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Successive cambia development in *Avicennia marina* (Forssk.) Vierh. is not climatically driven in the seasonal climate at Gazi Bay, Kenya

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Abstract

This study is intended to provide early access to recent findings on the formation of the successive cambia of *Avicennia marina* (Forssk.) Vierh. in Kenya. The non-annual character of the growth layers was demonstrated by using three trees from a cambial marking experiment and three trees from a plantation of known age. The respective number of growth layers produced during one year was on average a half and three. Considering 28 stem disks of trees at three study sites, differing in local site conditions, growth layer development was shown to be strongly correlated with stem diameter ($R^2 = 0.84$, $p < 0.0001$, $n = 31$). However, an additional influence of the site conditions was also demonstrated (homogeneity-of-slopes model test: $F = 54.72$, $p < 0.0001$, $n = 28$). With increasing salinity and/or inundation class the width of the growth layers decreased. The significance of these variations in growth layer width offer interesting perspectives. The larger proportion of xylem in comparison with phloem in trees with wide as opposed to narrow growth layers may provide extra mechanical strength. On the other hand, the larger fraction of living tissue (phloem and parenchyma) in trees with thin growth layers may be beneficial for the water balance of the tree. Next to the non-annual nature of the growth layers and their networking pattern, more than one cambium was found to be simultaneously active. We conclude that classical dendrochronological methods (ring width measurements) should not be applied to *A. marina* (from Kenya).

Introduction

In contrast to trees of temperate regions, tropical trees are usually believed to show no growth rings. Regardless of a seasonal climate, growth rings are not always anatomically distinct in the tropics (Détienne, 1989; Jacoby, 1989; Sass et al., 1995; Worbes, 1995; Verheyden et al., 2004b) and if so, they do not necessarily imply an

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annual growth rhythm (Worbes, 1989; Stahle, 1999). However, these difficulties have not hindered applying dendrochronology to several tropical species (e.g., Gourlay, 1995; Pumijumnong et al., 1995; Eshete and Stahl, 1999; Worbes, 1999; Segala Alves and Angyalossy-Alfonso, 2000; Callado et al., 2001; Enquist and Leffler, 2001; Brienen and Zuidema, 2005; Heinrich and Banks, 2005; López et al., 2005) including the mangrove species *Rhizophora mucronata* (Verheyden et al., 2004a, b, 2005) and *Rhizophora mangle* (Menezes et al., 2003) and a species of the genus *Diospyros* (Duke et al., 1981). Moreover, many other tropical tree species are still to be investigated (Worbes, 2002). The current study examines the growth layers of the most widely distributed mangrove, *Avicennia marina* (Forsk.) Vierh. (Tomlinson, 1994; Duke et al., 1998).

The mode of secondary growth in *Avicennia* is rather uncommon and occurs via successive cambia (Studholme and Philipson, 1966; Zamski, 1979; Carlquist, 2001). They give rise to an alternating pattern of light coloured phloem and darker xylem, resulting in clearly visible growth layers (Fig. 1a). Nevertheless, no attempts have been made so far to unveil environmental factors affecting the development of a new cambium and only few investigations have dealt with the periodicity of growth layer formation (Chapman, 1944, 1947; Gill, 1971). Furthermore, these studies presented contradictory and inconclusive results. While Chapman (1944) assumed that the rings of *A. nitida* Jacq. were semi-annual, he later suggested that they could be annual (Chapman, 1947). This new assumption, however, was based on a single tree. Gill (1971) observed two to six 'rings' in 1-year-old shoots, and showed a relationship between 'ring' numbers and stem diameter,

but not age. He concluded that the development of successive cambia in *A. germinans* (L.) L. is probably endogenously controlled and is not environmentally influenced. However, firm conclusions can not be made since the study was partially based on branches that have been shown to behave differently in several species (Fegel, 1941; Zimmermann and Potter, 1982; Cherubini et al., 2003).

