of *R. mucronata* is related to the seasonal climate at Gazi Bay. Second, we will explore the chemical composition of the intervessel pits of *R. mucronata* and *A. marina*. Pit membranes as well as pit chambers and pit canals will be addressed as a first step toward the clarification of intervessel pit chemistry and its internal variation, which to date remains largely unknown.

Materials and Methods

Study Sites and Sample Collection

Study sites are located in the mangrove forest of Gazi Bay (39°30′E, 4°25′S), situated approximately 50 km south of Mombasa, Kenya. The climate along the Kenyan coast is characterized by a bimodal distribution of the precipitation. A distinct dry season (December–March) is followed by a long (April–July) and a short rainy season (October–November). During the wet season, the rivers Mkurumuji and Kidogoweni provide an important freshwater source for the mangroves of Gazi Bay (Kitheka, 1997). Samples were collected in February 2006 from two *A. marina* trees (Table 1, accession numbers 3886 and 3887) and in June 2006 from eight *R. mucronata* trees comprising two trees from four sites each (Table 1, accession numbers 3870–3885). Wedges of the outermost wood of about 10 × 3 ×

4 cm³ were excised at approximately 1.3 m height with a handsaw and immediately stored in 50% alcohol.

Sample Preparation and Analysis

From each *R. mucronata* wood sample, a broad growth ring was chosen to separate the wood formed during the rainy season from the wood formed during the dry season (Table 1, pair and impair accession numbers, dry and rainy season, respectively). The distinction between seasons is based on a corresponding change in vessel density (Verheyden et al., 2004; Schmitz et al., 2006). Wood samples were trimmed into cubes of $1 \times 5 \times 1$ mm³, dehydrated in an acetone series (30–100%), infiltrated with Spurr's epoxy resin through a series of propylene oxide/resin mixture and embedded at 70°C. Longitudinal sections of around 100 nm thickness were made with an ultramicrotome (Ultracut E, Reichert-Jung) using a diamond knife and stained with a 1% potassium permanganate solution for TEM analysis with a Philips CM 12 at an accelerating voltage of 60 kV. Photographs were taken from one or two positions showing intervessel pits. Thickness of the pit membranes, of the vessel and pit canal linings (Fig. 1a, L_v and L_{pc}, respectively), and of the intervessel walls (Fig. 1b, W) was measured with the image analysis software AnalySIS 3.2 (Soft Imaging System GmbH, Münster, Germany). Linings were defined as thin, electron dense layers that bordered vessel lumina including intervessel pits. They were measured for each vessel of the intervessel pit pair. T-tests for dependent samples and Pearson correlation analyses were performed in STATISTICA (StatSoft, Inc., 2006, data analysis software system, version 7.1, www.statsoft. com) after transforming the data with an inverse function, when data were not normally distributed. For the samples with two measuring positions, data were averaged per sample.

For cellular UMSP, unstained sections of 1 μm thickness were transferred to quartz slides, immersed in a drop

Table 2. Quantitative Description of the Intervessel Pits of *R. mucronata* Wood Sampled in the Dry and Rainy Season as Measured on Longitudinal Sections (Fig. 1a,b).

Intervessel pit character[a] (μm)	Median (range)	SD[b]	n	P value[c]
PM thickness D	0.5 (0.2–0.7)	0.1	10	<0.05[d]
PM thickness R	0.4 (0.2–0.7)	0.1	9	
Vessel lining	0.1 (0.0–0.6)	0.1	38	<0.01
PC lining	0.06 (0.03–0.25)	0.05	38	
Intervessel wall thickness	11 (8–14)	2	19	

[a]Intervessel pit membrane thickness of wood formed in the dry (D) and rainy (R) season, thickness of the lining of vessel lumen and pit canal (PC), and wall thickness in between a vessel pair.
[b]Standard deviation.
[c]Significance value of a *t*-test for dependent samples. PM thicknesses were averaged per tree and per season; vessel lining and PC lining data were transformed via the inverse function to comply with the normality assumption.
[d]$n = 8$, values of samples with more than one measurement were averaged.

of non-UV absorbing glycerine, and covered with a quartz cover slip. Scanning profiles were made with a ZEISS UMSP 80 at a constant wavelength of 280 nm (representing the absorbance maximum of lignin) using an ultrafluar 100:1 objective. Data were recorded and processed with the software program APAMOS (Zeiss). For sample 3870 an additional scanning profile was made at 560 nm to obtain more details about deposits. The scan program digitizes rectangular tissue portions with a local geometrical resolution of 0.25 μm^2 and a photometrical resolution of 4,096 grayscales converted into 14 basic colors (Koch & Kleist, 2001). In addition, point measurements (diameter of 1 μm) covering a wavelength range from 240 to 700 nm were made in 1-nm steps of pit membranes, pit canals, pit canal deposits, vessel walls, and vestures using the program LAMWIN (Zeiss). From the resulting spectra, mean curves were calculated after removing outliers from the dataset.

