Stiffness gradients in vascular bundles of the palm *Washingtonia robusta*

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Palms can grow at sites exposed to high winds experiencing large dynamic wind and gust loads. Their stems represent a system of stiff fibrous elements embedded in the soft parenchymatous tissue. The proper design of the interface of the stiffening elements and the parenchyma is crucial for the functioning of the stem. The strategy of the palm to compromise between stiff fibre caps and the soft parenchymatous tissue may serve as a model system for avoiding stress discontinuities in inhomogeneous and anisotropic fibre-reinforced composite materials. We investigated the mechanical, structural and biochemical properties of the fibre caps of the palm *Washingtonia robusta* at different levels of hierarchy with high spatial resolution. A gradual decrease in stiffness across the fibre cap towards the surrounding parenchymatous tissue was observed. Structural adaptations at the tissue level were found in terms of changes in cell cross sections and cell wall thickness. At the cell wall level, gradients across the fibre cap were found in the degree of orientation of the microfibrils and in the lignin level and composition. The impact of these structural variations in the local material stiffness distribution is discussed.

Keywords: palms; gradients; micromechanics; tensile stiffness; cell wall; lignin composition

1. INTRODUCTION

A multitude of hurricane reports remarkably show that palms are able to withstand severe storms with high dynamic loads. The strategy of the palm in coping with these high bending stresses is of particular interest from a biomechanics as well as biomimetics perspective. The remarkable mechanical performance of the palm is the result of its specific structural organization. As in a multitude of biological systems (Speck *et al.* 1996; Spatz *et al.* 1997; Fratzl 2003; Aizenberg *et al.* 2005; Fratzl & Weinkamer 2007), different levels of structural hierarchy can be distinguished in the palm stem. Structure–function relationships have already been investigated at the macroscopic scale by correlation of mechanical properties and vascular bundle distribution across the stem (Rich 1987b). However, adaptive strategies in palms at the ultrastructural and biochemical levels have not yet been investigated. At these levels of hierarchy, the mechanical constraints and underlying principles of the embedding of stiff vascular bundles in the soft parenchymatous tissue are of particular interest. Analysing the micro- and nanostructural changes across individual vascular bundles leads, on the one hand, to a deeper understanding of the biomechanics of the palm, and on the other hand, the knowledge gained may serve as a source of

bioinspiration for the design of advanced technical fibre-reinforced composites.

The monocotyledonous palms possess vascular bundles that are unequally distributed across the stem and are embedded in the parenchymatous tissue. A vascular bundle consists of conducting elements and a fibre cap, which is stiffer by far than the surrounding parenchymatous tissue and determines to a large extent not only the overall but also the local stiffness of the plant. Hence, the local densities and material properties are closely related to the distribution of vascular bundles in the trunk. The fibre cap can make up 90% of the cross-sectional area of the vascular bundle and can reach diameters of up to 2 mm (Rich 1987a). However, the anatomy of a single vascular bundle can change considerably across the stem. Individual vascular bundles change their radial position while running up in a screw-like fashion and in a radial zigzag through the stem. Cell wall thickness and lignification of the fibres of a given bundle as well as the diameter of the fibre cap vary with regard to its relative position (Rich 1987a; Tomlinson 1990). In consequence, individual vascular bundles do not belong to a specific bundle ‘type’, but the anatomy of a vascular bundle is determined by its actual position in the stem. Accordingly, Waterhouse & Quinn (1978) defined radial zones of characteristic bundle patterns across the palm trunk.

Rich (1987b) examined the macroscopic properties of four different palm species by performing bending tests and density measurements on samples taken from different positions along the palm stems. He found a

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Figure 1. (a) Cross section of the peripheral region of the palm stem. The exodermis, which is approximately 2 mm thick, is missing at the edge of the trunk on the r.h.s. of the section. (b) Cross section of a vascular bundle where the peripheral fibre cells are thin-walled. (c) Radial-longitudinal section of a vascular bundle similar to the one shown in (b). F, fibre cells; Ph, phloem; X, xylem; P, parenchyma.

gradual increase in the stiffness and density from the top to the base of the stem and from the centre to the periphery of a given stem cross section within individual palm trunks. Pronounced radial gradients were reported showing differences in the stiffness of up to three orders of magnitude. Since the highest bending moments occur in the periphery of the base of the stem, the palm can be considered as optimized to resist high wind loads due to an efficient variation in the material properties along and inside the stem. In this respect, the mechanical design of palms is comparable to deciduous trees and conifers.