More than two decades after the study of Gill (1971), this paper is aimed at giving early access to our recent findings on secondary growth in *A. marina* of Gazi Bay (Kenya). The potential of the growth layers for age determination was evaluated by counting growth layers on stem disks of trees from a cambial marking experiment and of plantation trees of known age. In addition, the influence of local site conditions on growth layer formation was examined by using samples of the outermost wood as well as stem disks, from sites differing in salinity and inundation class.

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), which covers about 710 ha (UNEP, 2001) and is situated approximately 50 km south of Mombasa, Kenya. During the wet season, the rivers Mkurumuji and Kidogoweni provide an important freshwater source for the mangroves (Kitheka, 1997). Sampling was performed in nine sites, differing in salinity and inundation class (Fig. 2a, Table 1). Soil water salinity at approximately 20 cm depth ranged
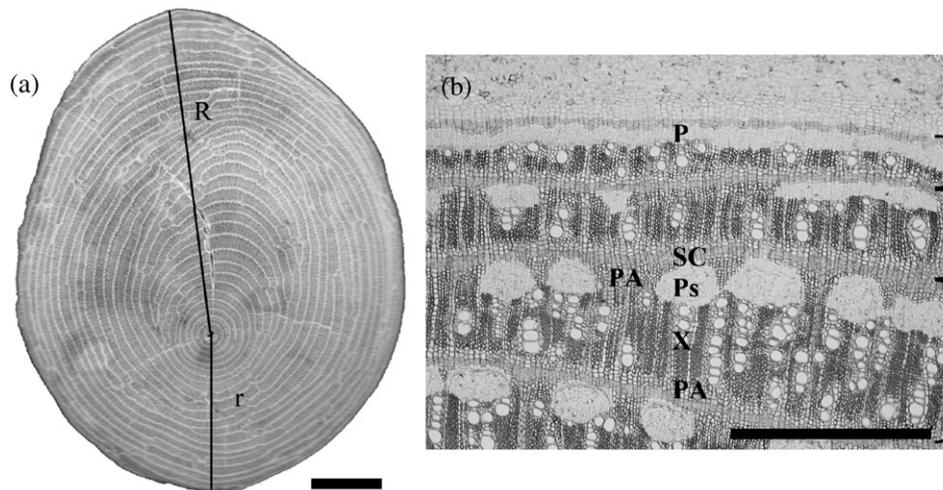

Fig. 1. (a) Asymmetric stem disk of an *Avicennia marina* tree showing the pattern of darker xylem bands and lighter coloured phloem bands formed by successive cambia. Scale bar = 1 cm. (b) Transverse section of the outer part of a wood sample showing the growth layers and their constituent tissues. Light microscope, scale bar = 1 mm. P phloem band, PA parenchyma, Ps phloem strand, *r* minimum radius, *R* maximum radius, SC sclereids, X xylem band. Brackets designate the growth layers.
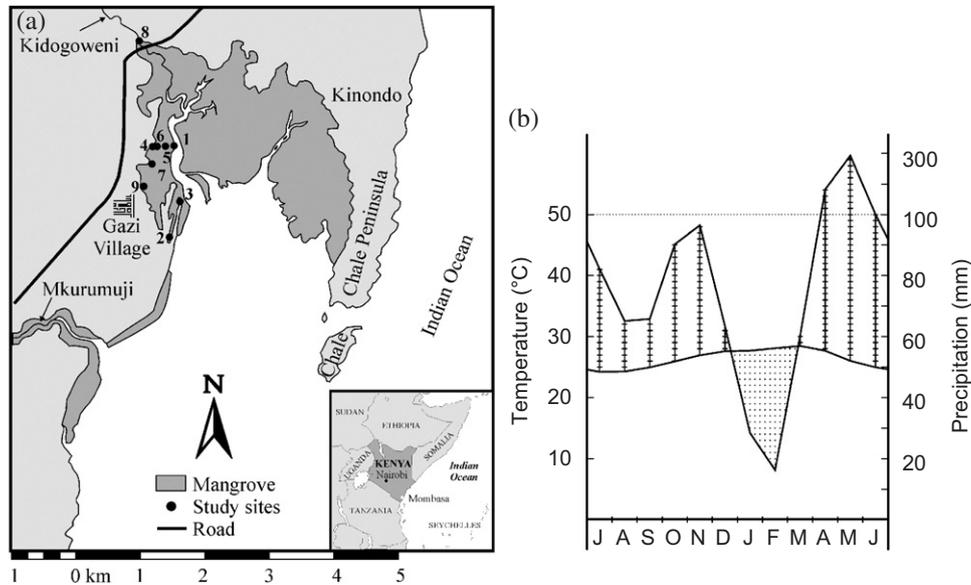
Fig. 2. (a) Location of the nine study sites in Gazi Bay, Kenya. Zoom: Gazi is situated approximately 50 km south of Mombasa, adapted from Dahdouh-Guebas et al. (2002). (b) Climate diagram of Mombasa (39°36′E,4°0′S) adapted from Lieth et al. (1999), showing the long (April–July) and short (October–November) rainy season and one distinct dry season (January–February). Precipitation scale is reduced to 1/10 above the horizontal line.

