

Peculiar tension wood structure in *Laetia procera* (Poepp.) Eichl. (Flacourtiaceae)

Julien Ruelle · Masato Yoshida · Bruno Clair ·
Bernard Thibaut

Received: 10 June 2006 / Revised: 5 October 2006 / Accepted: 16 January 2007 / Published online: 21 February 2007
© Springer-Verlag 2007

Abstract Tension wood of *Laetia procera* (Poepp.) Eichl. (Flacourtiaceae), a neo-tropical forest species, shows a peculiar secondary wall structure, with an alternance of thick and thin layers, while opposite wood of this species has a typical secondary wall structure (S1 + S2 + S3). Samples for the study of microstructural properties were collected upon the estimation of growth stresses in the living tree, in order to analyze the correlation of the former with the latter. Investigation using optical microscopy, scanning electron microscopy and UV microspectrophotometry allowed the description of the anatomy, ultra-structure and chemistry of this peculiar poly laminate secondary wall. In the thick layers, cellulose microfibril angle is very low (i.e., microfibril orientation is close to fibre axis) and cellulose microfibrils are well organized and parallel to each other. In the thin layers, microfibrils (only observable in the inner layer) are less organized and are oriented with a large angle relative to the axis of the cell. Thick layers are lightly lignified although thin layers show a higher

content of lignin, close to that of opposite wood secondary wall. The more the wood was under tensile stress, the less the secondary wall was lignified, and lower the syringyl on guaiacyl lignin units' ratio was. The innermost layer of the secondary wall looks like a typical S3 layer with large microfibril angle and lignin occurrence. The interest of this kind of structure for the understanding of stress generation is discussed.

Keywords Tension wood · Tropical rainforest species · UV microspectrophotometry · Scanning electron microscopy · Cellulose microfibril angle

Introduction

In order to restore their verticality after accidental leaning, to maintain the branch at a given angle or to change axis orientation to reach the canopy for better access to light trees are able to bend progressively their trunk or branches by a very active mechanical action driven by cambial activity (Sinnott 1952). This reorientation is associated with the formation of a peculiar type of wood, called reaction wood. In gymnosperm species, reaction wood is formed on the lower side of the tilted axis (compression wood), while in angiosperm species it is formed on the upper side (tension wood). Whatever the species considered, the process of axis reorientation is always based on circumferential heterogeneity in cambial region (cambial zone, differentiating and maturing zone) activity occurring at three distinct structural levels:

- at the macroscopic level, the division and differentiation of the cambial initials is controlled differently

Communicated by H. Cochard.

J. Ruelle (✉) · B. Thibaut
UMR EcoFoG, Campus agronomique,
BP 709, 97387 Kourou Cedex, French Guiana
e-mail: ruelle_j@kourou.cirad.fr

M. Yoshida
School of Bioagricultural Sciences, Nagoya University,
Chikusa, Nagoya 464-8601, Japan

B. Clair
Laboratoire de Mécanique et Génie Civil (LMGC),
UMR 5508, CNRS, Université Montpellier 2,
Place E. Bataillon, cc 048,
34095 Montpellier Cedex 5, France

- between the upper and the lower side of the trunk. This can lead to an eccentric growth that causes the reaction wood side to be often wider than the opposite side (Dadswell and Wardrop 1949; Almeras et al. 2005);
- at the mesoscopic level, proportions of the various cells types (fibres, vessels, ray and axial parenchyma) constituting secondary xylem can vary substantially between normal and reaction wood (Onaka 1949; Jourez et al. 2001; Ruelle et al. 2006);
 - at the microscopic level, fibres produced during the reaction process strongly differ structurally from normal fibres. This occurs through the modulation of various structural features: (1) secondary wall fibre thickness, that tends to increase in some species (Ruelle et al. 2006); (2) size and orientation (MFA) of secondary wall cellulose microfibrils: Washusen and Evans (2001) reported an increase of microfibrils size in tension wood and MFA is known to be lower in tension wood and larger (up to 45°) in compression wood (Timell 1986; Yoshida et al. 2000; Yoshizawa et al. 2000; Barnett 2004; Clair et al. 2006a); (3) organization and chemical composition of hemicellulose/lignin matrix surrounding cellulose microfibrils (Pilate et al. 2004; Gorshkova and Morvan 2006).

The mechanism allowing reorientation of the axis originates in structural modifications at the cell-wall level. Indeed, these micro-structural modifications induce a spontaneous tendency in wood to strain during its maturation process (Boyd 1977; Yamamoto 1998; Bamber 2001; Yamamoto et al. 2002). The maturation process of cell wall can be subdivided into steps from cell expansion, secondary wall formation and lignification to cell death (Plomion et al. 2001). During this process compression wood tends to swell and tension wood tends to shrink. This tendency is impeded because reaction wood is stuck to the core of old wood, resulting in a state of mechanical stress (compression or tension), called maturation stress. The asymmetry of longitudinal maturation stress around the circumference results in a bending moment generating a change in curvature and thus a reorientation movement (Archer 1986). The efficiency of this mechanism depends on the difference between the force acting on the reaction wood side and the force acting on the opposite side. The magnitude of the force acting on one side is the integral of the product of the area of maturing wood by the magnitude of the maturation stress in wood. The magnitude of maturation stress in wood, in turn, can be viewed as the product of the maturation strain by wood Young's modulus of wood. Finally, Young's modulus can be expressed as the product of

wood density by the specific modulus of wood material elasticity (Almeras et al. 2005).

All four of these biomechanical factors, i.e. maturation stress asymmetry and magnitude, Young's modulus of wood and specific modulus of wood material can be controlled through modulations of cambial activity at the above-mentioned levels. Eccentric growth controls the area of reaction wood and opposite wood. Proportions of each cell type in the xylem partly control the specific modulus of elasticity of wood. Fibre wall thickness controls wood density. Cellulose microfibril geometry and matrix composition partly control specific modulus of elasticity and directly control sign and magnitude of maturation strain (Okuyama et al. 1995; Yamamoto 1998). The mechanical effect of these structural modifications can be predicted by mechanical models acting at different levels. At the macroscopic level, reaction efficiency can be computed using beam theory and the principles of its application to a growing structure (Fournier et al. 1994a). Fournier and coworkers model (1994a) takes into account the effects of eccentric growth, modulus of elasticity and maturation strain (Almeras et al. 2005). At the mesoscopic level, the effect of wood anatomy (i.e. the proportions and organisation of the different cell types) can be predicted by homogenization procedures (Badel 1999). At the microscopic level, cell wall micromechanical models allow to predict wood specific modulus of elasticity and magnitude and sign of maturation strain (Yamamoto et al. 1998, 2002). These models take quantitative information about cell wall organisation at the microscopic and ultra-structural levels, cell wall chemical composition, and timing of cell wall differentiation and lignification as input data.