Although it is well accepted that the distribution of vascular bundles determines the macroscopic mechanical properties of the palm stem, the mechanical properties of the individual bundles have been only briefly considered up to now. The fact that the stiff fibre caps are embedded in a very soft parenchymatous tissue puts some interesting constraints on the mechanical design of the interface between both the tissue types. Owing to mechanical loading, high stresses are generated in the stiff fibres, whereas the stresses in the soft parenchyma cells stay rather low. Such inhomogeneous structures are prone to fail under external loads, because stress discontinuities are likely to occur at the interfaces.

One way of overcoming the addressed problem under the specific anatomical organization of monocotyledonous plants is to create a gradual transition of stiffness between the fibres of the vascular bundles and the parenchymatous cells. In the present study, we have investigated such transitions in terms of gradual changes in mechanical, anatomical, ultrastructural and biochemical properties across single fibre caps. Hereby, we concentrated on the most abundant type of vascular bundle.

2. MATERIAL AND METHODS

(a) *Plant material, conservation and sectioning*

All samples were taken from a 33-year-old individual of the palm *Washingtonia robusta* (Mexican fan palm), which had been grown in the greenhouses of the Botanical Garden of the University of Freiburg, Germany. The trunk was 10 m long with a base diameter of 40 cm. Polyethyleneglycol 2000

(PEG 2000) was used as embedding agent for preserving the material and for sectioning thin tissue slices. Sample blocks of approximately 1 cm³ were cut out of the peripheral zone of the stem at 5 m height within 2–6 cm distance to the edge of the trunk (figure 1a), which had a diameter of 30 cm at this height.

The sample blocks contained vascular bundles with fibre caps consisting of thick-walled fibres in the inner part and thin-walled fibres in the periphery (figure 1b). This is the dominant appearance form across the stem until approximately 2 cm away from the outer edge (figure 1a). The sample blocks were submerged in a 1 : 1 solution of PEG 2000 and water, and were kept at 60°C for 3 days until almost all water had been evaporated. For another day, they were put into pure PEG and kept at 60°C, before cooling down and hardening out at room temperature. The PEG blocks containing the samples were stored in a refrigerator.

(b) *Image evaluation*

For measurements of cell cross sections, cell wall thickness and cell wall area fraction of the fibres, 20 μm thick cross sections were cut with a rotating microtome, washed with water and stained with safranin. Grey-level images were taken and then analysed using the software IMAGEJ v. 1.38. Hereby, we defined four regions (figure 2a) to calculate average values and standard deviations.

(c) *Mechanical tests*

Tangential sections of 80 μm thickness were cut from the embedded sample blocks with a rotating microtome to prepare consecutive radial fibre strips of the fibre caps for mechanical tests (figure 2a). The sections were washed with water to dissolve the PEG and kept wet during the entire preparation and testing process. Fibre strips of approximately 3 mm length, approximately 120 μm width and approximately 80 μm thickness (figure 2b) were cut out of the microtome sections using a microlaser dissection device (PALM Microlaser Technologies GmbH, Germany, with a pulsed UV laser at 355 nm, CryLas GmbH, Germany). This highly sophisticated technique ensured a high quality and reproducibility of the small-sized samples. The strips were glued onto

Figure 2. (a) Three-dimensional sketch of a vascular bundle with red boxes marking the origin of the fibre strips for the mechanical test series and blue areas marking four defined regions to measure cell cross-sectional area, cell wall thickness and cell wall area fraction. (b) As-prepared fibre strip (inset, cross section of fibre strip).

a plastic foil and fixed in a microtensile testing stage. The applied strain was detected by video extensometry with black markers on the plastic foil at both ends of the sample (Burgert *et al.* 2003). The test length was 1.6–1.8 mm and the test speed was $2 \mu\text{m s}^{-1}$ (strain rate of $0.11\text{--}0.13\% \text{ s}^{-1}$). Force and elongation were recorded during the experiment. After sample rupture, small pieces of $30 \mu\text{m}$ length were cut out of the fibre strips with the microlaser at both sides of the position of failure. These pieces were flipped by 90° (figure 2b, inset), and the images of the cross sections were evaluated with the software mentioned above. The stresses applied to a sample during one experiment were calculated on the basis of the cross-sectional area of the entire fibre strip and the cross-sectional area of the pure cell wall.