Table 1. Sample collection sites and corresponding environmental data

Site[a]	Salinity[b] (‰)			Inundation class[c]	n[d]	Tw no.[e]
	Average	Min.	Max.			
1	28.5	20.9	34.6	1	8 (+8)	55924-26, 55928-32
2	29.3	26.6	34.0	4	10	
3	32.4	28.1	34.2	3	(3)	57798-80[f]
4	41.0	13.2	56.7	4	5	
5	51.0	44.4	61.0	2	10	
6	54.1	42.3	69.8	3	10	
7	54.1	42.3	106.0	3	(4)	55933-36
8	58.1	40.0	68.2	4	10	
9	61.5	46.5	79.9	3	10 + (3 + 8)	56737-39[g], 55895, 55898, 55901-03

[a]See Fig. 2a.
[b]Soil water salinity at approximately 20 cm depth.
[c]Inundation class according to Watson (1928) in (Macnae, 1968).
[d]Number of sampled trees with number of collected stem disks in parentheses.
[e]Accession number in the Tervuren wood collection (Belgium) of the studied stem disks.
[f]Plantation trees.
[g]Trees from the cambial marking experiment.

from 13.2‰ to 90‰ [data from this study and from Gillikin (2004), measurements carried out with a WTW P4 multiline conductivity meter, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany]. Depending on the topography and the tidal range of on average 3.8 m and exceptionally 4.1 m (Kenya Ports Authority tide tables for Kilindini, Mombasa), zones of different inundation classes can be defined. Inundation classes 1–4 correspond to an inundation frequency of 56–62, 45–56, 20–45 and 2–20 times a month, respectively (Watson, 1928 ; Macnae, 1968).

Three trees from a plantation (site 3, Fig. 2a) established in February 1992 were sampled in May 2005. The planted trees were growing under natural conditions and were enclosed within a natural forest. Wood disks were sawn at the base of the stem and from two branches at a bifurcation to sample branches of the same age. Another three trees (site 9, Fig. 2a) were marked using the pinning technique in October 1999 with a surgical needle of 1.2 mm diameter (Verheyden et al., 2004b) and felled in May 2002. The pinning method has been proven to be very effective for

examining radial growth in tropical trees (Shiokura, 1989). All wood samples are now part of the wood collection of the Royal Museum for Central Africa, Tervuren, Belgium (for accession numbers see Table 1). From the xylarium an additional set of 28 samples from 20 trees was selected from site 1, 7 and 9 corresponding to a seaward site, a sand flat and a landward site. Finally, in May 2005 the outermost wood of 59 trees located at site 1, 2, 4, 5, 6, 8, 9 was collected. Samples (on average $3 \times 1 \times 1$ cm) were taken at breast height with a handsaw and stored in formalin–acetic acid–alcohol (FAA) to preserve the cambial cells.

Climate description

The climate along the Kenyan coast is characterized by a bimodal distribution of the precipitation. A distinct dry season (January–February) is followed by a long (April–July) and a short rainy season (October–November) (Fig. 2b). The average temperature at the Kenyan coast ranges from 22 to 30 °C, with a mean relative humidity of 65–81% (annual averages of minima and maxima for Mombasa for the period 1972–2001, data from the Kenyan Meteorological Department, Mombasa, Kenya).