The structure of reaction wood fibres generally differs from that of normal wood fibres. In gymnosperms, compression wood fibres typically have a round shape, intercellular spaces and cracks in the cell wall (Timell 1986). Their wall is thick and heavily lignified, and the microfibrils are oriented at a wide angle with respect to the fibre axis. Among angiosperms, diversity in the form of tension wood fibres has long been recognized (Onaka 1949; Clair et al. 2006b). The most typical form of angiosperm tension wood is characterized by the development of a so-called gelatinous layer (G-layer). The G-layer is essentially made up of highly crystalline cellulose (Norberg and Meier 1966; Côté et al. 1969), with a very low microfibril angle (Fujita et al. 1974). However, several species do not develop G-fibres, while showing evidence of tension wood production (Fisher and Stevenson 1981; Clair et al. 2006b).

During an exploration of biomechanical strategies of tropical rainforest species, trees from *Laetia procera* species proved to be very efficient in restoring verticality after accidental leaning (Almeras et al. 2005). Moreover, dissymmetry in the magnitude of maturation strain was identified as the leading factor determining efficiency. The maturation strain of tension wood was especially high in this species and tension wood fibres presented a peculiar polylamellated structure. In order to check whether this macroscopic behaviour was related to characteristic structural features, tension wood and opposite wood were investigated using complementary techniques. In this way, optical and electronic microscopy, histochemical reaction and UV microspectrophotometry allowed us to obtain quantitative measurement about the structure and qualitative and semi-quantitative information about the chemical composition of the secondary wall and of the fibres.

Materials and methods

Plant material and sampling

Laetia procera (Poepp.) Eichler (Flacourtiaceae) is rare to locally frequent in primary and secondary neotropical forests, on sandy soil.

Five trees were selected in the same area in French Guyana, near Kourou. Their diameter at breast height (DBH) ranged between 19 and 28 cm (Table 1).

All trees were chosen because they were exhibiting a reorientation process after some accidental inclination. This was verified in situ by mechanical estimation of growth strain (GS). Experimentally, maturation strain can be estimated by releasing the longitudinal stress at the surface of wood and measuring the resulting strain, referred to as residual growth strain (GS). Growth strain is the sum of the maturation and support strains. At the periphery of the trunk, where measurements were done, support strain are due to the support of the newly formed layer and so are close to zero. Thus, growth strain (GS) is very close to maturation strain. In

the following text we will use GS, since it is what has been experimentally measured.

GS were evaluated using the “single hole” method (Fournier et al. 1994b; Clair et al. 2003; Almeras et al. 2005). This method gives the value of the displacement between two pins, hammered onto the trunk (after local debarking) at a 45 mm distance from each other. A 20-mm deep, 20-mm wide hole is drilled at the midpoint between the two pins. A displacement is measured (in μm) and converted into a strain (in %) using a calibration factor: 9.6×10^{-4} corresponding to a calibration made on *Eperua Falcata* (Fournier et al. 1994b), a tropical hardwood species with properties similar to those of *Laetia procera*.

Eight measurements (equally spaced around the circumference, i.e. every 45°) were performed at breast height on each tree. The first measurement position was located on the upper side of the leaning trunk. Two wood samples were taken, above and below the holes resulting from the measurement method, for anatomical and structural studies. Observations were made on both samples to ensure homogeneity of the studied wood (Fig. 1).

Methods

First, classical optical microscopy on stained sections (including Wiesner reaction) was used for all the five trees, in order to check if there were main differences between trees or if the variation, varied from one tree to another. Then, other techniques (Scanning electron microscopy, UV microspectrophotometry) were only applied to the tree with the highest contrast in GS between upper and lower part, Lp1 (Table 1).

Optical microscopy

Cross sections (thickness $24 \mu\text{m}$) were made with a sliding microtome using disposable razor blades (Feather N35). Sections were stained with safranin/fast green according to the protocol described by Yoshida et al. (2002). Safranin stains lignified tissues in red and

Table 1 Diameter at breast height (DBH, in cm), Growth Strain (microstrains) means value, standard deviation and number of positions used for upper and lower side for each tree

Trees	DBH (cm)	Tension wood (TW)		Opposite wood (NW)		Upper –lower side means
		Mean of the upper side	Number of positions	Mean of the lower side	Number of positions	
Lp1	19	$2,714 \pm 700$	3	666 ± 293	3	2,246
Lp2	23	$2,035 \pm 625$	2	515 ± 23	3	1,520
Lp3	28	$2,701 \pm 318$	3	666 ± 247	3	2,035
Lp4	22	$1,590 \pm 681$	3	582 ± 105	3	1,008
Lp5	26	$2,912 \pm 404$	3	416 ± 200	3	2,496

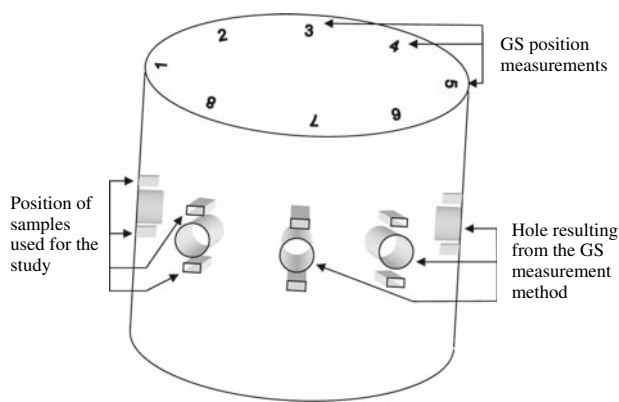


Fig. 1 Localization of specimen used for the experiments

fast green stains both lignified and un-lignified tissues in green.

