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Comparative Anatomy of Intervessel Pits in Two Mangrove Species Growing Along a Natural Salinity Gradient in Gazi Bay, Kenya

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• *Background and Aims* According to the air-seeding hypothesis, embolism vulnerability in xylem elements is linked directly to bordered pit structure and functioning. To elucidate the adaptive potential of intervessel pits towards fluctuating environmental conditions, two mangrove species with a distinct ecological distribution growing along a natural salinity gradient were investigated.

• *Methods* Scanning and transmission electron microscopic observations were conducted to obtain qualitative and quantitative characteristics of alternate intervessel pits in *A. marina* and scalariform intervessel pits in *Rhizophora mucronata*. Wood samples from three to six trees were collected at seven and five sites for *A. marina* and *R. mucronata*, respectively, with considerable differences between sites in soil water salinity.

• *Key Results* Vestured pits without visible pores in the pit membrane were observed in *A. marina*, the mangrove species with the widest geographical distribution on global as well as local scale. Their thick pit membranes (on average 370 nm) and minute pit apertures may contribute to reduced vulnerability to cavitation of this highly salt-tolerant species. The smaller ecological distribution of *R. mucronata* was in accordance with wide pit apertures and a slightly higher pitfield fraction (67 % *vs.* 60 % in *A. marina*). Nonetheless, its outer pit apertures were observed to be funnel-shaped shielding non-porous pit membranes. No trends in intervessel pit size were observed with increasing soil water salinity of the site.

• *Conclusions* The contrasting ecological distribution of two mangrove species was reflected in the geometry and pit membrane characteristics of their intervessel pits. Within species, intervessel pit size seemed to be independent of spatial variations in environmental conditions and was only weakly correlated with vessel diameter. Further research on pit formation and function has to clarify the large variations in intervessel pit size within trees and even within single vessels.

Key words: *Rhizophora mucronata*, *Avicennia marina*, intervessel pits, salinity, Kenya, pit membrane, vestures, ecological wood anatomy, cavitation vulnerability, xylem, field-emission SEM, TEM.

INTRODUCTION

Pits in xylem conductive elements fulfil an important role in vascular water transport in trees (Tyree and Zimmermann, 2002; Holbrook and Zwieniecki, 2005). Numerous investigations have evaluated the contribution of the structure of bordered pits and pit membranes to the efficiency and safety of sap ascent (e.g. Choat *et al.*, 2003, 2006; Wheeler *et al.*, 2005). However, detailed studies dealing with intra- and interspecific variation in intervessel pits, with respect to pit membrane as well as pit geometry, remain scarce (Sano, 2005; Domec *et al.*, 2006).

Mangrove forest is an interesting habitat in which to study intervessel pit characteristics along an ecological gradient because mangrove trees are subject to a salt stress that may change considerably even within a small area (e.g. Middelburg *et al.*, 1996; Marchand *et al.*, 2004). Mangrove trees growing at contrasting salinity levels have been shown to differ in cavitation vulnerability, suggesting a parallel variation in xylem structure

(Melcher *et al.*, 2001; Ewers *et al.*, 2004), including both vessel and intervessel pit characteristics. A cavitation-resistant xylem structure is one of the strategies plants may use to safeguard their water transport (Tyree and Sperry, 1989; Tyree and Ewers, 1991; Reich *et al.*, 2003). In particular, small conduit diameters are well known to cause a decrease in transport efficiency but also to provide greater hydraulic safety (Salleo and Lo Gullo, 1986; Mauseth and Plemons-Rodriguez, 1998; Corcuera *et al.*, 2004; Mauseth and Stevenson, 2004). In the mangrove *Rhizophora mucronata* (Rhizophoraceae) from Kenya, vessels produced in the dry season are slightly smaller than those produced in the rainy season (Verheyden *et al.*, 2004, 2005; Schmitz *et al.*, 2006). Regardless of the vessel diameter, small pit membrane pore diameters (Tyree *et al.*, 1994; Jarbeau *et al.*, 1995; Choat *et al.*, 2003; Sperry and Hacke, 2004) and/or small surface area of the intervessel pits (Orians *et al.*, 2004; Choat *et al.*, 2005; Wheeler *et al.*, 2005; Ellmore *et al.*, 2006; Hacke *et al.*, 2006) have been reported to increase the cavitation resistance of the water transport

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system. Vessel diameters in *R. mucronata* from Kenya only vary slightly in response to differences in soil water salinity (Schmitz *et al.*, 2006). Therefore, it is expected that variation in intervessel pit characteristics ensures cavitation resistance in *R. mucronata*. It is assumed that the functional significance of intervessel pits in this species is reflected in the ecological adaptation of its pit geometry.

The present study examines the ecological plasticity of intervessel pits in the mangrove species *R. mucronata* and *Avicennia marina* (Avicenniaceae) in Kenya. *Avicennia marina* grows under the most extensive range of environmental conditions (Clough, 1984; Ball, 1988; Hegazy, 1998; Matthijs *et al.*, 1999; Lopez-Portillo *et al.*, 2005; Ye *et al.*, 2005), both in terms of latitude and local distribution within mangrove forests (Duke, 1991; Duke *et al.*, 1998; Dahdouh-Guebas *et al.*, 2004). In contrast, the local distribution of *R. mucronata* is restricted to the seaward side of the forest and to riverine areas under moderate salinity (Table 1). The aim of this paper is to survey both qualitative and quantitative characteristics of intervessel pits, with electron microscopy techniques, in order to (*a*) compare intervessel pit anatomy between both species, and (*b*) to perform a within-species study, examining intervessel pits from sites differing in inundation class and salinity conditions. Three hypotheses with respect to ecological trends in pit morphology were tested: (1) individual pit size and/or pitfield fraction (% pit membrane area/vessel wall area within a pitfield) is smaller in *A. marina* compared with *R. mucronata*; (2) the size and surface area of intervessel pits shows a negative trend within each species with increasing salinity; and (3) pit membrane size is not correlated with vessel diameter.