RESULTS

Transmission Electron Microscopy

Intervessel pit membranes were thicker in the dry season wood compared to the rainy season wood in six of the eight studied *R. mucronata* trees (Table 2). The electron density of the pit membranes was similar in dry/rainy season wood (Fig. 1a/i, b/k, d/e) with the exception of three trees. Four out of five trees had pit membranes with a higher or similar electron density than the middle lamella in adjacent wall portions (Fig. 1a–b,g,i,k). The electron density was not strictly correlated with the average porosity of the pit membranes. While dark membranes were mostly opaque, some were very porous (Fig. 1c). The two *A. marina* trees had pit

membranes with a similar or lower electron density than the middle lamella areas (Fig. 1l). Next to these general observations, a large variability in intervessel pit properties was found between and even within trees. At one position of a dry season sample, several thin membrane-like layers were seen in the pit chamber (Fig. 1f). Two trees from which two positions were analyzed for dry or rainy season wood showed a considerable difference in pit membrane thickness between both positions with the average value being even larger in the rainy than in the dry season. Also the electron density, i.e., the intensity on a grayscale, contrasted between two positions within the dry season wood of two trees. One tree had pit membranes that were nearly transparent although the pit annulus was highly electron dense (arrows, Fig. 1d,e).

In *R. mucronata*, vessel lumen, pit canal, and pit chamber were lined by a dark staining substance (Fig. 1) with vessel linings somewhat thicker than linings of the corresponding pit border (Table 2). The electron density of the pit membranes (Fig. 1) was unrelated to the thickness of the corresponding linings. One sample had a relatively thicker lining (Fig. 1a) and very dark pit membranes. However, samples with a thin lining had pit membranes of variable electron density. Besides, three samples had a thin lining at one side of the intervessel pit and a thick lining at the other side (Fig. 1g,h). Next to the electron density of the pit membranes, pit membrane thickness was unrelated to thickness of vessel linings ($r^2 = 0.12$, $p =$ ns, $n = 19$) or pit canal linings ($r^2 = 0.19$, $p =$ ns, $n = 19$). On top of these linings, a membrane-like structure of similar electron density delineated the inner pit apertures in half of the samples ($n = 16$) with five samples being dry season wood (Fig. 1b). Not necessarily both sides of the vessel pair had their inner pit apertures lined by these membrane-like structures, but if one aperture was lined, they all were lined at that side of the vessel pair (Fig. 1g,i,k). The same was true for a similar lining at the outer pit aperture that appeared in only one sample (Fig. 1j). Fuzzy, granular material of a similar electron density as the linings bordered the vessel wall from pit membranes to vessel lumen in four samples (Fig. 1b,h,k). No lining or only a very thin lining was observed in the two studied *A. marina* trees. The vestures were clearly more electron dense than the corresponding vessel wall (arrows, Fig. 1l). Deposits of variable electron density (Fig. 1) filled the pit canals homogenously in all trees of both species. The pit chamber was not filled or was filled with a substance of lower electron density.