Wiesner reaction

Cross sections (thickness 12 μm) were made on opposite and tension wood specimens. Wiesner reaction was performed on these sections by pouring a few drops of 2% phloroglucinol ethanol solution on the section mounted on a glass-slide, adding one drop of 35% HCl and covering the section with a cover slip.

The Wiesner reactive reacts with coniferyl (G) and synapyl (S) aldehyde units in lignin. The higher the Klason-lignin content the stronger the intensity of the red coloration (Yoshizawa et al. 2000). This result provides us to do a comparison between the samples, i.e. a semi-quantitative analysis. As the coloration is not permanent, observation was performed during the 20 min after the beginning of the reaction.

UV microspectrophotometry

After dehydration through a graded ethanol series, the sections were embedded in epoxy resin. Thin cross sections (thickness 1 μm) were cut with a diamond knife, mounted on quartz microscope slides, overlaid with a drop of non-UV-absorbing glycerin, and covered with a quartz cover slip (Okuyama et al. 1998). The sections were observed under a microspectrophotometer (Zeiss MPM800). The scanning range of the wavelength was 250–350 nm, the step of the wavelength scanning was 1 nm, and the bandwidth was adjusted to 5 nm. UV absorption spectra were obtained at various locations inside the secondary wall of opposite and tension wood fibres using a beam spot of 0.5 μm diameter. The absorption spectra directly provide information on cell wall lignin content (Okuyama et al. 1998; Gindl 2002; Yoshida et al. 2002); the higher

the absorption, the more lignified the wall. Results from this technique can be used to make a comparison between specimens; i.e., a semi-quantitative analysis among samples.

Each measurement for one position (one part of fibre wall) was repeated eight times. For each specimen the absorption spectrum of the secondary wall was taken at least on five different fibres and averaged to determine the UV absorption spectrum of the specimen. The microspectrophotometer settings were: objective lens magnification 100 $\times$, program Lambdascan; (for more detailed information see Okuyama et al. 1998).

Field-emission scanning electron microscopy (FE-SEM)

Observations were made in both transverse and longitudinal planes. Sample geometries were $7 \times 5 \times 1$ and $5 \times 1 \times 7$ mm³, respectively (R $\times$ T $\times$ L). Samples were dehydrated through a graded ethanol series and then processed using the *t*-butanol freeze-drying method. In order to observe cellulose microfibrils of lignified layers, a lignin extraction treatment (NaCl 0.6%, CH₃COOH 0.13% in distilled water during 40 h) was performed on longitudinal sections. The dried samples were mounted on aluminium stubs and lightly sputter-coated with platinum. Samples were observed by FE-SEM (Hitachi, S-4500) at an accelerating voltage of 3 kV.

Microfibril angle (MFA) and diameter of cellulose aggregates were measured from direct observations by SEM on samples from tree Lp1. These measurements were made on ten pictures per sample with magnifications from 30,000 $\times$ to 70,000 $\times$ and on about 20 microfibrils per picture for MFA and on ten areas of about 5,000 nm² for the diameter of cellulose aggregates. Examples of images used for these measurements are shown in Fig. 2.

Statistical analysis

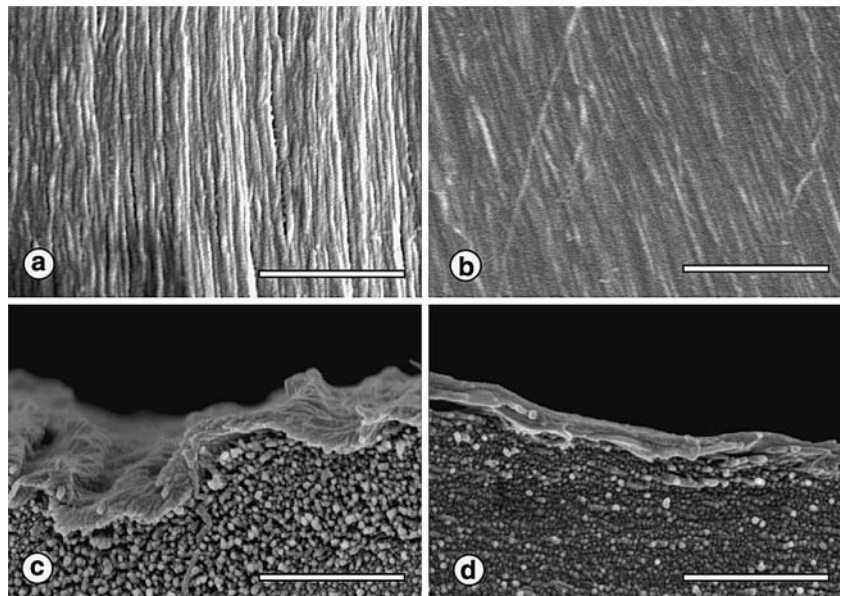
Results from MFA and cellulose aggregates measurements were compared to highlight significant differences between tension and opposite wood samples. We used the bilateral Student test to account for the significance of these results.

Results

Growth strain measurements

Measurements clearly show that wood located on the upper side exhibits much higher tensile growth strains

Fig. 2 Example of images used for the measurements of microfibril angle on tension wood (**a**), opposite wood (**b**) and cellulose aggregates diameter on tension wood (**c**), opposite wood (**d**). Scale bars: 500 nm



than wood located in all other position (Table 1). Measurements performed on the lower (opposite) side do not present significant difference in mechanical stressing with lateral position. Upper wood layer positions with very high growth strains are called tension wood (TW) in the next paragraphs while other positions are named opposite wood (OW) or lateral wood.

Considering data reported by Archer (1986) and more recent studies (Yoshida et al. 2000; Yoshizawa et al. 2000; Clair et al. 2006b), GS observed in tension wood are in the upper range of reported values.

Structure of the fibre wall

Polylaminate secondary wall of tension wood fibres

Optical microscopy observations after safranin/fast green staining show a homogeneous typical secondary wall in opposite wood fibres, while a peculiar polylaminate secondary wall structure is observed in tension wood fibres (Fig. 3). This structure consists of an alternance of thick and thin layers. This peculiar secondary wall stains as a typical gelatinous layer (G-layer), i.e. in green without any touch of red as in *Populus euramericana* (Jourez et al. 2001) or *Eperua falcata* (Satiat-Jeunemaitre 1986).