(d) X-ray diffraction

Synchrotron radiation at the μ -Spot beamline at BESSY II (Berlin, Germany) was used for scanning wide-angle X-ray diffraction (WAXD; Paris *et al.* 2007). Radial slices of the fibre caps of $40 \mu\text{m}$ thickness were scanned in dry condition with an X-ray beam diameter of approximately $10 \mu\text{m}$ and a step size of 10 or $15 \mu\text{m}$. Two-dimensional diffraction patterns of the crystalline cellulose were collected for each scanning step. The X-ray wavelength was 0.082 nm and the measuring time was 120 or 180 s for each frame. The area detector (MarMosaic 225, 3072×3072 pixels, with a pixel size of $73.2 \times 73.2 \mu\text{m}$; Mar USA, Evanston, USA) was placed at a distance of 46.2 cm, which allowed capturing the diffraction signal from the microfibrils with a reasonably high angular resolution. The azimuthal angle χ between the 002 reflections of the crystalline cellulose (figure 3) at $\theta = 6^\circ$ was used to determine the microfibril angle μ (Lichtenegger *et al.* 1998, 1999). If the cell walls are exactly perpendicular to the incoming beam, the microfibril angle is simply calculated as $\mu = \chi/2$. In the case of an uneven cell shape, $\chi/2$ gives a lower bound for the microfibril angle.

The degree of orientation of the cellulose microfibrils was determined according to a procedure originally introduced for mineral platelets in bone (Rinnerthaler *et al.* 1999). The background scattering and the scattering of amorphous cell wall material were subtracted before calculating this so-called ρ parameter. The amount of scattering from amorphous components was hereby estimated by radial integration between $7^\circ < \theta < 7.75^\circ$, which was outside of the 002 reflections of crystalline cellulose at $\theta = 6^\circ$. From the remaining total area

Figure 3. Typical intensity diagram after radial integration of a WAXD image. The arrows indicate the orientation of the longitudinal cell axis. The angle χ , shown on the r.h.s. of the graph, is taken for the determination of the microfibril angle μ . The grey area (A_{random}) is the fraction of randomly oriented microfibrils. Preferred orientation results in intensity peaks (A_{peak}). The area between 51° and 75° is shaded by the beam stop.

underneath the intensity curve (A_{total}), the area below the baseline of the peaks was taken as the scattering of randomly orientated microfibrils (A_{random} , grey area in figure 3). This area was subtracted to yield the area underneath the peaks being caused by microfibrils orientated parallel in the predominant direction (A_{peak}). The ratio $\rho = A_{\text{peak}}/A_{\text{total}}$ is defined as the degree of orientation, which varies between zero (no orientation) and one (all microfibrils are oriented).

(e) Thioacidolysis and gas chromatography–mass spectrometry

Thioacidolysis combined with gas chromatography (GC) and mass spectrometry (MS) of lignin-derived monomers was used to evaluate the lignin content and the structure (Lapierre *et al.* 1995) of the tissue fractions collected as follows. Approximately, 40 radial–longitudinal sections of the fibre caps with a thickness of $40 \mu\text{m}$ were taken for the analysis. Each section was divided into four fractions to accumulate approximately 2 mg material (dry weight) for each fraction. The first fraction contained the vessels of the vascular bundles. The thick-walled area of the fibre caps was

Figure 4. (a) Cell cross-sectional area, (b) cell wall thickness and (c) cell wall area fraction of four fibre caps in four regions across the fibre cap, respectively (figure 2). The error bars in (a,b) show standard deviations. The cell wall area fraction is calculated by dividing the cell wall area by the entire fibre cap area.

divided into two fractions (inner thick-walled fraction and middle thick-walled fraction); the fourth fraction contained outer thin-walled fibres. Duplicate analyses were performed for each fraction with the exception of fraction 4, which was analysed only once.