UV Microspectrophotometry

The UV absorbance (280 nm) of the pit membranes of both species ranged only from about 0 to 0.2 in both *R. mucronata* (Fig. 2a,f,g) and *A. marina* (Fig. 2b–d) while the vessel walls were characterized by UV absorbance values from about 0.2 to 0.4 (Fig. 2). Only at 1 of 11 positions of one sample were pit membranes of stronger absorbance than
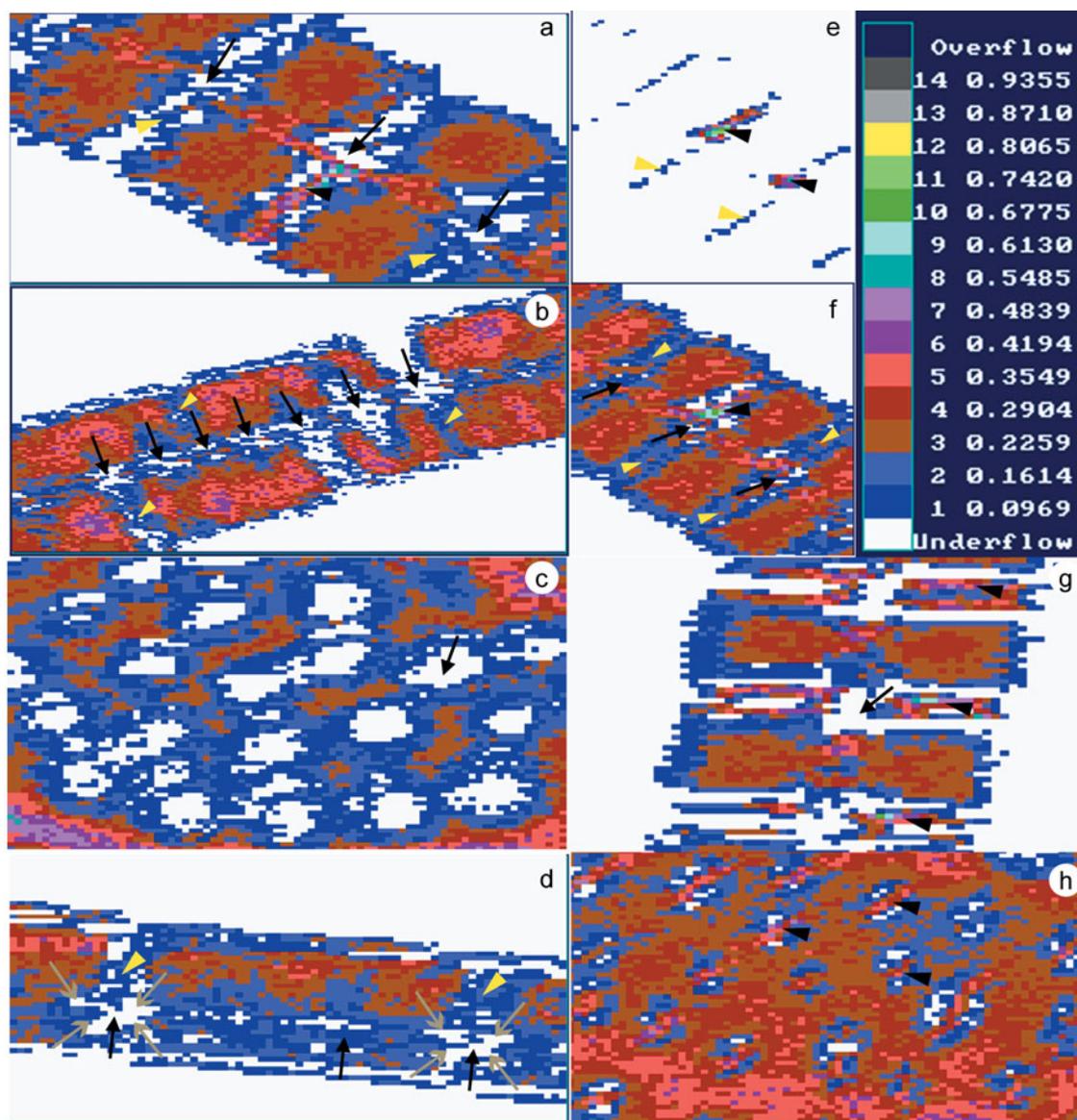
Figure 2. UV scanning profiles of intervessel pits of *R. mucronata* (**a, e–g**) and *A. marina* in longitudinal view (**b, d**) and surface view (**c, h**). In **b** pit canals are not always crossed by sectioning (see also Fig. 1l). (**c**) External side of the vessel, (**h**) lumen side of the vessel. Different colors correspond to different UV absorbance values as represented in the color legend. Images were taken at a wavelength of 280 nm (**a–d, f–h**) for lignin abundance and at 560 nm for deposits (see Fig. 3c). Black arrows, pit membranes; grey arrows, vestures; black arrow heads, high absorbent deposits in pit canals and pit chambers; yellow arrow heads, low absorbent pit canal deposits. Image magnification, 100×.

cell walls. Absorbance spectra of the pit membranes of both species showed a peak at around 280 nm and a second broad peak starting from about 580 nm up to 700 nm (Fig. 3a). Remarkably, these characteristic peaks were not found for one of the *R. mucronata* trees. No difference was observed between the spectral behavior of the pit membranes of *R. mucronata* wood formed during the dry season or during the rainy season. The vestures in *A. marina* were not or only slightly absorbing at 280 nm as clearly seen in the scanning profile (arrows, Fig. 2d). The absorbance spec-

tra of the vestures resembled more closely the spectra of the corresponding pit membranes than the vessel wall spectrum (Fig. 3b).