Observation of transversal and longitudinal sections by Scanning electron microscopy (SEM) confirms that the polylaminate structure of the secondary wall occurs in tension wood but not in lateral or opposite wood fibres (Figs. 4, 5). The number of thin layers has been counted on tension wood specimens; results are given in Table 2. There was an average of 5–6 thin layers with

thick layers between them. Thick layers are approximately ten times thicker than thin layers (Table 2).

Inner thin layer

Observations of longitudinal sections (Fig. 6a) also show a lignified layer inside the lumen of tension wood fibres; this inner layer allowed us to prospect the nature and the structure of thin layers observed in the polylaminate secondary wall. The aspect of this layer before (Fig. 6b) and after (Fig. 6c) lignin extraction treatment highlights its lignified feature and its large MFA. These features are typical of the S₃ layer commonly observed in the cell wall of opposite wood fibres (Fig. 7a).

Organisation of cellulose in the secondary wall

MFA was very low (close to fibre axis) in the thick layers of tension wood fibres and more than three fold larger (15–20°) in opposite wood ($P < 0.001$; Table 3). Unfortunately we were unable to accurately measure MFA in intermediate thin layer.

Diameter of cellulose aggregates is in the range of values reported by Fahlen and Salmen (2003) on *Picea abies*, i.e. between 18 and 23 nm, and is lower in opposite wood ($P < 0.001$) than in tension wood, respectively 18.4 and 21.9 nm.

Lignification features of tension wood fibres

Wiesner reaction

Intensity of the Wiesner reaction gives qualitative information on cell wall lignification features.

Fig. 3 Cross sections of tension wood (**a**) and opposite wood (**b**) from *Laetia procera* stained with safranin/fast green. Scale bars: 25 μm

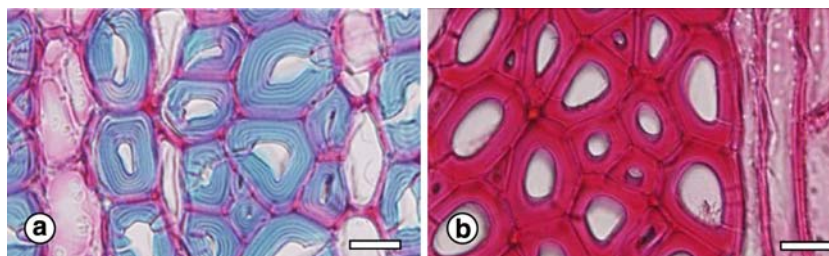


Fig. 4 Cross section of tension wood (**a**) and opposite wood (**b**) of *Laetia procera*, observed with SEM. Scale bars: 5 μm

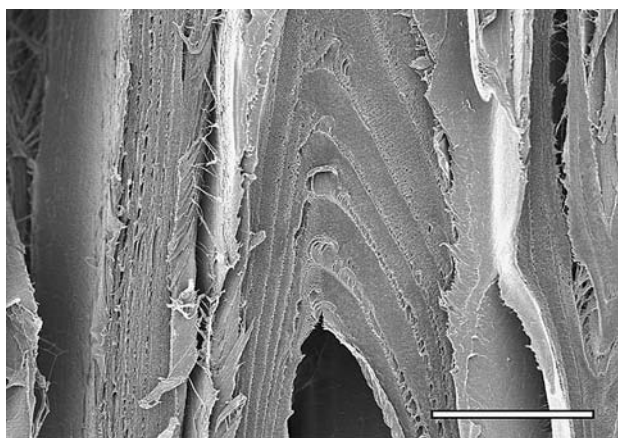
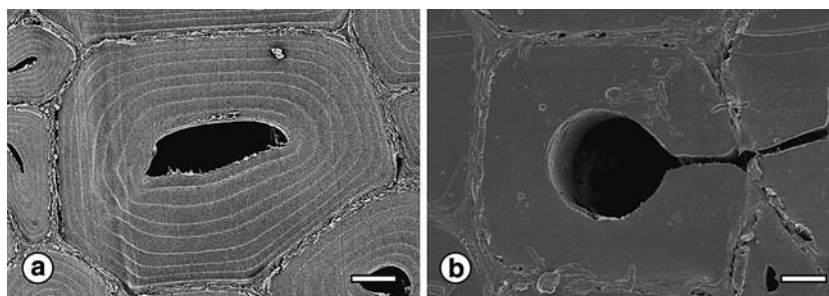


Fig. 5 SEM observation of the polylaminate structure of the secondary wall in tension wood fibre on a longitudinal section of *Laetia procera*. Scale bar: 15 μm

Polylaminate tension wood fibres appear less lignified than opposite wood fibres (Fig. 8). In TW fibres the reaction is stronger in the outer part and becomes weaker towards the centre. Some TW fibres have

stronger reaction intensity than others; some of them even show a lack of reaction on the polylaminate structure although the S_1 layer and the primary wall are stained in red by the reaction (Fig. 9). This could mean that various types of tension wood fibres can be observed.

Compared to typical G-layers, known to have very low lignin contents, the secondary wall of *Laetia* TW presents lignin within the thick layers and the coloration inside thin ones indicates a higher amount inside them.

UV microspectrophotometry

The average absorption spectra for the S_2 layer of a opposite wood sample (Lp1–5) and for the thick and thin layers of the secondary wall of a tension wood specimen (Lp1–2) are given in Fig. 10.

