

Bieke Lybeer · Joris VanAcker · Paul Goetghebeur

## Variability in fibre and parenchyma cell walls of temperate and tropical bamboo culms of different ages

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**Abstract** This study tries to clarify the conflicting results from previous studies on cell wall thickening in bamboo culms by applying light and transmission electron microscopy in combination with image analysis. It focused on both fibre and parenchyma wall thickness of both temperate (*Phyllostachys* spp.) and tropical (*Gigantochloa levis* and *Dendrocalamus asper*) bamboo species of different ages in the light of their suitability for the wood industry. The observations indicated a great heterogeneity in cell wall thickness and cell wall layering pattern of fibres within one culm. Nested design ANOVA's revealed a rising trend in wall thickness of late maturing fibres and parenchyma cells during the first year but significant wall thickening during later years could not be demonstrated. The high variability within one culm and between culms of the same age from 1 year on is partly masking a clear increased cell wall thickening at higher age. Nevertheless, the highest mean values for fibre wall thickness were recorded in culms of 44 months old or older, suggesting that some kind of late cell wall maturing can take place within one culm.

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### Introduction

Bamboos are one of the most important non-timber forest products and one of the more important agricultural non-annual plants in the world. During the last decade, increased knowledge and research about bamboo have had a tremendous economic impact and have given rise to many new industries and products. The suitability of bamboo culms for large-scale utilization as additional or alternative raw material for the wood processing industry in Europe has clearly been demonstrated (Van Acker et al. 2000). The composition of plant tissue in terms of cells with varying wall thickness and of proportion of thick-

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B. Lybeer · P. Goetghebeur  
Laboratory of Biology, Research Group Spermatophytes, Ghent University,  
K.L. Ledeganckstraat 35, 9000 Gent, Belgium

J. VanAcker · B. Lybeer (✉)  
Laboratory of Wood Technology, Ghent University,  
Coupure links 653, 9000 Gent, Belgium  
E-mail: Bieke.Lybeer@UGent.be

walled cells is likely to have a large effect on the utilization of the culms in the wood processing industry. Cell wall thickening of bamboo fibres and parenchyma cells does occur during culm elongation and early maturation, and is considered to continue for several years (Alvin and Murphy 1988; Liese and Weiner 1996, 1997; Murphy and Alvin 1997a, b). Such a prolonged maturation of bamboo culms is of considerable importance as it influences certain properties and consequently the processing and their utilization (Alvin and Murphy 1988; Liese 1998). Murphy and Alvin (1997a) demonstrated that culm density, and fibre wall thickness increase with age. In contrast, Van Acker et al. (2000) could not clearly show an impact pattern of age on the density. As culm density is correlated with cell wall thickness, no significant cell wall thickening should take place during later years. Moreover, the published quantitative information regarding cell wall thickness does not always show an unambiguous increasing cell wall thickness with age as is clear from Table 1 in Liese and Weiner (1996).

It is known that the maturation process of fibres is proceeding differently over the transverse section of a culm wall. Maturation starts at the outside of the culm wall and proceeds towards the inner side. Furthermore, it is influenced by the position of the fibres within the vascular bundle. Several authors (Alvin and Murphy 1988; Liese and Weiner 1996, 1997; Murphy and Alvin 1997a) described fibre maturation in bamboo species with type I vascular bundles. A vascular bundle type I consists of only one part: the central vascular bundle, with a supporting tissue of four sclerenchyma sheaths of nearly the same size (Liese and Grosser 2000). The fibres close to the vascular tissue are described as early maturing fibres and the fibres adjacent to the parenchyma as late maturing fibres (Murphy and Alvin 1997a). Murphy and Alvin (1997b) studied fibre maturation in the bamboo *Gigantochloa scortechinii* with types III and IV vascular bundles. A type III vascular bundle has a central vascular strand with four smaller fibre caps and an additional free fibre strand located at the protoxylem side. Type IV has a central vascular strand with four smaller fibre caps and in addition two free fibre strands, located at the phloem and protoxylem side. This type IV always occurs in combination with type III (Liese and Grosser 2000). Murphy and Alvin (1997b) paid special attention to aspects of fibre maturation in free fibre strands. They describe the fibres of the vascular fibre caps to thicken to almost their maximum during the first year of culm