MATERIALS AND METHODS

Study sites and sample collection

The study sites are located in the mangrove forest of Gazi Bay (39°30′E, 4°25′S), situated approx. 50 km south of Mombasa, Kenya. Sampling was done in May 2005 at seven sites for *A. marina* and five sites for *R. mucronata*. Study sites were chosen to represent locations with different salinity and inundation frequency (Table 1). Soil water salinity data were available from about five (one to ten) sampling dates in the rainy season (May 2005 and June 2006) and for the *A. marina* sites also from the dry season (February 2006, except from site 5). At each site, the soil water was collected in triplicate at approx. 25 cm depth with a punctured plastic tube connected to a vacuum pump and measured with a hand-held refractometer (ATAGO, Tokyo, Japan). Depending on the topography and the tidal range, zones of different inundation classes can be defined. Inundation classes one, two, three and four correspond to an area being inundated by, respectively, 100–76 %, 75–51 %, 50–26 % and 25–5 % of the high tides (Tomlinson, 1994). Samples were excised at approx. 1·3 m height with a hollow puncher, 3 mm in diameter, for the *R. mucronata* trees and a hand saw for the *A. marina* trees. Three trees were sampled per site and per species, except from sites 4 and 5 of *R. mucronata*, where five and six trees were sampled, respectively. For both species, additional samples were collected from two trees at two sites. The samples were immediately stored in 30 % alcohol until analysis. The range of tree circumferences (measured at the base of the tree) and tree height (calculated trigonometrically) was 4–135 cm and 1–7 m for *A. marina* and 12–33 cm and 3–7 m for *R. mucronata*, respectively (Table 1).

TABLE 1. *Environmental and tree characteristics of the three* Avicennia marina *and* Rhizophora mucronata *trees sampled at each site in the mangrove forest of Gazi Bay (Kenya)*

Site	Salinity (‰)*			Inundation class[†]	Tree characters (range)	
	Min.	Max.	Range		Circumference (cm)[‡]	Height (m)
Avicennia marina						
1	21	38	17	1	32–135	6–7
2	40	68	28	2	40–49	3–4
3	40	80	40	3	26–30	5–6
4	38	82	44	3	4–41	1–4
5	5	68	63	4	33–101	4
6	10	80	70	4	33–43	5
7	10	96	86	4	37–82	4–5
Rhizophora mucronata						
1	30	33	3	3	19–22	7
2	0	11	11	4	18–25	4–5
3	21	38	17	1	18–27	6–7
4	22	42	20	2	12–20	4
5	18	49	31	3	12–33	3–6

*Soil water salinity at 25 cm depth, representing spatial and temporal variations.

[†]Inundation classes 1–4 correspond to an area being inundated by respectively 100–76 %, 75–51 %, 50–26 % and 25–5 % of the high tides (Tomlinson, 1994).

[‡]Measured at the base of the tree.

Sample preparation and image analysis

Scanning electron microscopic (SEM) observations were carried out on three trees per site with a Hitachi cold field emission SEM S-4700 (Hitachi High Technologies Corp., Tokyo, Japan). Samples were trimmed into cubes of approx. 3 mm^3 and split tangentially. The blocks were dehydrated for 5 min in an ethanol series (50 %, 70 %, 90 %, 100 %) and air-dried. They were mounted on stubs with electron conductive carbon cement (Neubauer chemikaliën, Münster, Germany) and sputter coated with platinum using an Emitech K550 sputter coater (Emitech Ltd, Ashford, UK). The remaining eight trees, two from site 1 and site 3 for *A. marina*, and two from site 3 and site 5 for *R. mucronata* were cut into blocks of about 2 mm^3 for transmission electron microscopic (TEM) observations. The samples were dehydrated through a graded ethanol series (30 %, 50 %, 70 %, 90 %, 100 %). The ethanol was gradually replaced with LR White resin (London Resin Co., Reading, UK) over several days. The resin was polymerized at 60 °C and 1000 mmHg for 18–24 h. Embedded samples were trimmed and sectioned on an ultramicrotome (Ultracut, Reichert-Jung, Austria). Sections, 1 μm and 2 μm thick, were cut with a glass knife, heat-fixed to glass slides and stained with 0·5 % toluidine blue O in 0·1 M phosphate buffer. Resin-embedded material was prepared for TEM-observations by cutting ultra-thin sections between 60 nm and 90 nm using a diamond knife. The sections were attached to Formvar grids and stained with uranyl acetate and lead citrate using a LKB 2168 ultrostainer (LKB-Produkter AB, Bromma, Sweden). Observations were carried out using a JEOL JEM-1210 TEM (JEOL, Tokyo, Japan) at 80-kV accelerating voltage, and digital images were taken using a MegaView III camera (Soft Imaging System, Münster, Germany).

Anatomical measurements

Horizontal and vertical pit membrane diameters (Fig. 1D) of approx. 40 pits per vessel were measured on SEM images of *A. marina*. For images showing more than 40 pits, a labelled grid was used to randomly select 40 pits. Similarly, about 20 pits per vessel were examined for *R. mucronata* (Fig. 1G, H). Measurements were carried out on three to seven vessels per tree, with a total number of three trees per study site in order to examine a total number of 600 and 300 pits per site for *A. marina* and *R. mucronata*, respectively. As for *A. marina*, pit density (number of pits per vessel wall area) was measured on the same images, in quadrats comprising approx. 40 pits per vessel. The shortest and longest axis of the pit apertures were measured in surface view on SEM images, at the broadest point including the vestures. For both outer and inner pit apertures, three to seven random trees were measured, including around 300 pits for *A. marina* and 150 pits for *R. mucronata* (Table 2). Measurements were carried out manually with the image analysis software AnalySIS 3·2 (Soft Imaging System GmbH, Münster, Germany). Pit membrane area was calculated via the formula of the area of an ellipse and, together with the pit density, this allowed the percentage of pit membrane area per unit wall area in a pit field (hereafter referred to as pitfield fraction) to be calculated. With respect to *R. mucronata*, pit membrane areas were calculated via the formula of a rectangle and the sum was compared with the total wall area. SEM images of *A. marina* showing the full width of a vessel were used to determine the vessel diameter in comparison with the average pit membrane diameter. For *R. mucronata*, the horizontal pit membrane diameter was considered to be similar to the vessel diameter. Consequently, vertical pit diameters were used to evaluate the intraspecific variation of *R. mucronata* instead of individual pit membrane areas as used for *A. marina*. Intervessel wall thickness, pit membrane thickness and pit chamber depth (see Fig. 2) were measured on TEM images from four *A. marina* trees and three *R. mucronata* trees (from one tree no measurements could be made).