Sample preparation and microscopic analysis

In this study the following terms and definitions will be used (see also Fig. 1b): *xylem band* to designate the zone in between the phloem tissue and the sclereids. To the inside, this band also includes a few cell layers of parenchyma, which are the first derivatives of the new cambium. The term *phloem band* is used to designate a zone of phloem strands united in a band of parenchyma tissue and *growth layer* to designate one ontogenetic unit of parenchyma, xylem, phloem and sclereids following the terminology of Parameswaran (1980).

To investigate the annual nature of growth layer formation, growth layers were counted from three plantation trees of known age and from three trees from a cambial marking experiment. First, wood discs were air dried and sanded using a series of sandpaper from 100 to 1200 grit, to make the phloem and xylem bands clearly visible (Fig. 1a). For the wood disks of site 1, 3, 7 and 9 the number of growth layers was then counted along the maximum as well as minimal radius making use of a magnifying glass. Because of the networking pattern of the growth layers (Fig. 1a) the convention was applied that a growth layer is counted if it crosses a pencil drawn line from pith to bark along the maximum and minimum radius. For the calculation of the average number of growth layers formed per year, the measurement at the longer radius was used (see Worbes, 1989). The wound inflicted by the technique of cambial marking is visible as a datable scar, indicating the position of the cambium at the time of wounding. The number of rings from these cambial marks onwards was counted with the aid of a stereo microscope at 30 magnifications.

The samples preserved in FAA were cut into little blocks of approximately 8 mm sides with a scalpel, creating a transverse plane. Samples were washed in 50% ethanol and dehydrated in an ethanol series (50, 75, 90, 96, 100%) with the last step taking 48 h (Ruzin, 1999). Subsequently, they were soaked for 24 h with PEG 1500 (Pure, VWR International, Prolabo) at 60 °C and embedded with fresh PEG 1500 for another 24 h at room temperature. Samples were sectioned at 20 μm thickness with a sliding microtome (Microm), dehydrated and double stained with Safranin O (Merck) and Fast green FCF (C.I. 42053, Merck). Sections were mounted on slides with Canada balsam (Merck). The number of parenchyma, xylem, phloem and sclereid cells of the last growth layer were counted along three radial files per sample using digital image analysis software (AnalySIS Pro v.3, Soft Imaging System GmbH, Münster, Germany) with a microscope at a magnification of 125 times (Olympus). To standardize the measurement only xylem fibres were counted and neither vessels nor xylem parenchyma were considered. However, distinction between parenchyma and fibres was not always obvious. In addition, the width of the last as well as the previous growth layers was measured along three radial files at 25 magnifications from the beginning of the phloem band to the end of the xylem band, excluding the sclereids and the small parenchyma zone (Fig. 1b). This is justified since both the number of sclereids and parenchyma cells in each growth layer are not statistically different between sites (ANOVA test, F-values of 0.73 and 0.53, respectively, $df = 6$, $p > 0.05$). For comparison between and within trees, the width of the current growth layer was standardized since the stem of *A. marina* was often asymmetric (Fig. 1a), with narrower growth layers at the side of the smallest radius. The width of the last growth layer was divided by the average width of the preceding growth layers. Taking samples instead of disks obviously limited the data set. Between trees, variation could be considered but, within trees, variation could only be accounted for along the length of the 8 mm wide sample.

Statistical analysis

Simple linear regressions were performed to analyze the relationship between (i) number of growth layers and stem or branch diameters (all samples included in the analysis) and (ii) growth layer width and age (samples from plantation trees only). Time series of the growth layer width were compared to the growth layer number, as a measure of tree age. To test the

difference between slopes we used a homogeneity-of-slopes model. Via a *t*-test for dependent samples the difference in growth layer width was tested between the small and large sides of the asymmetric stem disks. The assumption of homogeneity of variances was tested via Levene's test. All statistics were executed in STATISTICA 7.0 (StatSoft Inc., Tulsa, USA).