**Table 1** Collected samples

Species	Origin	Age in months (the exponent gives the number of culms sampled of that particular age)	Total number
<i>P. viridiglaucescens</i>	Meise, Belgium	1 <sup>3*</sup> , 3 <sup>3</sup> , 6 <sup>3*</sup> , 9 <sup>3</sup> , 12 <sup>3*</sup>	15
<i>P. nigra</i>	Ghent, Belgium	1 <sup>2</sup> , 3 <sup>2</sup> , 6 <sup>2</sup> , 9 <sup>2</sup> , 12 <sup>2</sup>	10
<i>P. viridiglaucescens</i>	Prafrance,	8 <sup>2</sup> , 20 <sup>2</sup> , 32 <sup>2*</sup> , 44 <sup>2</sup> , 56 <sup>2</sup> , 68 <sup>2</sup> , 80 <sup>2</sup> , 92 <sup>2</sup> , 104 <sup>2*</sup>	18
<i>P. nigra</i>	France	8 <sup>2</sup> , 20 <sup>2</sup> , 32 <sup>2</sup> , 44 <sup>2</sup> , 56 <sup>2</sup> , 68 <sup>2</sup> , 80 <sup>2</sup> , 92 <sup>2</sup> , 104 <sup>2</sup>	18
<i>P. viridis</i>		8 <sup>1</sup> , 20 <sup>1</sup> , 32 <sup>1</sup> , 44 <sup>1</sup> , 56 <sup>1</sup> , 68 <sup>1</sup> , 80 <sup>1</sup> , 92 <sup>1</sup> , 104 <sup>1</sup> , 128 <sup>1</sup> , 152 <sup>1</sup>	11
<i>G. levis</i>	Real Quezon,	8 <sup>4*</sup> , 19 <sup>1</sup> , 21 <sup>2*</sup> , 22 <sup>1</sup> , 29 <sup>1</sup> , 31 <sup>1</sup> , 32 <sup>2</sup> , 39 <sup>2</sup> , 40 <sup>2*</sup>	16
<i>D. asper</i>	Philippines	8 <sup>4</sup> , 21 <sup>4</sup> , 29 <sup>1</sup> , 31 <sup>1</sup> , 32 <sup>1</sup> , 35 <sup>1</sup> , 41 <sup>1</sup> , 42 <sup>1</sup> , 43 <sup>1</sup> , 45 <sup>1</sup>	16
			104

Samples indicated with \* were used for TEM studies

growth (early maturing fibres) and the fibres of free fibre strands to keep their potential for continued wall thickening even in 3-year-old culms (late maturing fibres). Because the maturation process from the outside of the culm wall towards the inner side is apparently clear, the cell wall thickening of fibres and parenchyma cells in the middle of the culm wall was investigated here.

The conflicting results from the previous studies induced further detailed investigation of the progress and variability in cell wall thickness of bamboo culms. This study focused on both fibre and parenchyma wall thickness of both temperate and tropical bamboo species of different ages in the light of their suitability for the wood industry.

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## Materials and methods

### Plant samples

Samples of culms with different ages were taken of *Phyllostachys viridiglaucescens* (Carrière) A. and C. Rivière, *P. nigra* (Loddiges ex Lindley) Munro and *P. viridis* (Young) McClure in the Bambuseraie in Prafrance, France. Culms in the first year of development were harvested in the Belgian National Botanical Garden in Meise (*P. viridiglaucescens*) and in the University Botanical Garden, Ghent (*P. nigra*), Belgium. The tropical bamboo species *Gigantochloa levis* (Blanco) Merrill and *Dendrocalamus asper* (Schultes f.) Backer ex Heyne were sampled at a 4-year-old plantation in Real Quezon in the Philippines. All plant samples had been marked with the year and month of emergence giving precise age at the date of sampling (Table 1).

### Light microscopy

Transverse sections of 18–25  $\mu\text{m}$  from the 6th and 12th internode above the ground level were cut using a Microm-HM440E sliding microtome. After double staining with safranin and astrablue, dehydration and clearing, the sections were permanently mounted in Entellan (Merck, Darmstadt, Germany).

All observations were made with an AHBS-21 Vanox Olympus universal microscope using bright field illumination at a magnification of 400 $\times$ .

### Transmission electron microscopy

First, sections of 50  $\mu\text{m}$  of some samples of *P. viridiglaucescens* and *G. levis* (Table 1) were cut using a Microm-HM440E sliding microtome. From the middle of the culm wall of these sections, a small part containing a vascular bundle and parenchyma cells was dissected using a sharp scalpel. Then, these specimens were dehydrated in a graded series of acetone and subsequently washed in a graded series of alcohol and impregnated with the resin LR white<sup>TM</sup> (Polysciences, Eppelheim, Germany) through a series of alcohol/LR white mixture, followed by immersion in pure resin. Finally, transverse sections of 50 nm in thickness were cut with a Reichert Ultracut ultramicrotome using a diamond knife and mounted on formvar coated single dot copper grids. The sections were stained according to Donaldson (1992) with a 1% solution of potassium permanganate dissolved in double distilled water containing 0.1%

sodium citrate. The duration of on-grid-staining was 3 min, followed by washing twice in double distilled water. The sections were examined with a Jeol-1010 transmission electron microscope operating at 60 kV.

### Cell wall measurements

For the cell wall measurements the image analysis program analySIS 3.0 was used. For each characteristic, 25 measurements were recorded. Fibre tips, as identified by their distinctly reduced size, were excluded from the analysis.

For the temperate *Phyllostachys* species the fibre wall thickness and fibre diameter at xylem and phloem inner side (adjacent to the vascular tissue) and outer side (adjacent to the parenchyma) and parenchyma cell wall thickness were measured at the middle culm wall. In some sections, no clear difference in cell wall thickness and diameter between the inner and outer fibres could be observed. In that case, 50 measurements along a transect from the inner to the outer side were made.