Vessel grouping was measured in three *A. marina* trees at an additional site and five *R. mucronata* trees at site 1 (Table 1). The percentage of solitary vessels and the vessel grouping index were calculated. At both sites, average soil water salinity (32 ‰) and inundation class (class 3) were similar.

Statistical analysis

For statistical analyses raw data were used. One-way ANOVA analyses were performed to test the effect of different trees of the same site on individual pit size, pit membrane area in the case of *A. marina* and vertical pit membrane diameter in the case of *R. mucronata*. Since horizontal pit diameters of *R. mucronata* trees are related to the vessel diameters, pit areas are inappropriate for intraspecific comparison. Sites were ordered according to the salinity range of the site instead of the average salinity that is not experienced by the tree. Pearson and Spearman R correlation coefficients were calculated between the diameters of intervessel pits and xylem vessels of *A. marina* and *R. mucronata* respectively. *t*-Tests for independent variables, with unpooled variances, were carried out to compare pit characteristics between the study species. When the assumption of normality was not met a Kolmogorov–Smirnov two-sample test was executed instead.

RESULTS

SEM and TEM observations revealed the presence of intervessel pits with vestures in *A. marina* (Figs 1C–F and 2A–C). The vestures were not extensively developed but they were consistently present in all intervessel pits. They adopted a lip-like, unbranched form although irregular forms were seen sporadically. Occasionally, vestures were observed at the lumen side of the vessel, sometimes extended as horizontal wall thickenings near inner pit apertures (Fig. 1E). Aspirated pits were seen in both SEM and TEM images but, with the pit chambers in both species relatively shallow (Table 2), it was difficult to determine whether pit membranes were truly aspirated or not (Figs 1F and 2C). *R. mucronata* has non-vestured intervessel pits (Figs 1G, H and 2D–F).
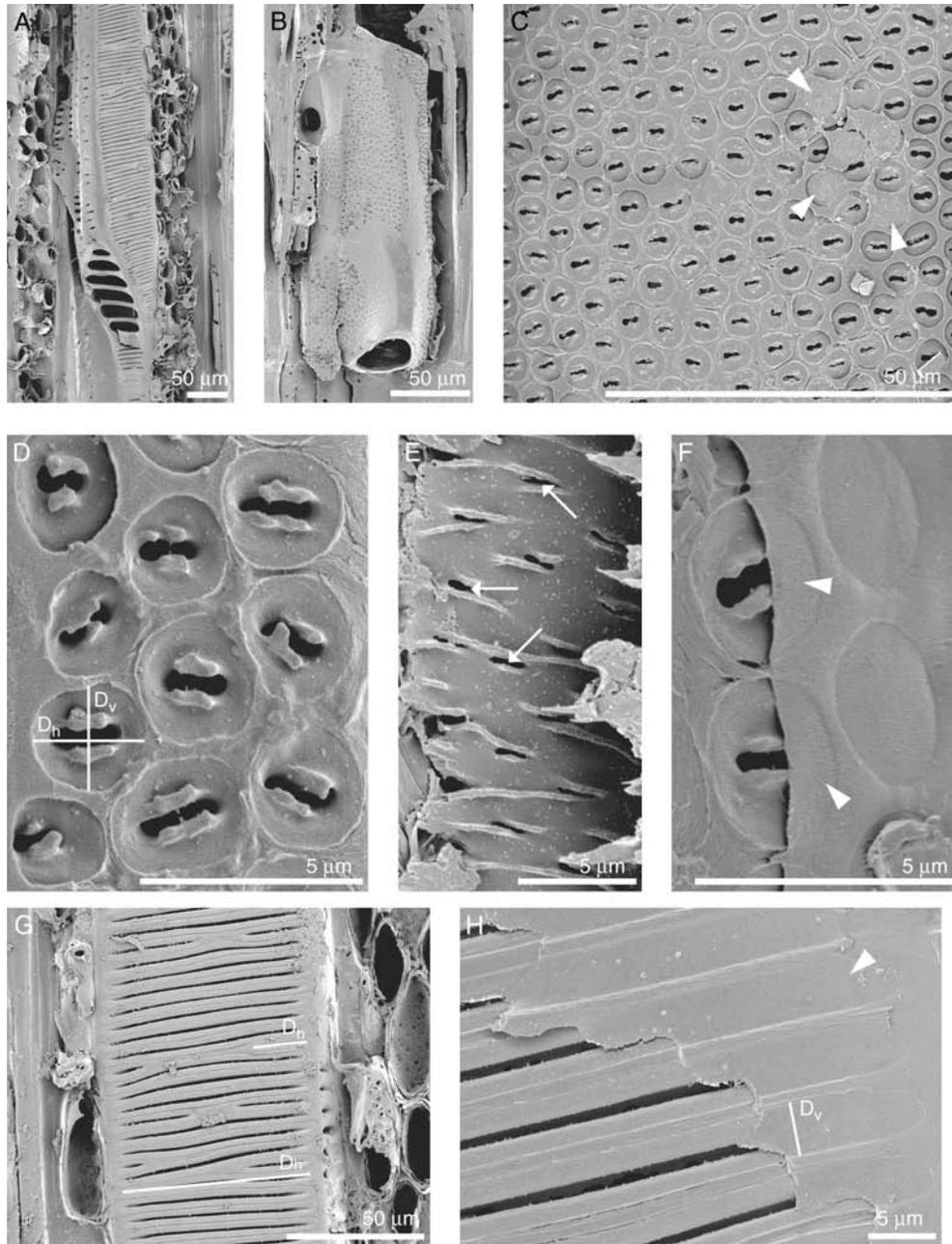
FIG. 1. Scanning electron micrographs of intervessel pits of *Avicennia marina* (B–F) and *Rhizophora mucronata* (A, G, H) in surface view. (A) Vessel element of *R. mucronata* showing scalariform intervessel pitting and a scalariform perforation plate with five bars. (B) Vessel element of *A. marina* showing alternate intervessel pitting and a simple perforation plate. (C) Detail of intervessel pits of *A. marina* with detached pit membranes showing outer pit apertures, surrounded by lip-like vestures. Arrowheads indicate pits with intact pit membranes. (D) Lip-like vestures protruding from the outer aperture into the pit chamber (D_v, vertical pit diameter; D_h, horizontal pit diameter). (E) Vestures at the lumen side of the vessel, more or less extended to horizontal wall thickenings. Arrows indicate inner apertures. (F) Vestures in their hypothesized role as supporters of the pit membrane (arrowheads), here only covering half of the pit chamber. (G) Part of a vessel element of *R. mucronata* showing scalariform pits (D_h, horizontal pit diameter). (H) Detail of scalariform pits with outer pit apertures partly covered with the pit membranes (arrowhead) (D_v, vertical pit diameter).