In tension wood, the average absorption, and therefore polylaminate layer lignin content, is lower than in the S_2 layer of opposite wood. Actually absorbance values in tension wood specimen ranges

Table 2 Values and standard deviation of the various measurements on fibre secondary wall of tension wood specimen from Lp1 tree

Tree	Specimen	GS (μstrain)	Thick layer thickness (μm)	Thin layer				Thin layer thickness/thick layer thickness
				Thickness (μm)	Number			
					Min	Mean	Max	
Lp1	1	3,504	1.38 ± 0.26	0.08 ± 0.03	4	5.30	8	0.06
	2	2,170	1.31 ± 0.15	0.13 ± 0.03	5	6.00	7	0.10
	3	2,467	1.44 ± 0.52	0.15 ± 0.03	4	5.13	7	0.11

Fig. 6 Longitudinal section of *Laetia procera* tension wood. **a** Observation of the lignified layer inside the lumen (scale bar: 15 μm). **b** Detail of the image a (scale bar: 1 μm). **c** Detail of the inner layer view from the lumen after a lignin extraction treatment (scale bar: 1 μm). Microfibril angle in the inner layer is very large

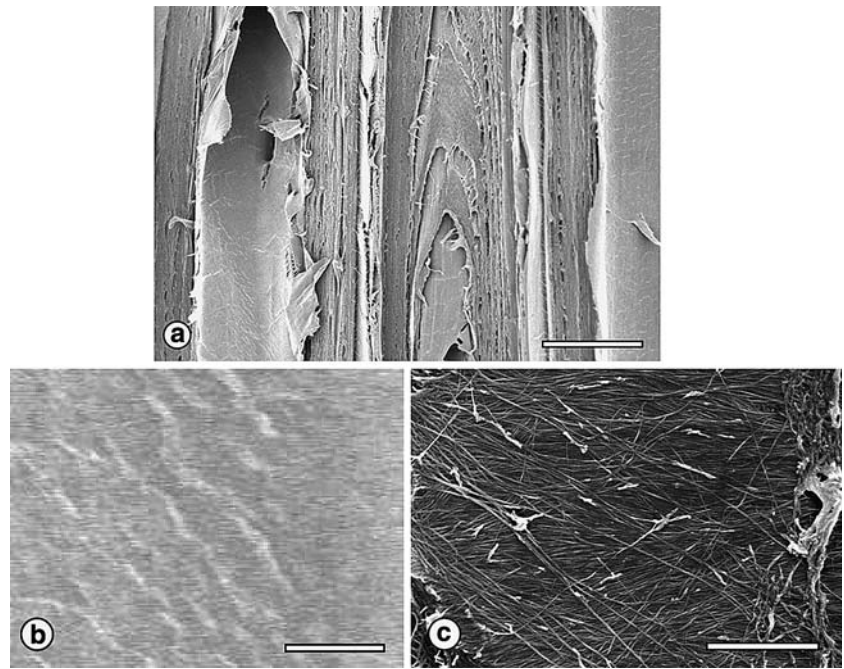
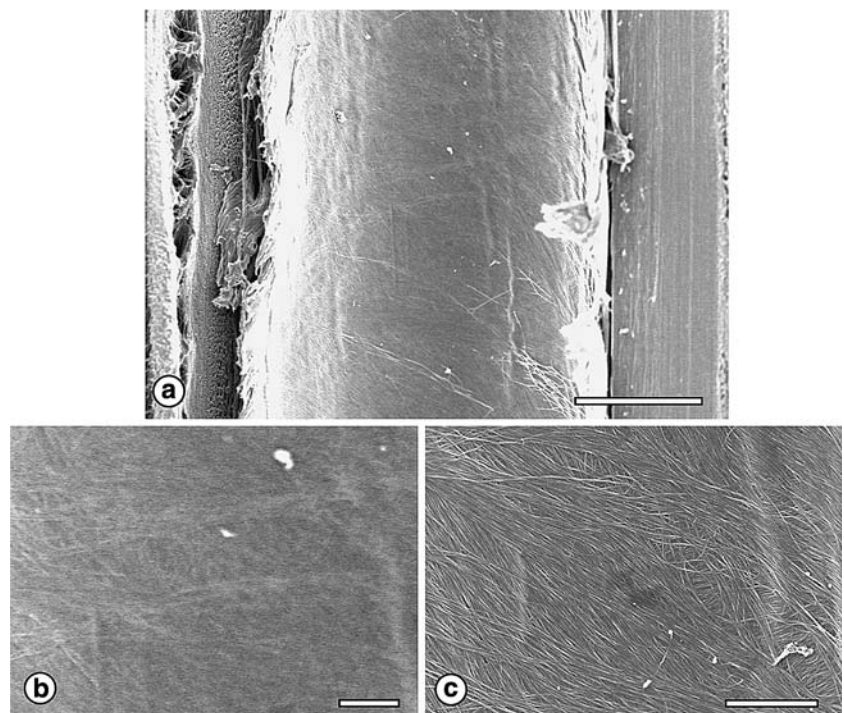


Fig. 7 Longitudinal section of *Laetia procera* opposite wood. **a** Observation of the lignified layer inside the lumen (scale bar 5 μm). **b** Detail of the image a (scale bar 1 μm). **c** Detail of the inner layer view from the lumen after a lignin extraction treatment (scale bar 1 μm). Microfibril angle in the inner layer is very large



from 0.15 to 0.24 at 280 nm and from 0.14 to 0.22 at 270 nm. In opposite wood absorbance values are higher than in tension wood and ranges from 0.34 to 0.53 at 280 nm and from 0.31 to 0.47 at 270 nm. Moreover the average absorption of the whole fibre at 270 and 280 nm decreases with increasing growth strain values (Table 4; Fig. 11).

UV microspectrophotometry also shows that lignification of TW is stronger in secondary wall thin layers

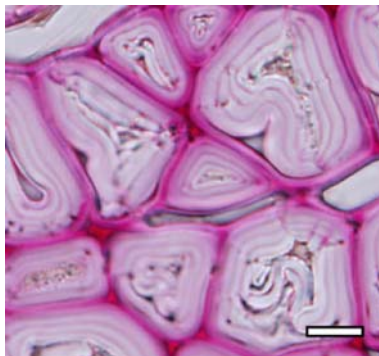
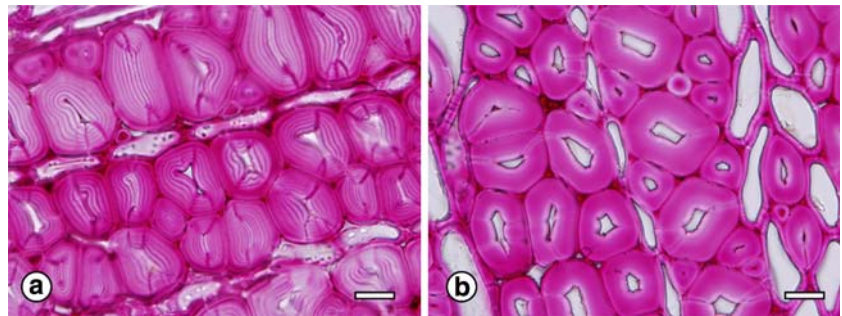
than in the thick layer. Thus, intermediate thin layers are chemically different.