The tropical species *D. asper* and *G. levis* have vascular bundles with free fibre strands. For these species the fibre wall thickness and fibre diameter at xylem and phloem side adjacent to the vascular tissue and fibre wall thickness and fibre diameter in the free fibre strands as well as the parenchyma cell wall thickness were measured at the middle of the culm wall.

In all samples, the percentages of parenchyma cells in the middle of the culm wall were analysed.

### Statistical analyses

The measurements were statistically analysed using SPSS 11.0. First, the data were described using box- and scatter-plots. Nested design ANOVA's were performed to test whether age had a significant impact on cell wall thickness. This method eliminates the variability in cell wall thickness of the individual culms from the effect of age on the different culms. For most ANOVA analyses a logarithmic transformation of the values was necessary.

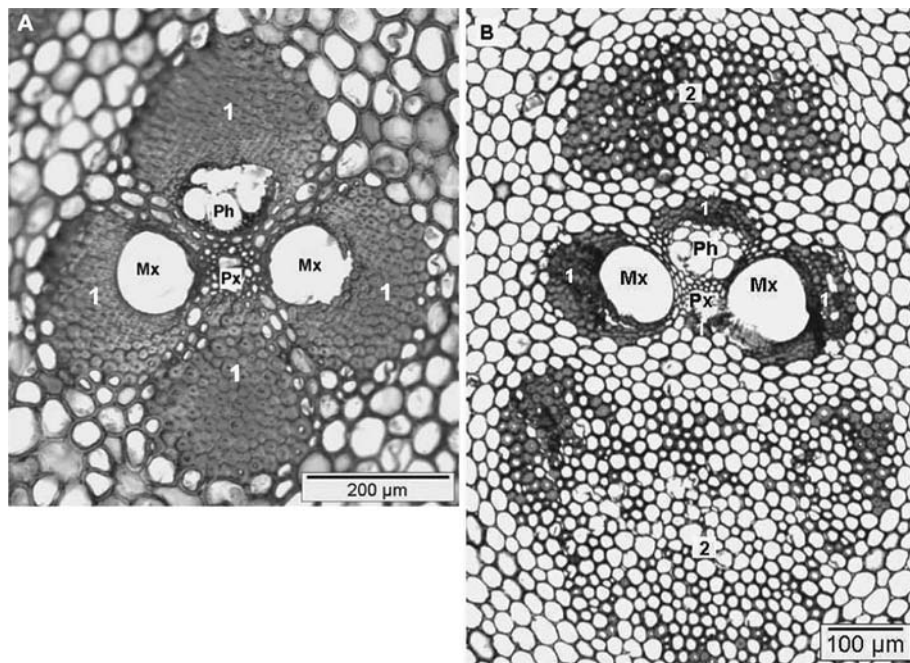
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## Results

### Cell wall thickness at different ages

The *Phyllostachys* species have type I vascular bundles (Fig. 1a) as has been described for the genus by Liese and Grosser (2000). Some minor differentiation between the species is present. *P. nigra* has epidermis cells with dark contents and symmetric vascular bundles with equally large fibre caps. Parenchyma cells that are interspersed between fibres in the supporting tissue can characterize *P. viridiglaucescens*. *P. viridis* can be recognized by the presence of sclereid hypodermis cells. *G. levis* and *D. asper* have types III and IV vascular bundles (Fig. 1b). *G. levis* has notably more type III bundles. In *D. asper*, both bundle types are equally present. Liese and Grosser (2000) described the genera *Dendrocalamus* and *Gigantochloa* to have type III or IV vascular bundles.