The present observation of 105 vessels in *A. marina* and 75 vessels in *Rhizophora mucronata*, which include around 4200 and 1500 intervessel pits, respectively, showed that no pores were present in the pit membranes (Figs 1E, H and 3A–C). Occasionally, small pores were observed in the pit membranes of *A. marina* but these were interpreted as artefacts due to sample preparation (Fig. 3D).

Quantitative analysis of the pit geometry of both species, as based on SEM and TEM observations, demonstrated that the pitfield fraction, the vertical and horizontal pit diameter, the individual pit membrane area, the pit apertures, the pit chamber depth and the intervessel wall thickness are smaller in *A. marina* compared with *R. mucronata* (Table 2). The vessel diameter was only slightly correlated

TABLE 2. *Comparison of quantitative intervessel pit characteristics of* Avicennia marina *and* Rhizophora mucronata

Intervessel pit characters	*A. marina*	*n*	*R. mucronata*	*n*	*P*-value[†]
Pitfield fraction (%)[‡]	60 ± 8 (39–91)	105	67 ± 4 (54–75)	79	<0·0001*
Vertical pit diameter (μm)	3·0 ± 0·4 (1·5–5·2)	4359	3·4 ± 0·5 (1·8–5·6)	1919	<0·0001*
Horizontal pit diameter (μm)	3·0 ± 0·5 (1·4–6·8)	4359	45 ± 17 (4–85)	1561	<0·001
Pit membrane area (μm^2)	7 ± 2 (2–15)	4359	136 ± 87 (0–357)	1827	<0·001
Pit membrane thickness (μm)	0·37 ± 0·08 (0·23–0·61)	129	0·3 ± 0·1 (0·1–0·5)	83	<0·001
Min. chamber depth (μm)[§]	0·09 ± 0·05 (0–0·19)	55	0·15 ± 0·05 (0·07–0·30)	74	<0·0001*
Max. chamber depth (μm)[§]	0·28 ± 0·08 (0·16–0·57)	120	0·7 ± 0·1 (0·4–1)	53	<0·001
Intervessel wall thickness (μm)	7 ± 2 (4–11)	108	9 ± 1 (7–11)	70	<0·001
Shortest axis of inner aperture (μm)	0·6 ± 0·1 (0·3–0·9)	307	1·4 ± 0·3 (0·7–2·4)	135	<0·001*
Longest axis of inner aperture (μm)	1·6 ± 0·3 (0·9–2·9)	307	36 ± 9 (13–56)	135	<0·001*
Shortest axis of outer aperture (μm)	0·5 ± 0·2 (0·2–1·7)	301	0·9 ± 0·2 (0·3–1·7)	149	<0·001
Longest axis of outer aperture (μm)	1·9 ± 0·4 (0·5–3·0)	301	46 ± 19 (7–68)	149	<0·001

Values are means ± s.d. with the minimum and maximum values in parenthesis.
[†]Significance value of a *t*-test (*) or Kolmogorov–Smirnov test from independent samples depending on the normality of the data.
[‡]Percentage of the pit membrane area per vessel wall area within a pitfield.
[§]Measured as illustrated in Fig. 2A, D.

to the horizontal pit diameter in *A. marina* (Pearson: $r^2 = 0.29$, $P < 0.01$, $n = 29$) and to the vertical pit diameter in both *A. marina* (Pearson: $r^2 = 0.13$, $P = 0.06$, $n = 29$) and *R. mucronata* (Spearman R: $r^2 = 0.06$, $P < 0.0001$, $n = 996$). The thickness of *A. marina*'s pit membranes showed a unimodal distribution and exceeded those of *R. mucronata*. A clear bimodal pattern was observed in the latter species (Figs 2E, F and 4). Furthermore, the pit membrane was observed to be more electron dense in *R. mucronata* as opposed to *A. marina* (Fig. 2A, D). The pit chamber of both species studied was remarkably shallow, with a pit channel ending in a constriction in *R. mucronata* (Fig. 2E, F) or with vestures in *A. marina*. Consequently, one could distinguish a minimum and a maximum pit chamber depth (Fig. 2A, D and Table 2).

The distribution of the individual pit membrane area and vertical pit diameter in *A. marina* and *R. mucronata*, respectively, showed a wide range of variation (Fig. 5). Mean individual pit membrane area differed significantly between *A. marina* trees within a single site (Table 3 and Fig. 5A). Within sites, mean vertical pit diameters also differed significantly between *R. mucronata* trees (Table 3 and Fig. 5B).