The analysis was performed together with 0.05 mg of nonadecane, heneicosane and docosane as internal calibration standards. During this procedure, the β -O-4 bonds within the lignin polymer were cleaved. After the reaction, the lignin-derived monomers were extracted as described by Lapierre *et al.* (1995). The combined organic extracts were concentrated to approximately 0.2 ml. Ten microlitres of the sample were silylated by 50 μl BSTFA and 5 μl pyridine prior to injection in a DB-1 Supelco capillary column with helium as carrier gas. The flow rate of the gas was kept constant at 1 ml min⁻¹. The GC was operated from 40 to 180°C at a rate of 30°C min⁻¹, and then from 180 to 260°C at a rate of 2°C min⁻¹. It was combined with an ion trap mass spectrometer (Varian Saturn 2100) operating in the electron impact mode (more than 0.70 eV) with ions detected in the 50–600 m/z range.

(f) UV microspectrophotometry

To resolve the topochemical distribution of lignin and other cell wall phenolics with high spatial resolution, a microspectrophotometer (UMSP 80; Zeiss, Germany) was used to determine the lignin content semi-quantitatively by the absorbance in UV light (Koch & Kleist 2001). Small sample blocks (1 × 1 × 5 mm) were embedded in Spurr, according to Koch & Kleist (2001), to prepare 1 μm thick cross sections for the measurements. Absorbance spectra of the cell walls were taken from 240 to 400 nm at different radial positions within the fibre cap with a spot diameter of 1 μm . The wavelength of the maximum absorbance was then used for two-dimensional scans across the fibre caps with a step size of 0.25 μm using the scan program Automatic Photometric-Analysis of Microscopic Objects by Scanning (APAMOS; Zeiss).

3. RESULTS

(a) Cell cross-sectional area, cell wall thickness and cell wall area fraction

Cell cross-sectional area, cell wall thickness and cell wall area fractions were determined within four vascular bundles in four different regions, respectively (figure 4). Regions 1–3 covered the thick-walled fibres (figure 1b), whereas region 4 covered the outer thin-walled fibres. The

mean cell cross-sectional area increased towards the outermost position (figure 4a), yet the scattering was very high. The wide variability of cell cross-sectional areas can be partly explained by the fusiform cell shapes. Tissue cross sections contained fibres that were cut close to their ends as well as towards their central region, respectively. Hence, different cell cross-sectional areas might be found for fibres of the same shape and size. The trend towards smaller cross sections from regions 3 to 4 might be explained by a retarded expansion of the fibres at the periphery of the cap, which has already been reported for other palm species (Rich 1987a; Tomlinson 1990).

The mean cell wall thickness was nearly constant from regions 1 to 3, but decreased by a factor of approximately 2.5 in region 4. The variation in the cell wall thickness within one region was considerably lower than that of the cell cross-sectional area, which supports the explanation of the variability of the latter. Both parameters influence the distribution of cell wall area fractions across the fibre cap. A pronounced decrease from the inner to outer part was found for the four fibre caps (figure 4c).

(b) Mechanical tests

Two stiffness parameters across the fibre caps were analysed. The tissue stiffness was determined by calculating the stresses on the basis of the entire cross-sectional area of the tissue sample. The cell wall stiffness was calculated on the basis of pure cell wall area. By determining both parameters, it was possible to distinguish between variations at the tissue level (cell wall area fraction) and variations at the ultrastructural and biochemical levels of the cell wall. A gradual decrease in tissue stiffness from the centre to the periphery of the fibre cap was found for all four tested fibre caps (figure 5a). The values decreased from 400 to 500 MPa for fibre strips close to the phloem, and to 5 to 20 MPa for strips close to the parenchymatous tissue.

The gradient in tissue stiffness correlated with the decrease in cell wall area fraction across the fibre cap (figure 4c). Additional variations at the cell wall level, which enhanced this gradient, became obvious when plotting the data of cell wall stiffness (figure 5b). The values gradually decreased from 1100 to 2100 MPa close to the phloem, and to 20 to 50 MPa close to the parenchyma. Within the thick-walled part of the fibre cap, the values decreased by a factor of 1.5 to 3. Hence, variations at the tissue level can only partly explain the

Figure 5. (a) Tissue stiffness of fibre strips of four different fibre caps versus their radial position. (b) Cell wall stiffness versus radial position.

decrease in stiffness of the fibre strips. The additional difference in the stiffness across the fibre caps must therefore be caused by variations in the mechanical properties of the cell walls. This indicates that alterations exist in the ultrastructure and biochemical composition of the cell walls. Mean fracture strains of the fibre strips within the individual bundles were in the range of $5.4 \pm 1.8\%$ to $10.3 \pm 2.8\%$. No trend in the values of fracture strain was visible across the individual fibre caps.