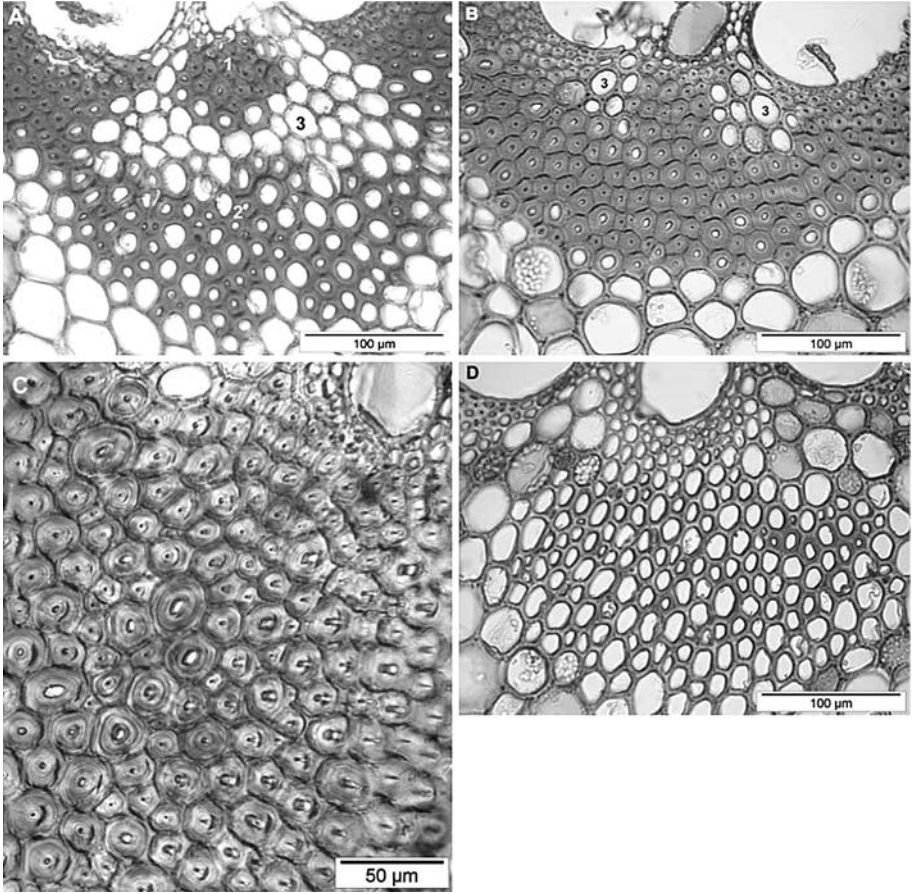
Figure 2 shows xylem fibre caps from the inner towards the outer side of a 3-month-old, a 6-month-old, a 20-month-old and a 44-month-old sample of



**Fig. 1** a Type I vascular bundle of *P. viridiglaucescens* with four fibre caps (1) surrounding the vascular bundle. b Type IV vascular bundle of *G. levis* with four smaller fibre caps (1) adjacent to the vascular tissue and two free fibre strands (2), located at the phloem and protoxylem side. *Mx* metaxylem, *Px* protoxylem, *Ph* phloem

*P. viridiglaucescens*. In the fibre cap of a 3-month-old culm the difference between inner and outer fibres is evident, with more mature fibres, characterized by a thicker cell wall, close to the vascular tissue (Fig. 2a). In the older bamboo culms this difference in cell wall thickness between inner and outer fibre fades. As maturing is taking place over several years it is expected that fibres of older samples have a thicker cell wall and a smaller lumen than fibres of younger samples. Nonetheless, exceptions have been observed. The fibres of a 6-month-old and a 20-month-old sample have a thicker cell wall and a smaller cell lumen than the fibres of a 44-month-old sample (Fig. 2b, c, d). Furthermore, a large variability in fibre wall diameter is present within one section and even within one fibre cap, which implies a large variability in potential of continued cell wall thickening (Fig. 3). A greater variability and a thinner cell wall are observed in free fibre strands of *G. levis* and *D. asper*.

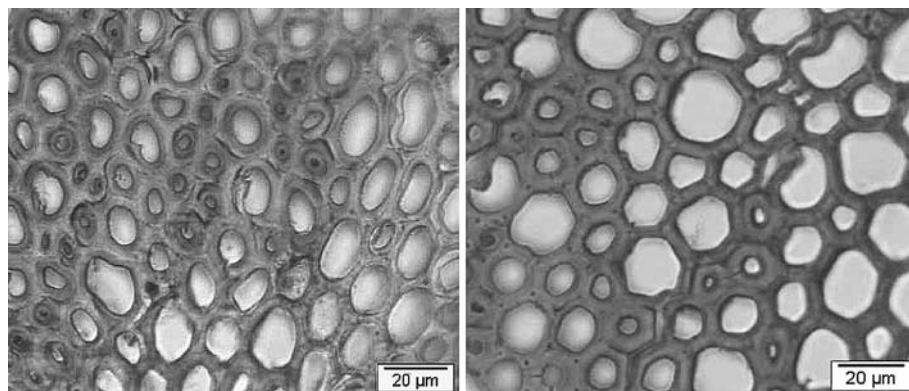
Using box- and scatter-plots it was concluded that both location and species are responsible for several differences between the samples. The samples from Prafrance have longer and wider internodes with a broader culm wall than the samples from Belgium. *P. viridiglaucescens* and *P. nigra* from Prafrance also have higher values for most cell characteristics than the Belgian samples. In general, *P. viridiglaucescens* has higher values than *P. nigra* and *P. viridis* is comparable to *P. viridiglaucescens*. *D. asper* and *G. levis* did not differ significantly. Furthermore, a significant difference for the measured characteristics of internode 6 and 12 could not be observed. There is no correlation between cell



**Fig. 2** Fibre cap at the protoxylem side from the inner towards the outer fibres of a 3-month-old (a), a 6-month-old (b), a 20-month-old (c) and a 44-month-old culm (d) of *P. viridiglaucescens* illustrating the unexpected outcome that younger samples can have thicker fibre walls than older samples. In the section of the 3-month-old culm, there is a clear difference in cell wall thickness between the inner early maturing fibres (1) and the outer late maturing fibres (2). Parenchyma cells (3) that are interspersed between the fibres characterize *P. viridiglaucescens*

wall thickness and thickness of the culm wall. For further analyses, the samples were analysed per origin and per species.