DISCUSSION

Vestured pits in Avicennia marina

As far as is known, this is the first time vestured pits have been observed in *A. marina*. Due to the rudimentary nature of the vestures, it is not surprising that probably most previous studies overlooked the presence of this feature (Meylan and Butterfield, 1973; Matthew and Shah, 1983; Krishnamurthy and Sigamani, 1987; Sun and Suzuki, 2000). The vestures appeared as lip-like projections associated with the outer aperture and pointed into the pit chamber (Figs 1D and 2A). At the lumen side of the vessel the vestures seemed to narrow down the inner pit apertures and to extend the pit canal (Figs 1E and 2B). Moll and Janssonius (1920) reported that the numerous

bordered pits in vessel walls of *A. alba* were needle-like and that the inner pit apertures showed a needle-like form. As *Avicennia* is a member of the Lamiales (Schwarzbach and McDade, 2002), the discovery of vestures in *A. marina* is especially noteworthy since this character is only known in some genera of the Oleaceae (Jansen *et al.*, 2001). Mathew and Shah (1983) reported vestured pits in few genera of Verbenaceae, but not in *A. marina*. Their observations are, however, not convincing and most likely represent pseudo-vestures. SEM images of *A. germinans* from the Tervuren wood collection (RMCA Tervuren) and light microscopic observations of sections from the Jodrell slide collection (RBG Kew) suggested that vestured pits are present in other species of *Avicennia*. However, vestures are difficult to detect using a light microscope due to their small size and minute pit apertures. Therefore, SEM observations are required to confirm their occurrence.

The observation of vestures in *A. marina* is in accordance with the overall confinement of vestured pits to xeric or warm environments (Jansen *et al.*, 2003, 2004*a*). This may be related to the functional significance of vestures, as first formulated by Zweypfenning (1978) and later supported by ecological studies (Jansen *et al.*, 2003, 2004*a*). Inherently large pit membrane pores and especially an increased porosity or even rupture of the pit membrane have been suggested to be the cause of air-seeding (Hacke and Sperry, 2001; Choat *et al.*, 2003; Sperry and Hacke, 2004; Wheeler *et al.*, 2005). As an increased porosity may result from excessive stretching upon pit-aspiration, it is suggested that adaptations preventing the pit membrane from deflecting are extremely important in view of cavitation resistance. Zweypfenning's hypothesis states that vestures could provide such advantage by offering mechanical support to stretched pit membranes (Zweypfenning, 1978). The funnel shaped pit channel could offer a similar advantage to the pit membranes of *R. mucronata* (Fig. 2E, F and Table 2). However, the shallow pit chamber and especially the thickness of the pit membrane itself could also play a substantial role in the prevention of excessive pit membrane
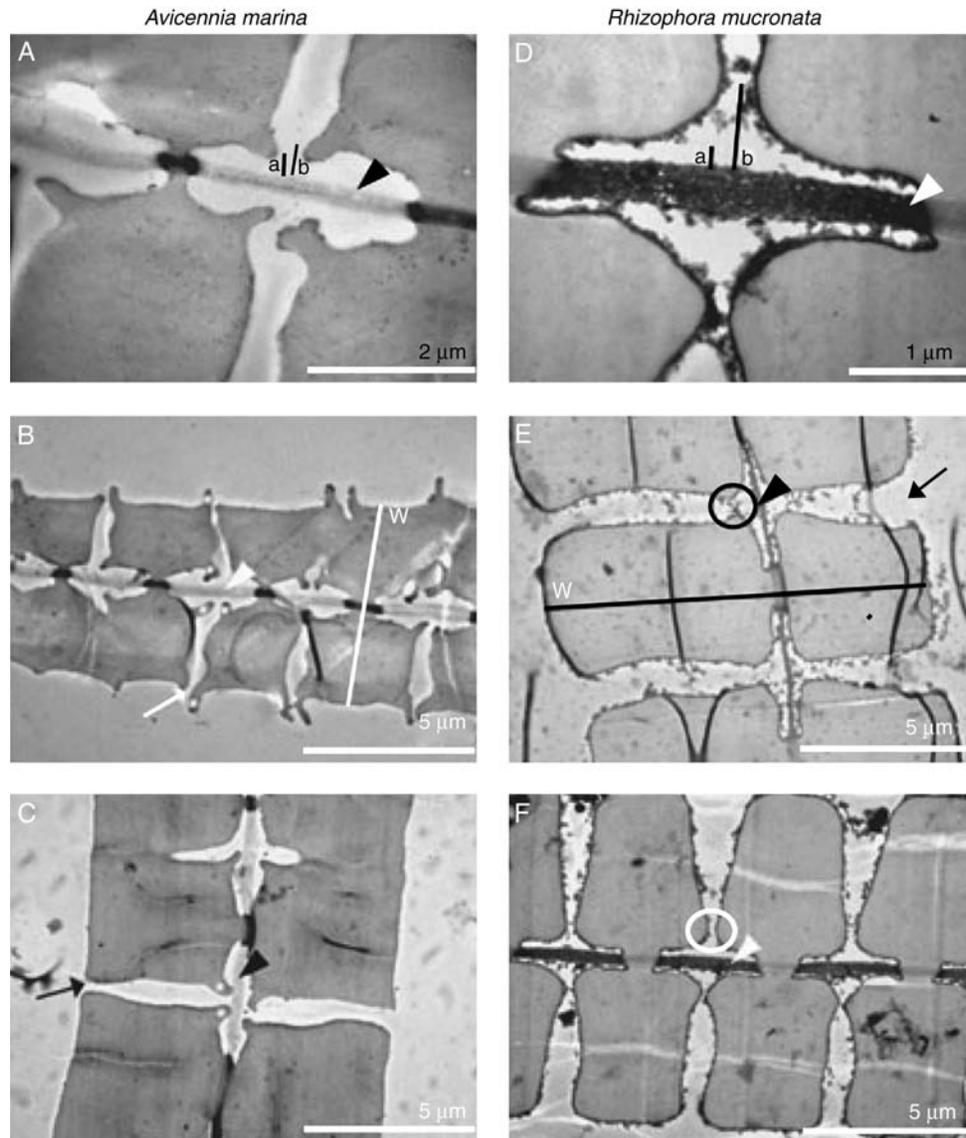
FIG. 2. Transmission electron micrographs of longitudinal sections of intervessel pits of *Avicennia marina* (A–C) and *Rhizophora mucronata* (D–F). (A) Intervessel pit of *A. marina* showing rudimentary vestures and pit membrane of low electron density. (B) Vestures extending from the outer apertures into the pit chamber and from the inner apertures into the vessel lumen. Note the transparent pit membranes. (C) Vestures in their hypothesized role as supporters of the pit membrane. (D) Intervessel pit of *R. mucronata* showing pit membrane and vessel wall lining of high electron density. (E, F) Overview of intervessel pits of *R. mucronata* with electron dense pit membranes, a dark lining of the entire secondary wall, shallow pit chambers and a constriction of the pit channels near the outer apertures. Note the difference in pit membrane thickness between (E) and (F). Arrowheads indicate pit membranes; arrows indicate inner apertures; circles indicate pit canal constrictions. *, vestures; a, minimal pit chamber depth; b, maximal pit chamber depth. w, intervessel wall thickness.