Results

On average half a growth layer was formed per year in the trees from the cambial marking experiment of site nine and three growth layers in the trees of the 13.25-year-old plantation (Fig. 2a, Tables 1 and 2). From these plantation trees, growth layers were also counted in pairs of bifurcating branches. Along the maximum branch radius, two of the three branch pairs had an unequal number of growth layers. Along the minimum branch radius all three pairs of branches showed a different growth layer count (Table 2). The incongruence in growth layer formation both between and within trees was also pointed to by a microscopic analysis. The radial increment of the current growth layer of 59 trees of seven sites (Table 1, 1–2, 4–6 and 8–9) was measured. Using width instead of cell count was justified because of the high correlation between both characters ($r^2 = 0.96$, $n = 189$, $p < 0.0001$). The standardized width of the current growth layer (see Materials and methods) differed both within sites and trees (Fig. 3). The within tree variation (Fig. 4a) explains the large standard

deviation for some trees. Furthermore, the formation of a new growth layer can be started before completion of the previous one (Fig. 4b).

The number of growth layers was shown to be strongly correlated to the radius of the wood disks (Table 3). The larger the stem or branch, the more growth layers were counted from pith to bark (Table 2), despite the similar age of the three stem disks and of each pair of branches. Likewise, less growth layers were counted at the minimum than at the maximum radius of the wood disks (Fig. 1a, Table 2). This highly significant relationship between stem size and number of growth layers in the plantation trees was supported by analyzing an extra 28 wood disks from three other sites in the mangrove forest of Gazi Bay (Table 3, Fig. 5). Correlation coefficients between stem radius and growth layer count further increased when the data were grouped according to sampling site (Table 3). Furthermore, the slopes of the regression lines for the three sites separately were found to be significantly different (Table 3, Fig. 5). The associated growth layer width, ranged between 0.69 ± 0.09 mm (site 7) and 1.20 ± 0.34 mm (site 1) on average and did not show a correlation with age. No trend was observed from juvenile to mature wood in none of the ring width chronologies of the three plantation trees (R^2-values of, respectively $= 0.031$, 0.11 and 0.000093, $p \geqslant 0.05$). As shown by the three plantation trees and the 28 stem disks mentioned above, the width of the growth layers also differed within one tree. The width at the minimum branch radius was smaller than at the maximum branch

Table 2. Number of rings in three stem disks of known age collected from a plantation and in three trees from a cambial marking experiment

Tw number[1]	Min. radius (cm)	Number of growth layers[2]	Max. radius[4] (cm)	Number of growth layers[2]	Time period (yr)	Number of growth layers/yr[3]
Site 3, Plantation						
57798	**3.7**	**37**	**6.2**	**50**	**13.25**	**3.8**
branch1	1.2	16	1.6	21		
branch2	1	13	1.2	14		
57799	**3**	**30**	**4.3**	**37**	**13.25**	**2.8**
branch1	1.1	14	2.1	19		
branch2	1.2	16	2.4	22		
57800	**2.9**	**27**	**4.7**	**42**	**13.25**	**3.2**
branch1	1.1	17	1.5	19		
branch2	1.1	15	1.5	19		
Site 9, Cambial marking						
56737			**4.4 (0.19)**	**1.5**	**2.58**	**0.6**
56738			**2.7 (0.15)**	**1.0**	**2.58**	**0.4**
56739			**4.8 (0.10)**	**1.5**	**2.58**	**0.6**