In the pit canals of both species, deposits were regularly observed with a low absorbance at 280 and 560 nm (yellow arrow heads, Fig. 2a–b,d–f). The absorbance spectra of these low absorbent deposits showed a peak at 280 nm and a broad peak between 540 and 700 nm (Fig. 3c). A second type of relatively high absorbent deposits was detected in the pit canals and/or pit chambers of *R. mucronata* sections
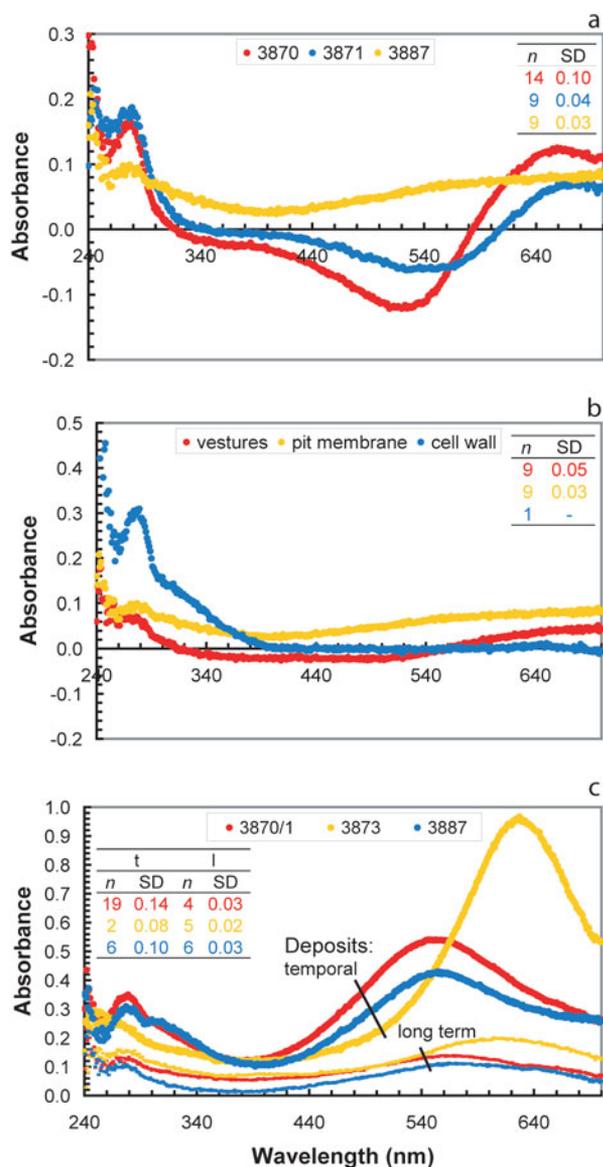
Figure 3. Mean curves of absorbance spectra of (**a**) intervessel pit membranes; (**b**) pit membranes, corresponding vestures and vessel cell wall of tree 3887; (**c**) high absorbent deposits of pit canals and pit chambers shrinking or even disappearing after some hours, and low absorbent pit canal deposits present for at least the entire study period of several days as seen in UV scanning profiles (Fig. 2). Accession numbers 3870, -71, -73, *R. mucronata*; 3886–3887, *A. marina*. In **c** values of 3870 and 3871 were averaged because it concerns dry and rainy season wood of the same tree, respectively, and curves did not differ considerably. *n*, number of pit membranes, vestures, or deposits of which the absorbance spectra were used for the average curve; *SD*, standard deviation.

analyzed shortly after sectioning (black arrow heads, Fig. 2g). Their absorbance spectra showed two high peaks, one at 280 nm and one at around 540 or 620 nm (Fig. 3c). However, in sections studied later these highly absorbing deposits were only seen sporadically (black arrow heads,

Fig. 2a,e–f), and in a time period of hours they had shrunk to the size of droplets and were located in the outer corners of the pit chambers, or in the inner aperture as observed from the vessel lumen in *A. marina* (Fig. 2h) or they had fully disappeared.