stretching in both *A. marina* and *R. mucronata* (Fig. 2). Pit membranes are composed of a number of microfibrillar layers (Schmid and Machado, 1968; Sperry and Hacke, 2004; Sano, 2005) perforated by most likely tortuous rather than straight intervessel pathways (Choat *et al.*, 2004). Since SEM images only show surface structures, the openings occasionally observed in pit membranes of *A. marina* are most likely artefacts resulting from sample preparation (Fig. 3D). Splitting of the wood samples might have removed one of the microfibrillar sheets of the pit membrane, rendering the membranes more sensitive to damage from preparative handlings such as dehydration. The artefactual nature of the pit membrane pores is supported by their irregular and inconsistent distribution: they are completely absent in many large pit fields (Fig. 3C) and restricted to particular areas (Fig. 3D). Furthermore, the finding of thick, non-porous pit membranes in the two species studied corresponds with previous observations. The thick pit membranes of *Fraxinus* are less likely to show pores than the thinner pit membranes of *Betula*, *Salix* and *Ulmus* (Sano, 2004, 2005; Choat *et al.*, 2006). The main reason why pores in the pit membranes could
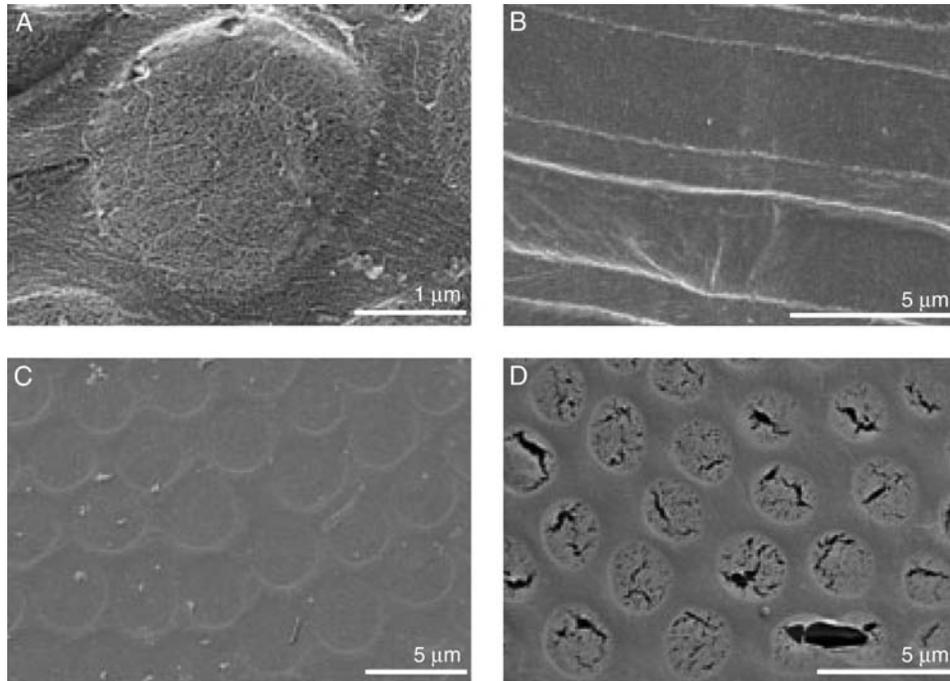
FIG. 3. Scanning electron micrographs of intervessel pits of *Avicennia marina* (A, C, D) and *Rhizophora mucronata* (B) in surface view. (A, B) Detail of non-porous pit membrane of *A. marina* (A) and *R. mucronata* (B). (C) Porous pit membranes are completely absent in large pit fields of *A. marina*. (D) Artefactual pores occur in restricted pit field areas damaged by sample preparation.

not be seen with TEM is most likely due to the thickness of TEM sections (60–90 nm), which is much larger than the majority of the pit membrane pores.