(c) X-ray diffraction

Scanning WAXD was used to measure the mean orientation and the degree of orientation of the cellulose microfibrils in the cell walls. Three independent line scans (different samples) across the fibre caps revealed values between 30° and 80° for the angle χ , which represents the angle between the 002 reflections of the cellulose crystalline units of the two adjacent cell walls (Lichtenegger *et al.* 1998, 1999). The thin-walled fibres of the outer parts of the vascular bundles collapsed upon drying. Therefore, no measurements could be taken from this area, and only the thick-walled fibres were examined. The angle $\chi/2$ represents the microfibril angle μ , if the irradiated cell walls are exactly perpendicular to the incoming beam; otherwise the microfibril angle would be larger than $\chi/2$ (Paris & Müller 2003). As the fibre cells were of uneven shape (figure 1a), the cell walls were, in many cases, not exactly perpendicular to the incoming beam resulting in a large scatter of the data and also in a possible underestimation of the microfibril angle. Nevertheless, the values for $\chi/2$, as shown in figure 6a, can be considered as a fair approximation of μ , which should in particular reflect a possible gradient in the microfibril angle across the fibre caps. The three line scans indicated that the cellulose microfibrils were orientated at a rather large angle to the longitudinal axis of the cell. The values for the microfibril angle varied between 15° and 40° , and no obvious trend in the microfibril orientation across the fibre cap was observed.

However, the degree of orientation of the microfibrils, ρ , showed a clear trend across the inner part of the thick-walled fibres in two of the three line scans (figure 6b,c). In the third line scan (figure 6d), the values first slightly decreased and then increased again. This increase can be partly explained by additional azimuthal peaks at 90° and 270° , which were measured in all line scans at greater

distances from the phloem (figure 3). These maxima indicated a partly horizontal orientation of the microfibrils and can contribute considerably to the values of ρ . The dotted lines in figure 6b–d indicate the position where the first horizontal orientation appeared.

(d) Lignin study by thioacidolysis/GC-MS

Besides the orientation of the microfibrils, the structure and the chemical composition of the matrix (hemicelluloses, pectins, lignin and other phenolics) may influence the mechanical properties of the cell wall. The available amount of tissue fractions was very low (approx. 2 mg for each fraction). It was therefore not possible to determine their lignin content using the conventional gravimetric or spectrometric methods that are sample demanding and require a preliminary solvent extraction step. A method to unequivocally characterize lignin in such non-extracted samples is thioacidolysis, which specifically yields *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) monomers from monocotyledonous lignin units only involved in β -O-4 bonds (referred to as uncondensed lignin units). The H-monomers released by the vascular bundles were recovered in very low relative amount (molar yield of 0.1–0.3% of the total yield of thioacidolysis monomers) and were therefore neglected in the following. Even though the structure of native lignins may affect thioacidolysis yield, this yield is primarily dependent on the lignin content of the sample. Accordingly, the variations in thioacidolysis (S+G) yield suggested a decrease in lignification across the fibre cap (figure 7). The yield was the highest in the vessels with a total amount of $193 \mu\text{mol g}^{-1}$. The inner thick-walled fibre fraction with a (S+G) yield of $118.9 \mu\text{mol g}^{-1}$ seemed to be, by a factor of 1.7, more lignified than the middle thick-walled fraction ($70.5 \mu\text{mol g}^{-1}$). These three fractions were weighed to an error of 0.1 mg and subjected to duplicate analysis. The total amount of uncondensed units of lignin has, therefore, to be taken with an intrinsic error of approximately 10%. The fourth fraction containing the outer thin-walled fibres could not be weighed. With the assumption that its dry weight was approximately 1 mg, it gave a thioacidolysis yield that was two orders of magnitude smaller than that of the other three fractions. This low yield suggested a very low level of lignification.