Plantation: [1]Accession number in the Tervuren wood collection, samples were taken at the base of the stem and from a dichotomous branching; [2]Growth layers were counted as they crossed a drawn line from pith to bark at the max. and min. radius; [3]Calculated from the growth layers counted at the maximum radius.
Cambial marking: [2]Counted from the cambial mark (parenchyma band) to the bark. [4]Maximum radius of the stem disk, in parentheses the distance cambial mark-bark.
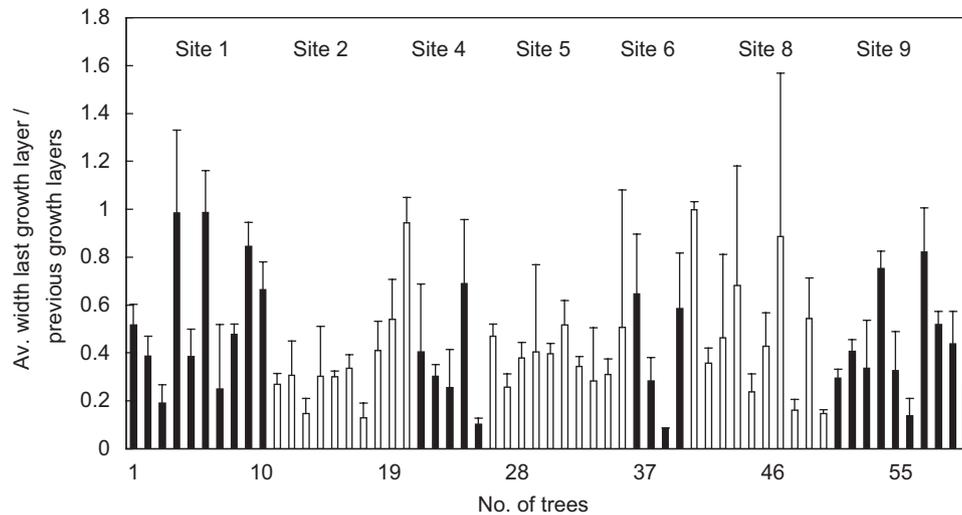
Fig. 3. The developmental stage of the last growth layer is illustrated for 63 trees of seven study sites. To exclude site-related differences the ratio was taken of the width of the last growth layer, averaged over three radial files, to the mean width of the previous growth layers. The different sites are ordered according to increasing salinity and separated by the use of alternating dark and white bars. Error bars correspond to standard deviations.

radius in asymmetric stem disks ($t = -5.64$, $df = 30$, $p < 0.0001$).

Discussion and conclusions

The growth layers in *A. marina* from Kenya were shown to be non-annual (Table 2). This finding indirectly demonstrates that the development of new cambia in *A. marina* is not controlled by the seasonal climate at the study site (Fig. 2b). In contrast, in the mangrove *R. mucronata* Lam., growing at Gazi Bay, the seasonality causes annual rings (Verheyden et al., 2004b, 2005). Also in other tropical trees with a single cambium, annual ring formation is primarily governed by rainfall seasonality or a period of inundation (Jacoby, 1989; Borchert, 1999). But, in *A. marina* the aclimatic growth rhythm is confirmed by the disparity in ring numbers in the even-aged pairs of branches (Table 2). Additional evidence is given by the widely fluctuating developmental stage of the current growth layer of 59 trees in Gazi (Fig. 3). If climate directed the production of new cambia, the last layer should have been in the same developmental stage in all trees. However, even along the circumference of a single stem this is not the case (Figs. 3 and 4a), resulting in a growth-layer network (Fig. 1a). The cause may be an irregular distribution of assimilates and hormones as mentioned before in *Dryobalanops sumatrensis* (J.F. Gmelin) Kosterm. (Sass and Eckstein, 1995). This can be due to an irregular flowering in the branches of one tree (Clarke and Myerscough, 1991; Clarke, 1992). The differences within one site can thus be explained by the combination of an endogenous

control on growth layer formation and an uneven-aged forest.