Intervessel pit morphology of two mangrove species

When comparing overall pit architecture of *R. mucronata* and *A. marina* the first conspicuous difference is their pit type (Fig. 1A, B). The minute alternate intervessel pits of
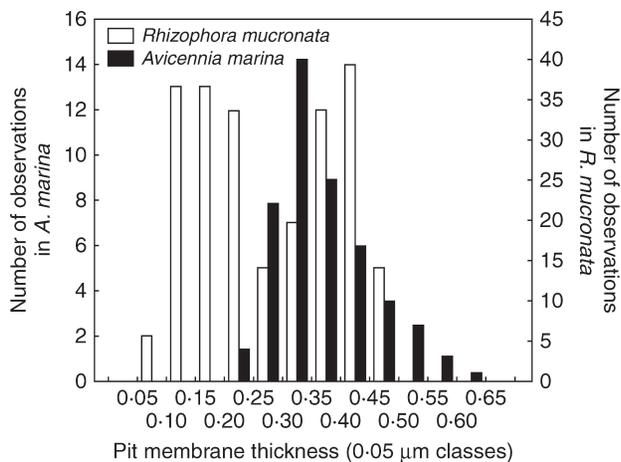

FIG. 4. Distribution of intervessel pit membrane thickness in *Rhizophora mucronata* and *Avicennia marina*. Data are from 83 pits from three trees of *R. mucronata* and from 129 pits from four trees of *A. marina*.

A. marina, as opposed to *R. mucronata*'s scalariform pitting, resulted in a slightly smaller pitfield fraction in *A. marina* (Table 2). A small pitfield fraction implies a lower cavitation risk (Hargrave *et al.*, 1994; Choat *et al.*, 2003, 2004), since the occurrence and size of inherently large pit membrane pores is thought to increase with the total pit membrane area per vessel (Wheeler *et al.*, 2005). However, because of the much higher percentage of solitary vessels in *R. mucronata* than in *A. marina* (79 ± 6 *vs.* 33 ± 24, $t = -28.4$, d.f. $= 127$, $P < 0.0001$) and the lower vessel grouping index (1.25 ± 0.08 *vs.* 2.0 ± 0.6, $t = 21.9$, d.f. $= 359$, $P < 0.0001$), *R. mucronata*'s overlapping pitfield area between neighbouring vessels may be smaller. It is thus possible that the intervessel pit membrane area of the entire vessel network is much higher in *A. marina*.

Structural differences in the pit micromorphology between both species could be interpreted as alternative solutions to cope with the saline mangrove environment. Inner and outer pit apertures were comparatively smaller in *A. marina* than in *R. mucronata* (Table 2). The minute pit apertures are related to the bordering vestures and result in an extended compartmentalization of the water transport system, minimizing conductivity loss from expanding embolisms (Ellmore *et al.*, 2006). In addition, small intervessel pits reduce the actual sealing area of the pit membrane and thus increase the air seeding pressure (Wheeler *et al.*, 2005). Pit membranes in *A. marina* were generally thicker, increasing on one side the hydraulic resistance (Choat *et al.*, 2006), but on the other side decreasing the vulnerability to cycles of cavitation and refilling (Hacke *et al.*, 2001). These are likely to occur in
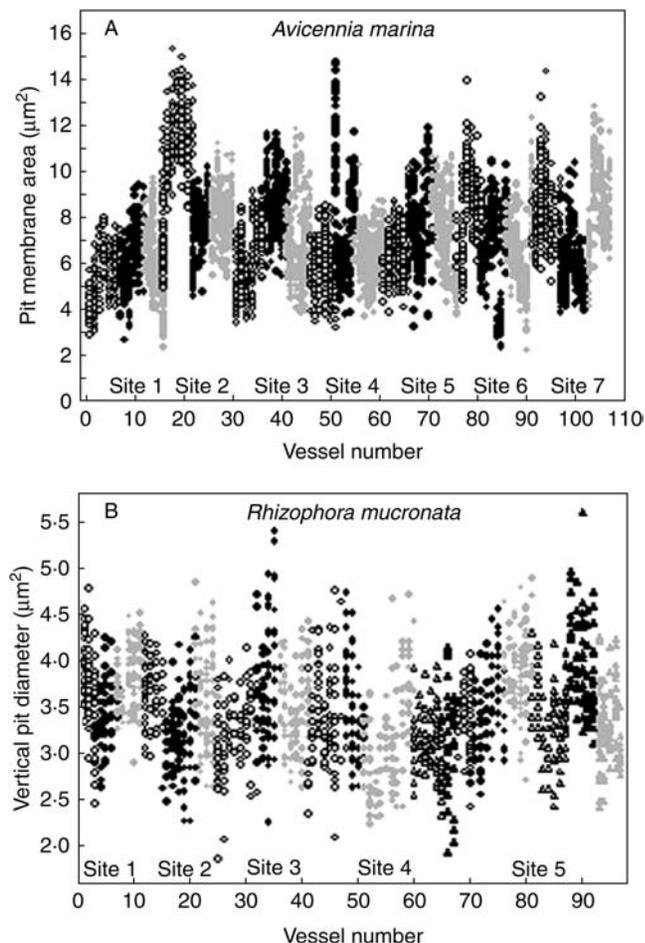
FIG. 5. Distribution of intervessel pit size within vessels and trees along a natural salinity gradient. (A) Pit membrane area of the alternate pits of *Avicennia marina* in three trees for all seven sites with increasing salinity range (see Table 1). (B) Vertical pit diameter of *Rhizophora mucronata* in, respectively, three (sites 1–3), five (site 4) and six (site 5) trees for five sites with increasing salinity range (see Table 1). The horizontal diameter of the majority of the scalariform pits corresponds to the vessel diameter, making comparison of the pit membrane area inappropriate. The different trees on each site are indicated by distinct point markers. On average 100 and 200 pits were measured per *A. marina* and *R. mucronata* tree, respectively, with three to seven vessels studied per tree.

TABLE 3. *Results of one-way ANOVAs, testing for differences in pit membrane area between* Avicennia marina *trees and vertical pit diameter between* Rhizophora mucronata *trees within the studied sites (see also Table 1)*

Factor	Site	*A. marina*		*R. mucronata*	
		F-value	*n*	*F*-value	*n*
Tree	1	95·83*	3	24·88*	3
	2	122·41*	3	51·77*	3
	3	75·82*	3	36·35*	3
	4	39·14*	3	37·03*	5
	5	195·41*	3	62·08*	6
	6	181·33*	3		
	7	42·60*	3		

* $P < 0.0001$.