Nested design ANOVA's revealed that age does not have a significant influence on cell wall thickening in both early maturing fibres close to the vascular tissue and late maturing fibres adjacent to the parenchyma in *Phyllostachys* species or fibres of free fibre strands in *G. levis* and *D. asper* of the middle of the culm wall (Table 2). Only the parenchyma cell wall thickness during the first year of development is significantly different between 1-, 3- and 6-, 9-, 12-month-old culms for *P. nigra* and between 1-, 3-, 6- and 9-, 12-month-old culms for *P. viridiglaucescens* as is proved by a Scheffé test ( $P < 0.01$ ). The variability in cell wall thickness within one culm and between culms of the same age is so large that due to the overlap in wall thickness no significant difference can be demonstrated. Although the fibre wall thickening



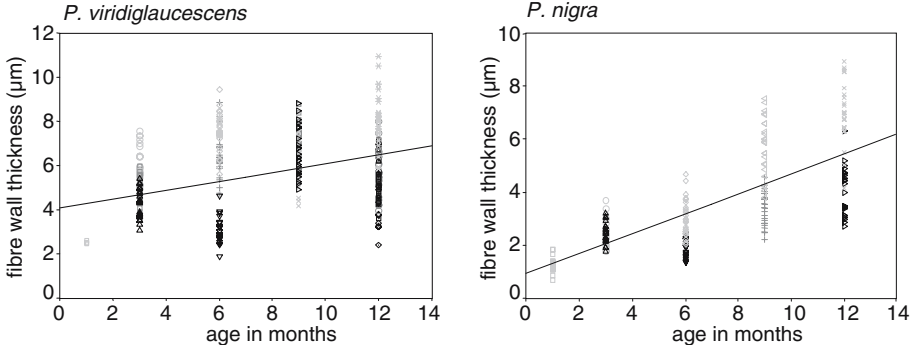
**Fig. 3** Outer fibres at the xylem side of an 8-month-old *P. viridiglaucescens* (left) culm and fibres of a free fibre cap of an 8-month-old *G. levis* (right) culm showing the great variability in cell diameter and cell lumen size within the same fibre cap

**Table 2** *P*-values of nested design ANOVA with factors age and code comparing the influence of age on parameters fibre wall thickness and parenchyma cell wall thickness

Parameters	Species	<i>P</i> -values of samples up to 12 months	<i>P</i> -values of samples from 8 months on
Fibre wall thickness, xylem inner side	<i>P. viridiglaucescens</i>	0.012	0.390
	<i>P. nigra</i>	0.141	0.450
	<i>D. asper</i>	–	0.094
	<i>G. levis</i>	–	0.867
Fibre wall thickness, xylem outer side	<i>P. viridiglaucescens</i>	0.074	0.567
	<i>P. nigra</i>	0.099	0.510
	<i>D. asper</i>	–	0.253
	<i>G. levis</i>	–	0.693
Fibre wall thickness, phloem inner side	<i>P. viridiglaucescens</i>	0.083	0.296
	<i>P. nigra</i>	0.561	0.218
	<i>D. asper</i>	–	0.250
	<i>G. levis</i>	–	0.949
Fibre wall thickness, phloem outer side	<i>P. viridiglaucescens</i>	0.127	0.296
	<i>P. nigra</i>	0.565	0.218
	<i>D. asper</i>	–	0.080
	<i>G. levis</i>	–	0.935
Parenchyma cell wall thickness	<i>P. viridiglaucescens</i>	0.000	0.543
	<i>P. nigra</i>	0.005	0.133
	<i>D. asper</i>	–	0.059
	<i>G. levis</i>	–	0.889

The samples of *Phyllostachys nigra* and *P. viridiglaucescens* younger than 12 months old are harvested in Belgium, the samples older than 8 months are harvested in France. From *Gigantocloa levis* and *D. asper* only culms of 8 months old and older were sampled

is not significant during the first year of development, an upwards trend from a 1-month-old culm towards a 12-month-old culm in outer fibres (i.e. fibres adjacent to the parenchyma) at xylem and phloem side from *P. viridiglaucescens* and *P. nigra* can be observed (Fig. 4). This upwards trend is not found



**Fig. 4** Scatter-plots visualizing the nested design ANOVA's during the first year of development (*P. viridiglaucescens* and *P. nigra*) from late maturing fibres at the xylem side. Each *symbol* corresponds to measurements of one culm. From each culm, 25 late maturing fibres at the xylem side were measured

in the inner fibres, because they have already thick cell walls and a small lumen in 3-month-old culms (early maturing fibres). The tropical bamboo species *D. asper* and *G. levis* could not be studied in detail during the first year of development as no material younger than 8 months old was sampled. Table 3 compares mean fibre and parenchyma wall thickness of the different species of different ages. From this table it can be concluded that the highest values were recorded in culms of 44 months or older for the *Phyllostachys* species. One-way ANOVA reveals that mean parenchyma and fibre wall thickness of *P. nigra* and *P. viridiglaucescens* culms older and younger than 44 months are significantly different ( $P < 0.05$ ). In the recorded values of the mean wall thickness of *D. asper* and *G. levis* no increasing trend can be observed. From these tropical species no culms older than 40 months could be sampled. The measurements also display a high variability in cell diameter of the fibres. The cell diameter is independent of the age, which is logic as the cells already have their size in the bud. In early maturing fibres, 46% of the variation in cell wall thickness can be accounted for by the variation in the cell diameter ( $r^2 = 0.46$ ). In late maturing fibres, there is a very low correlation ( $r^2 = 0.05$  for samples 12 months old or younger and  $r^2 = 0.18$  for samples older than 12 months).