Besides the gradient in lignification, there was a major change in the syringyl : guaiacyl (S : G) ratio from the

Figure 6. WAXD line scans along radial-longitudinal sections of three different fibre caps showing (a) $\chi/2$ (half of the measured angle between the cellulose 002 reflections) as approximation for μ and (b–d) the degree of orientation of the cellulose microfibrils for the same line scans as in (a). The dotted lines indicate the radial position where the additional horizontal orientation of cellulose microfibrils appeared.

inner to the middle section of the fibre caps. The lignin in the vessels and fibre cells next to the phloem seemed to be of a similar composition with an S : G ratio of 2.5 : 1 and 2.4 : 1, respectively, whereas the ratio changed to 4.9 : 1 for the thick-walled fibres in the middle section. The outer thin-walled fibres showed a ratio of 3 : 1, being slightly higher than that in the vessels and the inner thick-walled fibres. The values of the S : G ratios showed differences of less than 5% in the duplicate analysis.

In addition to the lignin-derived H-, G- and S-monomers, thioacidolysis of the palm vessels and the thick-walled fractions of the fibre caps released substantial amount of *p*-hydroxybenzoic acid (in the 200–400 $\mu\text{mol g}^{-1}$ yield range, with a low reproducibility in the duplicate analysis), which is known to be associated with palm lignin via ester linkages (Sun *et al.* 1998; Suzuki *et al.* 1998; Kuroda *et al.* 2001). From the thin-walled fibre fraction, only a small amount of *p*-hydroxybenzoic acid could be detected. As thioacidolysis does not quantitatively cleave ester bonds, the results concerning these ester-linked phenolic acids can only be considered qualitatively. The amount of *p*-hydroxybenzoic acid released from the lignified fractions (vessels and thick-walled fibre caps), nevertheless, suggests that the samples consist of at least 2.7–5.5% *p*-hydroxybenzoate esters (as weight percentage of the dry samples). Weak amounts offerulic acid (in the 1–5 $\mu\text{mol g}^{-1}$ range) and trace amounts of *p*-coumaric acid were detected as well.

(e) UV microspectrophotometry

By means of scanning UV microspectrophotometry, it was intended to evaluate the topochemical distribution of cell wall phenolics (i.e. lignin, *p*-hydroxybenzoate esters)

Figure 7. Yield of lignin-derived (S+G) monomers after thioacidolysis in $\mu\text{mol g}^{-1}$ (bar graph; black bars, G-monomers; grey bars, S-monomers), and syringyl/guaiacyl (S : G) ratio of vascular bundle fractions (line graph). The two bars per fraction represent the results of duplicate analysis. The values of the S : G ratio represent the mean of the duplicate analysis. For the thin-walled fraction, only one analysis could be conducted. The content of this fraction is given by assuming a sample weight of 1 mg (see text). G, guaiacyl; S, syringyl; i., inner; m., middle; o., outer.

across the fibre cap with high spatial resolution. Figure 8a shows the averaged spectra of five measurements at three different radial positions in the fibre cap. An absorbance maximum at 262 nm was observed. The spectra are a superposition of the absorbance pattern of the lignin with an absorbance maximum usually occurring between 270 and 282 nm (Fergus & Goring 1970) and of the *p*-hydroxybenzoate esters that absorb most strongly between 244 and 255 nm (Doub & Vandenbelt 1947;

Figure 8. (a) Absorbance spectra from three different radial positions in the fibre cap. Each spectrum represents the calculated average of five single spectra taken at each radial position. Black line, position close to the phloem; dark grey line, middle position; grey line, position close to non-lignified thin-walled fibres. (b) Composite image of a toluidine blue-stained cross section and two-dimensional UV absorbance scans taken of an unstained cross section at 262 nm.

Fink *et al.* 1978; Sircar *et al.* 2007). The maximum absorbance decreased from the central to peripheral positions. Neither a peak shift nor a major change in the shape of the absorbance spectra was found. Hence, the absorbance at 262 nm can be directly considered as a relative measure of the lignin and *p*-hydroxybenzoate ester content.

Two-dimensional scans were performed at 262 nm with a step size of 0.25 µm to map the absorbance across the fibre cap (figure 8b). A gradual decrease in the absorbance from values of up to 0.8, close to the phloem, to values of approximately 0.4 for the outer thick-walled fibres was observed. The thin-walled fibres showed hardly any absorbance higher than the background values. This gradient in the content of cell wall phenolics across the fibre cap is in agreement with the thioacidolysis results.