An internal control of cambial differentiation is in agreement with the observations by Gill (1971) and is supported by our study. The number of growth layers was found to be correlated to the diameter of the stem disk of both planted (Table 2) and naturally grown trees of three sites (Fig. 5). Moreover, the disappearance of growth layers at the slow growing side of asymmetric stems (Table 2) confirms the strong influence of the diameter on the production of new cambia and thus growth layers. An influence of endogenous factors on growth layer formation was reported before in *Cupressus sempervirens* L. (Liphschitz et al., 1981). In *A. germinans* growth control was suggested to be exclusively endogenous based on the finding of a common growth layer width of about 1.30 mm (Gill, 1971). On the contrary, this study on *A. marina* demonstrated a between-sites variation in the growth layer width (Fig. 5) and the number of yearly formed growth layers (Table 2). The incongruence between both studies can probably be attributed to the larger sample set of this investigation ($n = 28$ vs. 10), representing three study sites instead of one. The influencing local site conditions, presumably salinity and/or inundation frequency, may interact with phenological processes (Duke, 1990; Borchert, 1999; Ochieng and Erftemeijer, 2002). In an ongoing study on the phenology of *A. marina* in Gazi Bay, differences in leaf loss were found between sites (V.W. Wang'ondu, personal communication). A site dependent phenology, related to differences in the cambial activity and/or the active period of one cambium (Paliwal and Prasad, 1970; Borchert, 1999), might thus explain the discrepancy in width and number
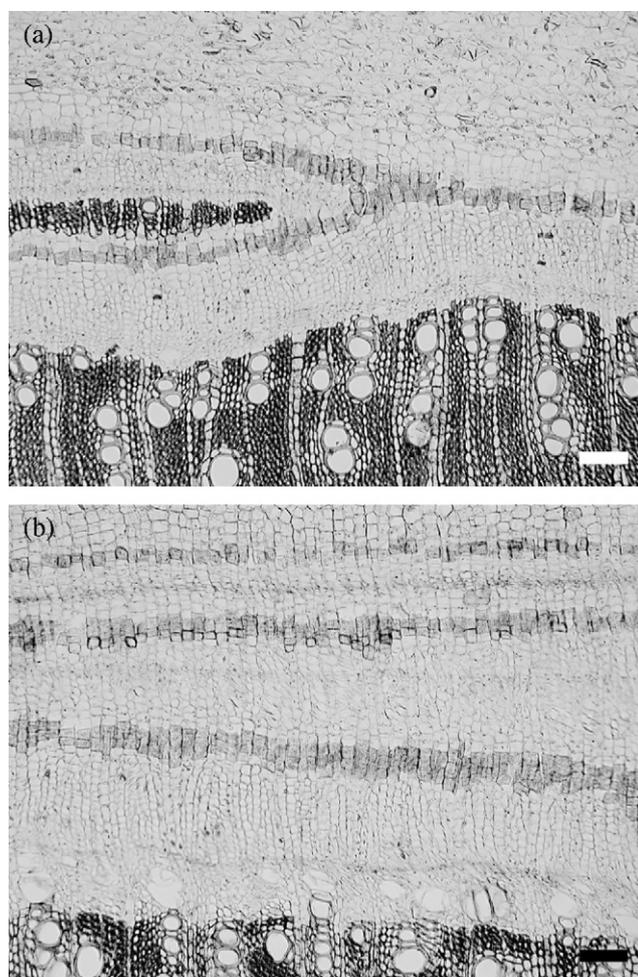
Fig. 4. Transverse sections of the outermost wood of two *Avicennia marina* trees. (a) A new cambium has been formed at the left side of the picture but not yet at the right side. (b) Three growth layers are being formed simultaneously implying a discontinuous time-axis. Light microscope, scale bar = 100 μm.

Table 3. Summary of the simple linear regressions of the number of growth layers in function of the maximum stem radius

Site	R^2 [a]	df	F	p
3, plantation	0.97	7	299.31	<0.0001
1, 7, 9	0.83	26	136.12	<0.0001
1, seaward	0.99	10	740.27	<0.0001
7, sand flat	0.92	6	79.03	<0.0001
9, landward	0.98	6	456.25	<0.0001
Site*max radius [b]		22	54.72	<0.0001

[a]Adjusted R^2.
[b]Homogeneity-of-slopes model test.

of yearly formed growth layers at different sites (Table 2, Fig. 5). Likewise, the regulation of the cambial activity in *Azadirachta indica* A. Juss. was mentioned to
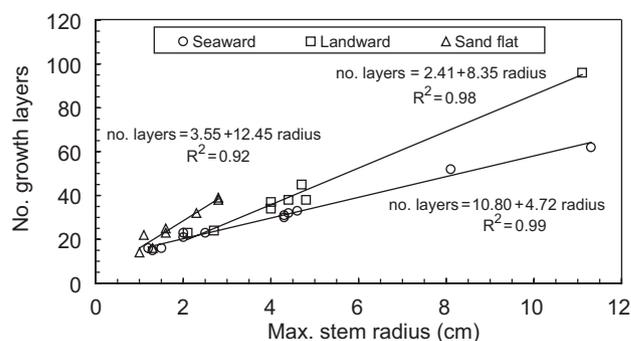