Besides the parenchyma wall thickness mentioned above, the proportion of parenchymatous tissue in the culm is of importance for the strength and consequently the utilisation of the culm. The mean value of the proportion of parenchymatous tissue in the middle of the culm wall at internode 6 is 56% with no significant difference between the species. As a result the proportion of vascular tissue and fibres is 44%.

### Cell wall layering

Folds are present in the sections (Figs. 5, 6, 7) due to the anatomical differences between parenchyma cells and fibres. Parenchyma cells have a larger diameter and cell lumen and a thinner cell wall compared to fibres, which have a smaller diameter, narrower lumen and a thicker cell wall. As a consequence, the impregnation of the parenchyma wall is easier than of the fibre wall, which is

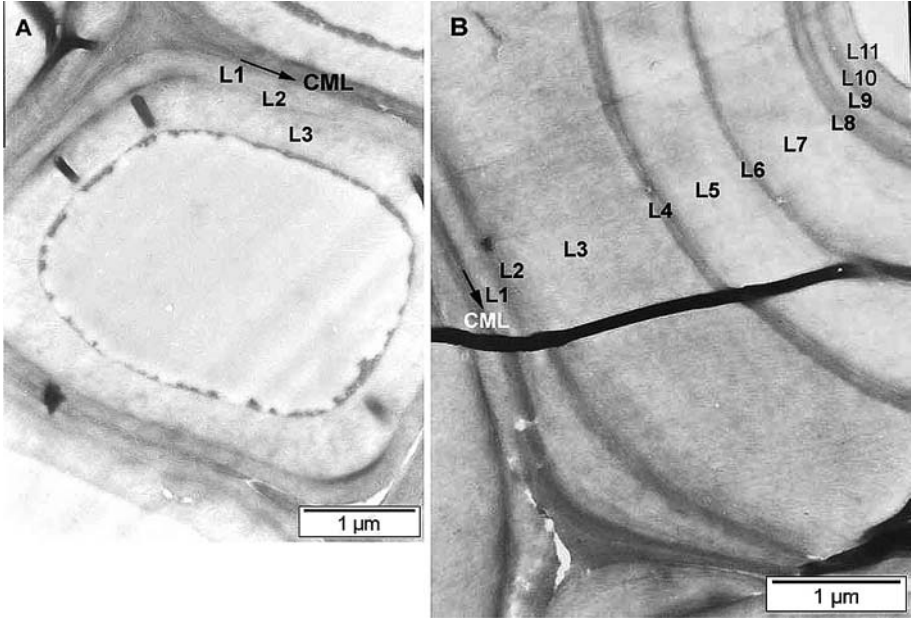


**Table 3** Comparison between mean fibre wall thickness at position xylem inner side (*xyin*), xylem outer side (*xyout*), phloem inner side (*phlin*), phloem outer side (*phlout*) and parenchyma wall thickness (*par*) of internode 6 of different species of different ages