4. DISCUSSION

Plants are able to adapt the mechanical properties of their tissues and cells in a variety of ways at different levels of hierarchy. Hence, to gain a better understanding of the principles of mechanical optimization, the underlying structures of the organism have to be studied at different levels of hierarchy. In the present work, we investigated the mechanical, micro- and ultrastructural and biochemical properties of the fibre caps of the palm *W. robusta* with high spatial resolution. The aim was to gain insight into the principles of functional optimization of the transition from the stiff supporting fibres to the soft parenchymatous tissue at the different structural levels. The investigated type of vascular bundle represents the majority of vascular bundles in the trunk taken; yet there exists another form of different appearance (figure 1a), which might possess a different kind of transition. In addition to this, the structure of palms is highly dynamic in time. With ageing, the fibre caps and the parenchymatous tissue become considerably stiffer as successive cell wall layers are deposited in the fibre cells, and the parenchyma gets lignified (Rich 1987a; Tomlinson 1990). We are therefore aware that our findings, which are based on just one organism, might represent only one out of many different strategies of optimization having evolved in different palm

species and plant families. Yet, our findings provide new insights into the mechanics of palm trees, and the micro- and nanostructural organizations of their stiffening elements represent an interesting concept to lower stress discontinuities under specific evolutionary constraints of anatomical organization.

A gradual decrease in tissue stiffness from the centre to the periphery of the fibre cap was measured (figure 5a). The outermost fibre strips showed stiffness values in the range of parenchymatous tissue measured also in other plant species (Niklas 1988). The way the gradient in stiffness is achieved became obvious by the determination of cell wall area fraction (figure 4c) and calculation of cell wall stiffness (figure 5b). Obviously, the gradual transition takes place at different levels of hierarchy including the amount of cell wall material as well as the ultrastructure and biochemical composition of the cell wall.

The investigation at the ultrastructural level by means of X-ray diffraction revealed relatively high values of the microfibril angle without a visible trend across the fibre cap. The large scattering of values might be partly due to the misalignment of cell walls in relation to the incoming beam. Yet, a trend in the microfibril angle would have been also detectable in this case. A gradual decrease in the values of ρ , which reflects the degree of orientation, was measured from the centre to the periphery of the fibre cap (figure 6b–c). It is obvious that for small microfibril angles, the axial tensile stiffness is directly related to the amount of oriented fibrils, and thus to ρ . This relation, though, cannot be simply transferred to the present system with its large microfibril angles.

The chemical analysis of the lignin revealed a decrease in lignification across the fibre cap. Thioacidolysis yields suggested that the lignin content of the inner fraction, next to the phloem, was 1.7-fold higher than that of the middle fibre fraction, further away from the phloem (figure 7). The outer thin-walled fibres seemed to be hardly lignified. As each fraction contained fibres from more than 30 different vascular bundles, and the duplicate analyses showed values within the weighing error of 10%, the differences seem to be reliable. The results may underestimate the total amount of lignin, yet they represent

a good estimate when comparing relative values with each other. Comparisons of lignin contents reflect correct ratios, if the fraction of β -O-4-bonds (or the degree of condensation) remains constant throughout the investigated samples.

The analysis has further revealed high S : G ratios with very low amounts of *p*-hydroxyphenyl units, which is in good agreement with prior investigations on lignins from other palm species, categorizing palm lignin as a syringyl-rich type of lignin (Gallacher *et al.* 1994; Sun *et al.* 1998; Kuroda *et al.* 2001). The cited measurements, though, represent bulk measurements that have not resolved differences at the cellular level. Our measurements allow the resolution of differences in the lignin content and the S : G ratio at the cellular level. We observed an increase in the S : G ratio from 2.4 : 1 to 4.9 : 1 within the thick-walled fibres from the inner to middle region of the fibre cap. The thin-walled fibres in the cap periphery showed again a lower S : G ratio of 3.0 : 1 (figure 7). The latter is consistent with the observation that the thin-walled fibres lacked most of the secondary cell wall layers found in the inner thick-walled fibres, and consisted mainly of middle lamella and primary cell wall, which have a lower S : G ratio in hardwoods (Fergus & Goring 1970).