Fig. 5. The number of growth layers as a function of the maximum radius of the asymmetric stem disks for three sites differing in environmental conditions. The three sites correspond to site 1 (seaward), 9 (landward) and 7 (sand flat) as given in Table 1.

be determined by endogenous as well as some external factors like water supply and temperature (Rao and Rajput, 2001). On the contrary, an additional age effect on growth layer width could be excluded, in contradiction to earlier reports on other tropical tree species (Akachuku, 1984; Worbes et al., 2003). The inconsistency can be due to the internal control on growth layer formation. But, an extensive investigation, including trees of old age, is needed for confirmation.

Growth layers become narrower (steeper slopes in Fig. 5) with increasing soil water salinity, from the seaward (site 1), over the landward site (site 9) to the sand flat (site 7) (Table 1). Although the average soil water salinity at the sand flat is not so much higher than at the landward side, there are considerable differences in environmental conditions explaining the disparity in growth layer width. First, the sand flat shows an increased salinity fluctuation (Table 1) which imposes a larger stress on tree growth compared to a constant salinity (Lin and Sternberg, 1993). Second, in comparison to the sand flat the soil at the landward site has a higher loam fraction (Matthijs et al., 1999) making the substrate more effective in water storage. The functional significance of thin growth layers under high salinity conditions may be a higher proportion of living tissue, in this case phloem and parenchyma. Parenchyma sheaths may offer an improved regeneration capacity of the tree as do the phloem strands that are dispersed throughout the stem (Carlquist, 2001). They have been shown to serve as transporters of the auxin hormone that could promote vascular regeneration after wounding (Aloni, 2004). This is especially important for the *A. marina* trees of the more saline sites since die back is a common phenomenon in trees subjected to drought stress (Carlquist and Hoekman, 1985) that can be caused by a high salinity (Sperry et al., 1988; Tyree and Sperry, 1989; Hacke and Sperry, 2001). Additionally, the large proportion of phloem and parenchyma

tissue may offer an advantage for water transport. That is because they have been put forward as actors in the process of embolism repair. Although the underlying mechanism is not yet clear, the refilling of embolized vessels may be accomplished by a water flow from living cells (Holbrook and Zwieniecki, 1999; Tyree et al., 1999; Stiller et al., 2005). Another benefit that is gained from the increased number of phloem bands per surface area involves the higher degree of compartmentalization. Sectoriality may diminish the spread of embolisms (Orians et al., 2004; Ellmore et al., 2006). In this study *A. marina* trees at the seaward fringe of the mangrove forest were found to have wider growth layers, implying a smaller proportion of parenchyma and phloem tissue and a larger fraction of fibres. It is likely that this provides the tree with an enhanced mechanical strength to withstand tidal force and strong winds (Sun and Suzuki, 2001). At the same time the increased fibre proportion could offer an alternative safety mechanism against cavitation (Jacobsen et al., 2005).

Finally, the finding that more than one cambium can be active simultaneously (Fig. 4b) implies that no dormancy takes place between the formation of two successive growth layers. This is consistent with the frequent observation of a continuous cambial activity in tropical trees (see Sass et al., 1995; Rao and Rajput, 2001; Verheyden et al., 2004b). Two active cambia at the same time were reported before in *A. germinans* and *A. resinifera* Forst. f. (Zamski, 1979). The resulting discontinuous time axis together with the network of non-annual growth layers impedes the application of conventional dendrochronological methods and simple age determinations on *A. marina* from Kenya. The growth layers in this species turned out to be non-annual but related to stem size and local site conditions. More research is needed to confirm the hypothesis that the width and the yearly formed number of growth layers decrease with increasing salinity. The functional and/or mechanical significance of this trend present an extra research question. Finally, the annual variation in the yearly formed growth layers at a specific site has to be addressed to elucidate if growth layer count can still be used to give at least an estimation of tree age.

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