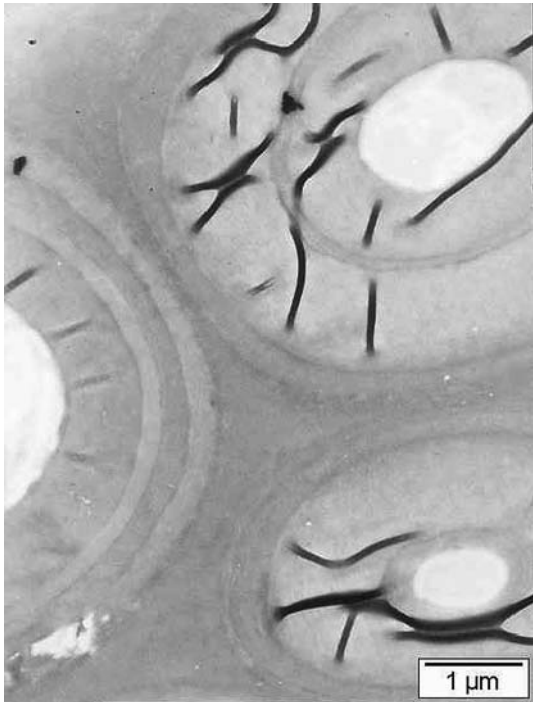
Species	Origin	Age (months)	Xyin ( $\mu\text{m}$ )	Xyout ( $\mu\text{m}$ )	Phlin ( $\mu\text{m}$ )	Phlout ( $\mu\text{m}$ )	Par ( $\mu\text{m}$ )	
<i>P. viridiglaucescens</i>	Belgium	3	4.78	5.00	5.08	5.08	2.89	
		6	5.99	5.58	4.92	4.92	4.73	
		9	6.55	6.55	6.04	6.04	4.23	
	France	12	5.85	5.97	4.68	5.50	4.19	
		8	6.53	5.45	6.02	6.02	4.24	
		20	7.43	6.53	6.03	6.03	4.67	
		32	6.35	5.19	5.70	5.70	4.63	
		44	7.89	6.54	5.43	5.43	4.30	
		56	9.61	9.61	7.50	7.50	4.98	
		68	8.51	8.51	6.85	6.85	4.37	
	<i>P. nigra</i>	Belgium	80	9.12	9.12	6.59	6.59	5.85
			92	7.91	7.91	6.30	6.30	4.35
			104	9.56	9.56	6.80	6.80	4.42
<i>P. viridis</i>	France	3	3.67	2.46	3.58	3.58	2.22	
		6	3.90	2.52	3.92	3.92	3.37	
		9	4.74	3.43	4.20	4.20	3.08	
	France	12	5.29	5.52	4.28	5.26	3.50	
		8	4.09	2.31	3.99	3.99	2.76	
		20	4.77	4.39	4.43	4.43	3.30	
		32	4.57	2.95	4.12	4.12	3.30	
		44	6.77	6.00	5.32	5.32	3.55	
		56	6.92	6.92	5.81	5.81	3.66	
		68	6.22	6.22	4.89	4.89	4.02	
	<i>D. asper</i>	Philippines	80	5.98	4.75	4.93	4.93	3.67
			92	5.09	3.85	4.85	4.85	3.52
			104	4.31	3.84	4.17	4.17	3.10
8			5.57	2.60	4.87	3.31	3.02	
20			6.86	6.86	6.72	6.72	4.52	
32			5.77	5.43	5.56	5.56	3.50	
44			5.98	5.98	5.33	5.33	3.40	
56			5.76	5.76	5.08	5.08	4.22	
68			9.55	9.55	6.50	6.50	4.62	
80			3.18	3.51	4.79	4.79	4.10	
<i>G. levis</i>	Philippines	92	5.52	5.52	5.82	5.82	3.71	
		104	8.44	8.44	7.15	7.15	4.69	
		128	9.47	9.47	7.28	7.28	5.78	
		152	7.20	7.20	6.34	6.34	3.64	
		8	4.22	3.11	3.90	3.33	1.44	
		21	4.17	2.93	4.01	3.27	1.44	
		29–35	4.42	4.54	3.76	4.56	1.61	
		41–45	3.92	2.84	3.88	4.88	1.76	
		8	4.20	3.45	4.26	4.15	1.79	
		22	4.27	3.13	4.29	3.92	1.96	
<i>D. asper</i>	Philippines	29–32	4.56	3.14	4.20	3.35	1.79	
		39–40	4.36	3.77	4.31	4.60	2.18	

From the table it can be concluded that the highest values were recorded in culms of 44 months or older for the *Phyllostachys* species. In the recorded values of the mean wall thickness of *D. asper* and *G. levis* no rising trend can be observed

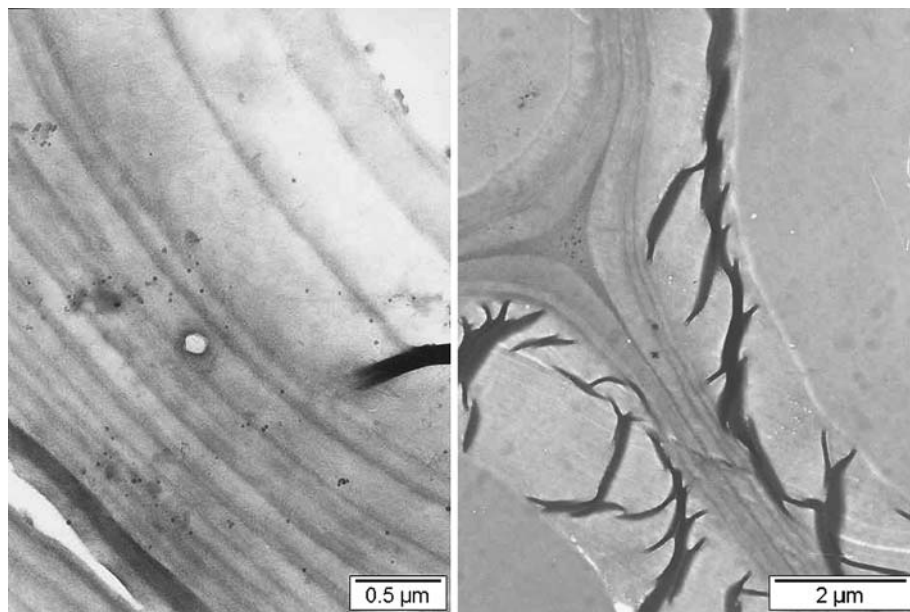
*xyin* inner fibres at the xylem side, *xyout* outer fibres at the xylem side, *phlin* inner fibres at the phloem side, *phlout* outer fibres at the phloem side, *par* parenchyma