The absorption ratio A_{280}/A_{260} markedly depends on the ratio of syringylpropane (S) to guaiacylpropane (G) units. Actually the decrease of this ratio corresponds to an increasing S/G ratio (Okuyama et al. 1998; Yoshida et al. 2002). This ratio for tension wood specimens is largely dominated by the properties of thick layers, because of the prominent proportion of

Table 3 MFA and diameter of cellulose aggregates (mean $\pm$ SD) for each sample from Lp1 tree

Tree	Type of wood	Specimen	GS (μ strains)	Average MFA ($^\circ$)/specimen	Average MFA ($^\circ$)/type of wood	Average diameter of cellulose aggregates (nm)/specimen	Average diameter of cellulose aggregates (nm)/type of wood
Lp1	TW	1	3504	3.1 ± 1.9	5.2 ± 3.1	22.8 ± 3.0	21.9 ± 0.8
		2	2170	8.6 ± 5.7		21.7 ± 2.2	
		3	2467	3.8 ± 2.3		21.3 ± 3.3	
	LW	4	-115	16.6 ± 4.8	17.5 ± 2.8	18.3 ± 1.6	18.4 ± 1.6
	OW	5	346	20.7 ± 5.3		20.4 ± 2.2	
	OW	6	922	16.2 ± 6.2		17.2 ± 1.9	
	OW	7	730	13.9 ± 4.8		16.4 ± 2.1	
	LW	8	461	19.9 ± 5.2		19.6 ± 1.6	

TW tension wood, OW opposite wood, LW lateral wood

Fig. 8 Result of the Wiesner reaction on transversal sections of tension (a) and opposite (b) specimen of *Laetia Procera*. Scale bars 20 μ m**Fig. 9** Detail of a transversal section of tension wood specimen, showing tension wood fibres with lack of Wiesner reaction. Scale bar 10 μ m

these layers compared to thin layers. According to our results, S/G ratio increases with growth strains, with a good relationship (Table 4; Fig. 12). This evolution has also been described in some other species differentiating or not a G-layer in their tension wood, such as eucalyptus (Baillères et al. 1995) or *Liriodendron tulipifera* (Yoshida et al. 2002).

Discussion

Observations made on *Laetia procera* tension wood show a polylaminate structure of the secondary wall with the alternance of lightly lignified thick layers, with

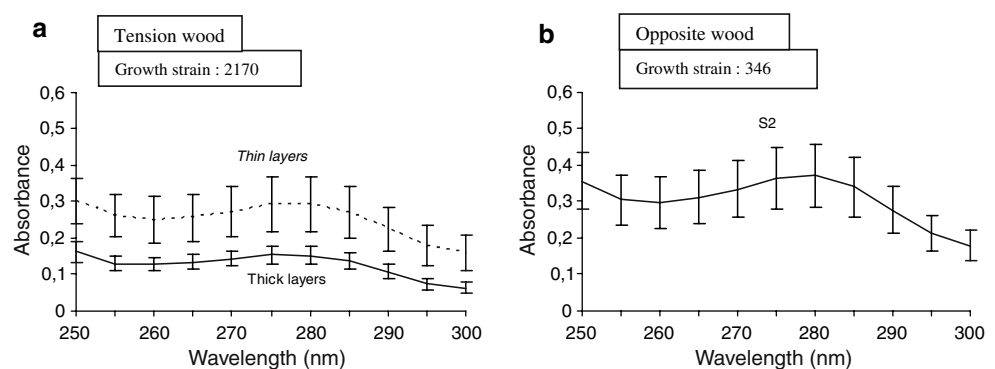
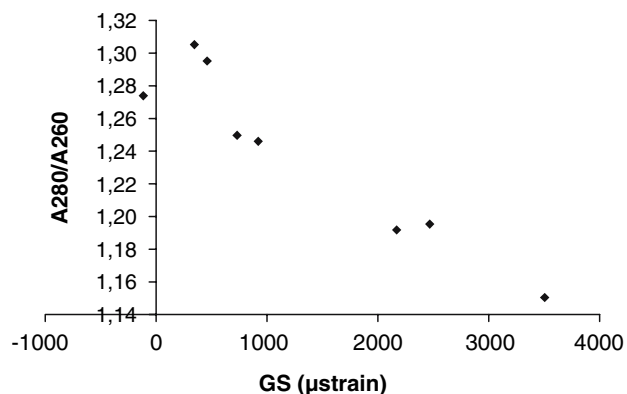
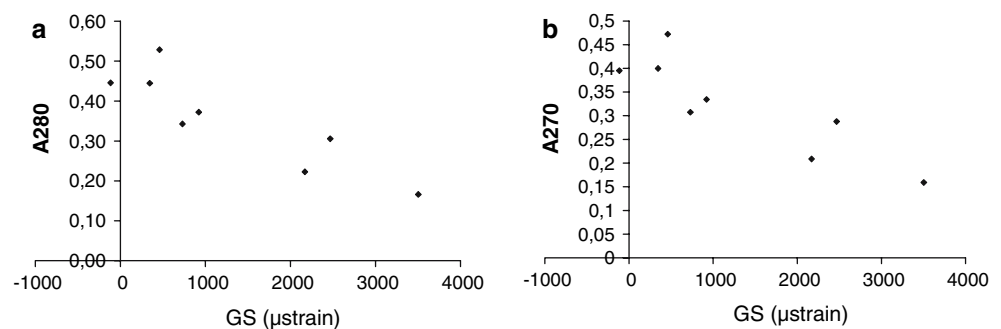
Fig. 10 UV absorption spectra of a tension wood (a) specimen (Lp1–2) and an opposite wood (b) specimen (Lp1–5) of Lp1 tree. Bars are standard deviations

Table 4 Absorbance at wavelengths 270 and 280 nm and ratio of absorbance A280/A260 of the various layers of specimen of *Laetia procera* n°1