A. marina since vestured pits may help in embolism repair (Jansen *et al.*, 2003), as do the abundant paratracheal parenchyma and the included phloem tissue (Holbrook and Zwieniecki, 1999; Tyree *et al.*, 1999; Salleo *et al.*, 2004; Stiller *et al.*, 2005; Salleo, 2006). The thickness of *R. mucronata's* pit membranes showed a bimodal distribution (Figs 2E and F and 4), which could be caused by, for instance, wound-induced depositions (Schmitt *et al.*, 1997; Frankenstein *et al.*, 2006) or by depositions due to seasonal variations (Wheeler, 1981; Sano, 2004). As far as is known, however, the wood samples collected were not from stems subject to any wounding. Seasonal changes are more likely. The average annual growth rate of *R. mucronata* in Gazi (Kenya) is 1.17 ± 0.73 mm year^{-1} (Verheyden *et al.*, 2004). Given that the differences in the pit membrane thickness were found in radial sections of 2 mm^2, the sections possibly contained both wood formed during the dry season and the rainy season. Alternatively, the pit membrane thickness may well be related to the thickness of the secondary cell wall. The present observations showed that vessels with a narrow diameter have thinner cell walls than large vessels (results not shown), but further research is needed to test if this is also associated with a difference in pit membrane thickness. The electron density of pit membranes in *R. mucronata* contrasted strikingly with the more transparent pit membranes of *A. marina* (Fig. 2A, D). This could be due to a different chemical composition of the pit membrane in both species. An electron-dense layer lining *R. mucronata's* vessel walls (Fig. 2E, F) suggested that the wood samples of this species are impregnated with substances characteristic of *Rhizophora*. Fresh material would be desired to see if the electron-dense layer on the vessel walls is also present after fixation. Further TEM observations would also be interesting to examine the chemical composition of the pit membranes (Bauch and Berndt, 1973; Coleman *et al.*, 2004).

As for many tropical trees, there is a lack of data on quantitative pit characters. In comparison to the few data on pit geometry in temperate trees, the intervessel pit anatomy of the two mangrove species studied suggests an increased hydraulic safety. The average pit chamber depth of the deciduous tree *Sophora japonica* is 0.84 μm and 0.2 μm, with and without inclusion of the vestures, respectively. *Fraxinus americana* has pits with an average pit chamber of 0.61 μm deep (Choat *et al.*, 2004). Compared with the mangrove species studied, these pit chambers are remarkably deep (Table 2). Schmid and Machado (1968) reported intervessel pit membranes $0.25–0.35$ μm thick in Leguminosae, with the membranes of air-dried samples as thin as $100–200$ nm. Pit membranes in vessels from temperate trees are generally <200 nm thick (S. Jansen, unpubl. res.). The relatively thick pit membranes of *R. mucronata* and *A. marina* (Table 2) suggest a considerable impact of pit membrane thickness on the hydraulic resistance of a tree (Choat *et al.*, 2006). When high safety is not of prime importance the formation of thick pit membranes would be unfavourable. Furthermore, the shortest axis of the outer pit apertures was remarkably smaller in the mangrove species studied (Table 2) than the

0·8–1·89 µm-sized apertures in *Ulmus laciniata* (Jansen *et al.*, 2004*b*). The longest axis of the outer pit apertures, 1·64–3·29 µm in *Ulmus laciniata* (Jansen *et al.*, 2004*b*), was wider in *R. mucronata* but generally shorter in *A. marina* (Table 2). Also, the pit aperture area of 2·3–3·9 µm^2 as reported for *Acer* and *Betula* species (Orians *et al.*, 2004) is larger than the 0·7 µm^2 as calculated for *A. marina* (Table 2). The shallow pit chambers, thick pit membranes and small pit apertures could outweigh the negative effect on the hydraulic safety of both mangrove species' comparatively large pitfield fraction (Table 2). The pitfield fraction of several temperate tree species (including evergreens) ranges from 9 % to 67 % (Orians *et al.*, 2004; Choat *et al.*, 2006; Ellmore *et al.*, 2006; Hacke *et al.*, 2006). These findings support the hypothesis that intervessel pit distribution is a compromise between hydraulic safety and efficiency (Sperry, 2003).

No intraspecific trends in intervessel pit size

The absence of an intraspecific trend in intervessel pit size with varying salinity conditions (Fig. 5) suggests that within species the ecological adaptability of the hydraulic architecture is restricted to vessel dimensions and vessel frequency. However, three trees per site are possibly insufficient to uncover a potential relationship between intervessel pit size and salinity because of the considerable variation within sites and trees. The potential difference in actual pit membrane pore sizes between and within the mangrove species studied could not be determined in this study. The natural porosity of the pit membranes remains to be verified since this character is closely related to cavitation vulnerability based on the air-seeding theory. Using fresh material, particle perfusion experiments should be performed in combination with air-seeding measurements to determine the size of the rare largest pores, which are responsible for cavitation (Choat *et al.*, 2003, 2004; Wheeler *et al.*, 2005).

Altogether, individual pit size and pit field fraction were smaller in *A. marina* than in *R. mucronata* and the diameter of the intervessel pits was only slightly correlated with the diameter of the corresponding vessels, as postulated in the Introduction. The hypothesis of a decreasing trend in pit size with varying salinity was rejected for both species. It is proposed that intervessel pit size and geometry are mainly determined by genetic factors with the absence of a phenotypic plasticity related to the widely fluctuating environmental conditions of the mangrove habitat. The minor decrease in vessel diameter of *R. mucronata* with increasing substrate salinity (Schmitz *et al.*, 2006) is thus not compensated for by a decrease in pit size offering a higher cavitation resistance. Therefore, the functional significance of the fluctuating vessel density should be addressed in future studies. Furthermore, there is need for additional comparative research, in combination with experimental tests, both between species and localities and within individual trees, to elucidate the adaptive and functional significance of the intervessel pits and their role in sap ascent.

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