DISCUSSION

Remarkable Variation of Pit Membranes within Species

As was hypothesized, the seasonal climate in Kenya seemed to influence pit membrane thickness in *R. mucronata* with slightly thicker pit membranes in the dry season wood than in the rainy season wood (Table 2). Although this seasonal effect suggests a functional significance, its relationship with hydraulic conductance and embolism vulnerability needs to be tested. Moreover, the seasonal difference does not correspond to the bimodal distribution observed in an earlier study (peaks around 0.15 and 0.40 μm; Schmitz et al., 2007*a*), indicating also factors other than season control pit membrane thickness. Tree size, linked with cambial age, seemed to be such an additional factor. While the mean pit membrane thickness recorded in the previous study, where younger trees were sampled, was 0.3 ± 0.1 μm (Schmitz et al., 2007*a*), it was 0.5 ± 0.2 μm in the present study. The formation of thicker pit membranes while growing taller might be an indication of the vessels' requirements for mechanical strength next to conducting efficiency (Sperry et al., 2006).

The differences in pit membrane thickness could not be strictly ascribed to the varying thickness of the deposits lining vessel lumina and pit borders. The coatings were unrelated to seasons, in contrast to former studies on different angiosperm tree species (Wardrop et al., 1963; Donaldson & Singh, 1990; Castro, 1991; Singh et al., 2002). All pits with an extremely dense and thick lining had an equally dense pit membrane. But a thin vessel lining was commonly present pointing to an ontogenetic origin such as the tertiary wall or protoplast residues (Wardrop et al., 1963; Schmid, 1965). The extremely thick linings might result from heartwood formation as was found in *Betula alleghaniensis* (Yang, 1978) but also from reactions to past wounding. Although the age of heartwood formation in *R. mucronata* in Kenya is currently unknown, heartwood encrustations are unlikely for three reasons. First, in *R. mucronata* from the Phillipines the sapwood extended over 3 to 5 cm (Panshin, 1932), which is the stem portion where the studied samples were taken. Second, the thickness of the linings of some vessel pairs was unequal (Fig. 1g,h). Third, a similar lining was found in *Carya tomentosa* surrounding pit chamber, pit canal, and vessel lumen in both sapwood and heartwood (Thomas, 1976).

Independent of pit membrane thickness, differences in pit membrane porosity were observed in *R. mucronata*. In

contrast to Schmid and Machado (1968), they do most probably not reflect different stages of pit development as samples were taken some distance from the cambium. More likely are (a)biotic stresses or increasing cambial age that could gradually lead to a degradation of the pit membranes via pectic enzymes secreted by xylem parenchyma (Barnett, 1981; Sperry et al., 1991; Choat et al., 2003). The remaining cellulose network, devoid of encrusting material (Sperry et al., 1991), would explain the increasing vulnerability to embolism with age (Melcher et al., 2003). While pit membranes could not be seen in 16% ($n = 19$) of the studied vessel pairs, their pit annulus, assumed to be an accretion of encrusting material (Schmid & Machado, 1968; Sano et al., 1999), was clearly observed (Fig. 1d,e). Therefore, the apparently transparent pit membranes are almost certainly ripped off (Fig. 1e) or destroyed as a preparation artifact. The appearance of dark dots in the pit membranes of one sample (Fig. 1d) and the loose membrane-like material in another (Fig. 1f) might be consistent with cracks in the pit membranes and layers peeling off from the pit membranes, respectively.

Exploration of Intervessel Pit Chemistry

Pit membranes of both *R. mucronata* and *A. marina* were found to have low but not zero lignin content (Fig. 2). Their nonlignified appearance in surface view under UV light (Fig. 2c), resembling earlier observations in *Aesculus hippocastanum* (Chaffey et al., 1997), might be explained by the presence of a pectinaceous coating as was observed in the sapwood of *Sapium sebiferum* (Pesacreta et al., 2005). The authors suggested that the coating is very thin, not identifiable via scanning electron microscopy, and thus is different from the previously mentioned dense coatings covering heartwood pits (e.g., Bonner & Thomas, 1972; Kininmonth, 1972; Thomas, 1976; Wheeler & Thomas, 1981). These pectic substances (Schmid & Machado, 1968; Morrow & Dute, 1999) or other nonphenolic, potassium permanganate reactive components such as lipids (Nemec, 1975; Hoffmann & Parameswaran, 1976; Robb et al., 1980) might penetrate pit membranes. This would explain the mostly darker appearance of pit membranes than cell walls after staining with potassium permanganate but their lower UV absorbance (compare Fig. 1a,l with Fig. 2a,b). In *A. marina*, the vestures showed the same reaction to both methods (arrows, Fig. 1l versus Fig. 2d), suggesting a mixed composition of lignin and polysaccharides. The lignin content (Fig. 3b), which is a possible reflection of their origin at the end of cell wall lignification (Parameswaran & Liese, 1977; Ohtani et al., 1984; Jansen et al., 1998), was in contrast to Ranjani and Krishnamurthy (1988) but in agreement with many other studies (Scurfield, 1970; Donaldson & Singh, 1990; Castro, 1991; Singh et al., 1999; Watanabe et al., 2006). The occasional observation of slightly stained pit membranes in *R. mucronata* (Fig. 1j) might be due to the activity of pectic enzymes with increasing cambial age. The