	Tree	specimen	GS (μstrains)	Thick layers			Thin layers			
				A280	A270	A280/A260	A280	A270	A280/A260	
Tension wood specimen	Lp1	1	3504	0.15	0.14	1.15	0.18	0.18	1.08	
		2	2170	0.15	0.14	1.19	0.29	0.27	1.17	
		3	2467	0.24	0.22	1.20	0.38	0.36	1.13	
Opposite and lateral wood specimen			S ₂ layers	A280	A270	A280/A260				
				4	-115	0.45	0.40	1.27		
				5	346	0.44	0.40	1.31		
				6	922	0.37	0.33	1.25		
				7	730	0.34	0.31	1.25		
				8	461	0.53	0.47	1.30		

Fig. 11 Absorbance at 280 nm (a) and 270 nm (b) versus growth strain values for specimen from Lp1 tree**Fig. 12** Absorbance ratio A280/A260 versus growth strain values of specimen from the tree Lp1

microfibrils almost aligned to the cell's main axis, and more lignified thin layers in which, it was not possible to measure microfibrils orientation. This kind of poly laminate structure has previously been observed in bamboo cells (Parameswaran and Liese 1976). In a recent study screening the anatomical diversity of tension wood among tropical dicotyledonous species (Clair et al. 2006b), similar features were observed in tension wood of an other Flacourtiaceae, *Casearia javitensis*.

Moreover Daniel and Nilsson (1996) observed this structure in a species from the Flacourtiaceae family, *Homalium foetidum*, but in their study its occurrence was not identified as a tension wood feature. Observation of this peculiar structure in tension wood fibres emphasises the idea exposed in Clair et al. (2006b) on the difficulty to classify tension wood structures.

The very low MFA observed in thick layers is similar to what is usually observed in tension wood fibres, with or without G-layer (Norberg and Meier 1966; Chaffey 2000; Ruelle et al. 2006). The microfibrils of the inner thin layer of these cells appear less organised and lie at a larger angle than those of thick layers. In their study on *Homalium foetidum*, Daniel and Nilsson (1996) observed differences in microfibril angle between thick and thin layers. These various observations lead us to hypothesise that cellulose organisation in the thin layers is (1) different from the one of thick layers and (2) similar to the one observed in the inner thin layer, so that all these thin layers look like successive S_3 layers between thick layers.

Prodhan et al. (1995) shows that G-layer in *Fraxinus mandshurica* is lignified, in the same way as in *Laetia procera*, with a higher content of lignin in the outer part of the G-layer. Similar observations were made by

Gierlinger and Schwanninger (2006), i.e., a higher lignification in the outer part of G layer, but in their work they found a very weak content of lignin in the rest of the G layer. These observations support the statement (Terashima 1990) that there is a lignification gradient from outer to inner part of the cell wall during fibre development. However the alternance of more lignified layers raises several questions about the development of the secondary wall and about its role in the very efficient strategy of reorientation observed in this species. In current fibre mechanics models, maturation strain associated to the end of the lignification process is assumed to occur simultaneously in the whole S₂ or G-layer. Moreover, it is known that the final pre-stressing values in a multilayered composite depend on the history of layer deposition (Yamamoto et al. 2002). This effect of successive step construction in a material on field of stresses is also true for various natural or man-made structures such as trees, bridges, etc. (Guitard et al. 1999; Malzbender 2004). It may be important to know whether each thick layer and associated thin layer is fully lignified successively or if the lignification process occurs after the deposition of all the layers.

This raises questions about the rhythm of secondary wall development. Hosoo et al. (2002, 2003) showed diurnal periodicity in the deposition of cell wall components on the innermost surface of developing cells. This seems to indicate that there is diurnal periodicity in cell wall formation, corresponding to the 24-h light-dark cycle. Question about relation between thick and thin layers alternance and circadian rhythm should be investigated, and young trees artificially inclined in controlled conditions can be a good material for such study, knowing that tension wood will be rapidly induced on upper part of the young tree (Jourez et al. 2001).

The multilayered tension wood has been, until now, only found in others genus of the Flacourtiaceae Family (Daniel and Nilsson 1996; Clair et al. 2006b). It should be interesting to investigate whether this feature occurs in the whole family.

Acknowledgments Many thanks to Tancrede Almeras for his critical review of this paper and Ivan Scotti for English corrections.

References

- Almeras T, Thibaut A, Gril J (2005) Effect of circumferential heterogeneity of wood maturation strain, modulus of elasticity and radial growth on the regulation of stem orientation in trees. *Trees* 19:457–467
- Archer RR (1986) Growth stresses and strains in trees. Springer, Heidelberg
- Badel E (1999) Détermination des propriétés élastiques et du retrait d'un cerne annuel de chêne dans le plan transverse: description de la morphologie, mesures des propriétés microscopiques et calculs d'homogénéisation
- Baillères H, Chanson B, Fournier M, Tollier MT, Monties B (1995) Structure, composition chimique et retraits de maturation du bois chez les clones d'eucalyptus. *Ann For Sci* 52:157–172
- Bamber R (2001) A general theory for the origin of growth stresses in reaction wood: how trees stay upright. *IAWA J* 22:205–212
- Barnett JR (2004) Cellulose microfibril angle in the cell wall of wood fibres. *Biol Rev* 79:461–472
- Boyd JD (1977) Basic cause of differentiation of tension wood and compression wood. *Aust For Res* 7:121–143
- Chaffey N (2000) Microfibril orientation in wood cells: new angles on an old topic. *Trends Plant Sci* 5:360–362
- Clair B, Ruelle J, Thibaut B (2003) Relationship between growth stresses, mechano-physical properties and proportion of fibre with gelatinous layer in chestnut (*Castanea Sativa* Mill.). *Holzforschung* 57:189–195
- Clair B, Almeras T, Sugiyama J (2006a) Compression stress in opposite wood of angiosperms: observations in chestnut, mani and poplar. *Ann For Sci* 63:507–510
- Clair B, Ruelle J, Beauchêne J, Prevost MF, Fournier M (2006b) Tension wood and opposite wood in 21 tropical rainforest species. 1. About the presence of G layer. *IAWA J* 27:329–338
- Côté WAJ, Day AC, Timell TE (1969) A contribution to the ultrastructure of tension wood fibers. *Wood Sci Technol* 3:257–271
- Dadswell HE, Wardrop AB (1949) What is reaction wood? *Aust For* 13:22–33
- Daniel G, Nilsson T (1996) Poly laminate concentric cell wall layering in fibres of *Homalium foetidum* and its effect on degradation by microfungi. *Recent Advances in Wood Anatomy*, New Zealand Forest Research Institute:369–372
- Fahlen J, Salmen L (2003) Cross-sectional structure of the secondary wall of wood fibers as affected by processing. *J Mater Sci* 38:119–126
- Fisher JB, Stevenson JW (1981) Occurrence of reaction wood in branches of dicotyledons and its role in tree architecture. *Bot Gaz* 142:82–95
- Fournier M, Baillères H, Chanson B (1994a) Tree biomechanics: growth, cumulative prestress, and reorientations. *Biomimetics* 2:229–251
- Fournier M, Chanson B, Thibaut B, Guitard D (1994b) Mesure des déformations résiduelles de croissance à la surface des arbres, en relation avec leur morphologie. Observation sur différentes espèces. *Ann Sci for* 51:249–266
- Fujita M, Saiki H, Harada H (1974) Electron microscopy of microtubules and cellulose microfibrils in secondary wall formation of poplar tension wood fibers. *Mokuzai Gakkaishi* 20:147–156
- Gierlinger N, Schwanninger M (2006) Chemical imaging of poplar wood cell walls by confocal Raman microscopy. *Plant Physiol* 140:1246–1254
- Gindl W (2002) Comparing mechanical properties of normal and compression wood in Norway Spruce: the role of lignin in compression parallel to the grain. *Holzforschung* 56:395–401
- Gorshkova T, Morvan C (2006) Secondary cell-wall assembly in flax phloem fibres: role of galactans. *Planta* 223:149–158
- Guitard D, Masse M, Yamamoto H, Okuyama T (1999) Growth stress generation: a new mechanical model of the dimen-