The two-dimensional UV scans revealed a gradual decrease in absorbance values reflecting the content of cell wall phenolics (i.e. lignin, *p*-hydroxybenzoate esters), with a twofold difference between the highest values found close to the phloem and the lowest values found close to the thin-walled fibres (figure 8). Despite the presence of *p*-hydroxybenzoate esters in significant amounts, the overall absorbance pattern should reflect the pattern of lignification, as the *p*-hydroxybenzoate esters are closely associated with the lignin and most probably show the same distribution. The results of the UV measurements are in good agreement with the differences in the levels of lignification observed in the chemical analysis. The differences in lignin content may be overestimated, though, as the chemical analysis revealed shifts in the S : G ratio, and the extinction coefficient is three times higher for guaiacyl than for syringyl (Fergus & Goring 1970).

It is well known for softwoods and hardwoods that changing the orientation of cellulose microfibrils in fibre cell walls is a powerful nanostructural tool to adjust the stiffness of tissues (Lichtenegger *et al.* 1999; Burgert 2006). Interestingly, the X-ray diffraction analysis revealed a constant and rather high microfibril angle (figure 6a). Consistently, the calculated cell wall stiffness of 1–2 GPa is considerably lower than that of wood tissues, which is in the range of 10–20 GPa (Reiterer *et al.* 1999). A reason might be the different growth forms of monocotyledonous and dicotyledonous plants, which put different mechanical constraints on the tissues. While dicotyledonous plants form wood tissues with rather moderate stiffness alterations (e.g. between earlywood and latewood) by cambial growth, monocotyledonous plants consist, to a large extent, of soft parenchymatous tissue, in which single vascular bundles with stiff fibre caps are embedded. Hence, the parenchyma sets limits in the possible design range of the fibre cap cells. The stiffness of the fibre caps should not dramatically exceed the stiffness of the parenchymatous tissue to avoid stress discontinuities under wind loads. Consistently, we found a rather high microfibril angle without considerable changes across the fibre cap. This orientation pattern also results in a high extensibility of the

stiffening tissue, which can be seen in the values for the fracture strain of the fibre strips. Thus, palms can cope with considerable deformations under wind loads.

However, the stiffness of the inner thick-walled fibres was up to two orders of magnitude higher than the reported values for the parenchymatous tissue. A mechanical gradient, which means a gradual transition in stiffness between fibres and parenchymatous tissue, was measured, which is most probably beneficial for avoiding stress discontinuities under the given growth conditions. Although we did not find changes in the orientation of the cellulose microfibrils, a further tool for stiffness adjustments was identified. The axial stiffness of cell walls with high microfibril angles highly depends on the shear modulus of the polymer matrix (Hull & Clyne 1996; Fratzl *et al.* 2004). The observed decrease in lignification towards the periphery of the fibre cap is a strong indication for the softening of the polymer matrix in order to achieve a gradient in axial stiffness. In addition, the guaiacyl monomers can better contribute to a strong three-dimensional lignin network, because they possess two possible free binding sites at the aromatic ring, whereas syringyl monomers possess only one. Hence, the decrease in the amount of guaiacyl units in the lignin structure can be regarded as a further strategy in matrix softening and thereby stiffness regulation for cell walls with high microfibril angle.

5. CONCLUSIONS

The embedding of vascular bundles with stiff fibre caps in the soft parenchymatous tissue in palms is of particular interest from a biomechanics as well as biomimetics perspective. A combination of gradients in density, structure and biochemical composition seems to be responsible for the measured gradual decrease in stiffness across the fibre cap. At the tissue and cellular levels, changes in cell cross-sectional area and cell wall thickness result in a decrease in density towards the periphery of the fibre caps. At the ultrastructural level, changes in the degree of orientation of the cellulose microfibrils were observed. The microfibril orientation, though, did not show any visible trend. At the biochemical level, a gradient in lignification and a change in lignin composition were observed. These variations in the biochemical level most probably have an effect on the shear modulus of the matrix, which in turn influences the cell wall stiffness. These findings point to an additional concept of how plants control and change the stiffness of their tissues besides the well-known strategy of changing the orientation of cellulose microfibrils in the secondary cell wall layers. Here, we have identified independent sources acting together at different levels of hierarchy to create a stiffness gradient, which is believed to be the key for the mechanical optimization of palms under the given biological constraints. These insights may serve as inspiration for a biomimetic transfer of the underlying principles to technical fibre-reinforced composites.

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