second peak in the absorbance spectra at around 660 nm, consistent with some softwood species analyzed by Bauch and Berndt (1973), could be a reflection of these encrustations (Fig. 3a).

In correspondence with this pectic coating, the deposits lining the pit borders in *R. mucronata* and less conspicuously also in *A. marina* could be polysaccharides (Fengel & Wolfsgruber, 1971; Castro, 1991). Although the ability to resist bacterial degradation of a similar layer in *Terminalia* suggested a high lignin (Singh et al., 2002) and tannin content (Donaldson & Singh, 1990). Aside from these linings, pit canals were bordered in a few trees by granular material (Fig. 1b,h,k) as found before in the pit canals of parenchyma cells (Wheeler & Thomas, 1981), fiber tracheids, and vessels (Yang, 1978). Lawn (1960) warned for granular precipitates as an artifact of potassium permanganate staining, but they could also be remnants of plasmalemma, tonoplast, or endoplasmatic reticulum (Scurfield, 1967). Of the same origin (Schmid, 1965; Scurfield, 1970) might be the membrane-like structures covering pit apertures (arrow heads, Fig. 1b,g,i–k), as found between two fiber tracheids of *Paulownia tomentosa* (Yang, 1986). Alternatively, it could be a layer of extractive material, protecting the tree against microbial attack and providing natural durability (Donaldson & Singh, 1990; Singh et al., 2002).

A similar function can be assigned to the deposits found to fill the pit canals in both studied species. The low absorbent deposits are most likely remnants of the highly absorbing ones after sample preparation given the more sporadic occurrence of the latter and the parallelism of their absorbance spectra (Fig. 3c). Next to protection against pathogens they could play a role in the water transport. Zimmermann et al. (1994, 2002) found mucopolysaccharides in xylem sap and attached to vessel walls in seedlings of *Rhizophora mangle*. Although they did not study intervessel pits and their findings are highly debated, these mucilages were proposed to play a major role in xylem sap flow by supporting the tension gradient in the water column. Whether the deposits found in *R. mucronata* and *A. marina* have a function in tree hydraulics or in contrast is an indication of nonactive vessels remains to be clarified.

CONCLUSION AND PERSPECTIVES

Several authors noticed the large variation in pit membrane structure and chemistry within species, trees, and even vessels (e.g., Bailey, 1957; Schmid & Machado, 1968; Bauch & Berndt, 1973; Sano & Nakada, 1998; Schmitz et al., 2007a). In this study, the hypothesis of seasonal changes causing differences in pit membrane thickness (Schmitz et al., 2007a) could only be partly accepted. The seasonal differences were weak and overshadowed by an enormous variability in electron density of the pit membranes. In addition, deposits were found filling pit canals and lining

pit borders, but they did not show a clear correlation with the variation of the pit membranes highlighting the importance of future research. Due to the time-demanding preparation of electron microscopic and UV microscopic studies, sample sets are usually small relative to the heterogeneous pit membrane structure and composition. This limitation urges for numerous studies, considering an extended number of trees and several positions within individual trees to gather enough data to get an overview of the common and variable characteristics. Next to an extensive number of replicates, a wide ecological spectrum should be considered, as well as the physiological status of the vessels. In this way, the potential role in tree hydraulics of the changes in pit membrane thickness and composition and of the deposits in pit canals can be elucidated. Besides the search for structure-function relationships, the resemblance in chemical composition of pit membranes and vestures as opposed to the cell wall added a new piece to the puzzle of their formation stimulating the expansion of the yet fragmentary knowledge.

Acknowledgments

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