- sional change of wood cells during maturation. *J Wood Sci* 45:384–391
- Hosoo Y, Yoshida M, Imai T, Okuyama T (2002) Diurnal difference in the amount of immunogold-labeled glucmannans detected with field emission scanning electron microscopy at the innermost surface of developing secondary walls of differentiating conifer tracheids. *Planta* 215:1006–1012
- Hosoo Y, Yoshida M, Imai T, Okuyama T (2003) Diurnal differences in the innermost surface of the S2 layer in differentiating tracheids of *cryptomeria japonica* corresponding to a light–dark cycle. *Holzforschung* 57:567–573
- Jourez B, Riboux A, Leclercq A (2001) Anatomical characteristics of tension wood and opposite wood in young inclined stems of poplar (*Populus euramericana* cv “Ghoy”). *IAWA J* 22:133–157
- Malzbender J (2004) Mechanical and thermal stresses in multi-layered materials. *J Appl Phys* 95:1780–1782
- Norberg PH, Meier H (1966) Physical and chemical properties of the gelatinous layer in tension wood fibres of Aspen (*Populus tremula* L.). *Holzforschung* 20:174–178
- Okuyama T, Takeda H, Yamamoto H, Yoshida M (1998) Relation between growth stress and lignin concentration in the cell wall: Ultraviolet microscopic spectral analysis. *J Wood Sci* 44
- Okuyama T, Yoshida M, Yamamoto H (1995) An estimation of the turgor pressure change as one of the factors of growth stress generation in cell walls. *Mokuzai Gakkaishi* 41:1070–1078
- Onaka F (1949) Studies on compression and tension wood. Wood research, Bulletin of the Wood research Institute, Kyoto University, Japan 24:1–88
- Parameswaran N, Liese W (1976) On the fine structure of bamboo fibres. *Wood Sci Technol* 10:231–246
- Pilate G, Chabbert B, Cathala B, Yoshinaga A, Leple JC, Laurans F, Lapierre C, Ruel K (2004) Lignification and tension wood. *C R Biologies* 327:889–901
- Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. *Plant Physiol* 127:1513–1523
- Prodhan AKMA, Ohtani J, Funada R, Abe H, Fukazawa K (1995) Ultrastructure investigation of tension wood fibre in *Fraxinus mandshurica* Rupr. var. *japonica* Maxim. *Ann Bot* 75:311–317
- Ruelle J, Clair B, Beauchêne J, Prevost MF, Fournier M (2006) Tension wood and opposite wood in 21 tropical rainforest species. 2. Comparison of some anatomical criteria. *IAWA J* 27:341–376
- Satiat-Jeunemaitre B (1986) Cell wall morphogenesis and structure in tropical tension wood. *IAWA Bull.* 7:155–164
- Sinnott EW (1952) Reaction wood and the regulation of tree form. *Am J Bot* 39:69–78
- Terashima N (1990) A new mechanism for formation of a structurally ordered protolignin macromolecule in the cell wall of tree xylem. *J Pulp Paper Sci* 16:150–155
- Timell TE (1986) Compression wood in gymnosperms. Springer, Heidelberg
- Washusen R, Evans R (2001) The association between cellulose crystallite width and tension wood occurrence in *Eucalyptus globulus*. *IAWA J* 22:235–243
- Yamamoto H (1998) Generation mechanism of growth stresses in wood cell walls: roles of lignin deposition and cellulose microfibril during cell wall maturation. *Wood Sci Technol* 22
- Yamamoto H, Kojima Y, Okuyama T, Abasolo WP, Gril J (2002) Origin of the Biomechanical properties of wood related to the fine structure of the multi-layered cell wall. *J Biomech Eng* 124:432–440
- Yamamoto H, Okuyama T, Yoshida M (1998) Growth stress generation and microfibril angle in reaction wood. Microfibril Angle in Wood. The proceedings of the IAWA/IUFRO international workshop on the Significance of microfibril angle to wood quality:225–239
- Yoshida M, Ohta H, Yamamoto H, Okuyama T (2002) Tensile growth stress and lignin distribution in the cell walls of yellow poplar, *Liriodendron tulipifera* Linn. *Trees* 16:457–464
- Yoshida M, Okuda T, Okuyama T (2000) Tension wood and growth stress induced by artificial inclination in *Liriodendron tulipifera* Linn. and *Prunus spachiana* Kitamura f. *ascendens* Kitamura. *Ann For Sci* 57:739–746
- Yoshizawa N, Inami A, Miyake S, Ishiguri F, Yokota S (2000) Anatomy and lignin distribution of reaction wood in two *Magnolia* species. *Wood Sci Technol* 34:183–196