**Fig. 5** Fibre from the inner side of a phloem fibre cap of a 1-month-old *P. viridiglaucescens* culm (a) with only few layers in comparison to a fibre from the inner side of a xylem fibre cap of a 3-year-old *P. viridiglaucescens* culm (b). *CML* compound middle lamella, *L* cell wall layer

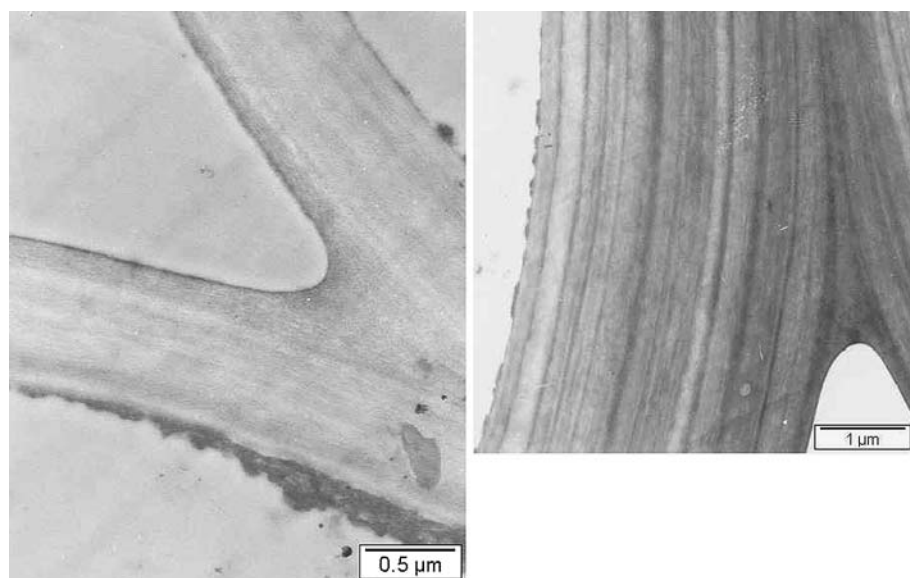


**Fig. 6** Variation in layering patterns as well as different number and thickness of layers within a phloem fibre cap of a 6-month-old *P. viridiglaucescens* sample



**Fig. 7** Comparison between late maturing fibres of a *P. viridiglaucescens* culm (3 years old) (*left*) with late maturing fibres of a *G. levis* culm (21 months old) (*right*). Late maturing fibres of *P. viridiglaucescens* culms have a thicker cell wall with more layers

demonstrated by the absence of folds in the parenchyma cells (Fig. 8). Fibres of vascular sheaths adjacent to the vascular tissue are smaller in diameter and thicker in cell wall than those of the free fibre strands. This is reflected in the



**Fig. 8** Parenchyma cell walls of a 1-month-old (*left*) and 3-year-old (*right*) sample of *P. viridiglaucescens* illustrating cell wall thickening due to deposition of cell wall layers

number of folds, which is greater in fibres adjacent to the vascular tissue. Test samples of impregnation with Spurr's resin (Spurr 1969) gave sections with even more folds, probably because it is more viscous than LR white resin.

The fibre and parenchyma wall is thickening due to the deposition of additional cell wall layers (Parameswaran and Liese 1976; Murphy and Alvin 1992, 1997a; Gritsch et al. 2004). Figure 5 shows fibres at the inner side of a phloem cap of a 1-month-old *P. viridiglaucescens* culm, with only few layers in comparison to the fibres at the inner side of a xylem cap of a 3-year-old *P. viridiglaucescens* culm with more cell wall layers, illustrating the cell wall thickening by deposition of new layers. All fibres have a S<sub>2</sub> wall with alternating narrow and broad layers. The narrow layers appear darker than broad layers after potassium permanganate staining, indicating higher lignin content. Different layering patterns as well as different number and thickness of layers can be present in fibres within one fibre cap (Fig. 6). The variability in layer thickness is most obvious in the broad layers; the narrow layers are more uniform in thickness.

Comparing the ultrastructure of the wall layering of early maturing fibres of *P. viridiglaucescens* with *G. levis* no difference can be observed. However, when comparing the late maturing fibres located at the periphery of the phloem and xylem cap of *P. viridiglaucescens* with the late maturing fibres in free fibre strands of *G. levis* it is observed that the late maturing fibres of *P. viridiglaucescens* culms have a thicker cell wall with more layers (Fig. 7). Early and late maturing fibres of *P. viridiglaucescens* are more uniform contrary to early and late maturing fibres of *G. levis*.

Parenchyma cell wall thickening due to deposition of cell wall layers is demonstrated in Fig. 8. The parenchyma cell wall is composed of different narrower layers with alternating lignin content. The number and thickness of layers is variable. The compound middle lamellae and cell corners are dark-stained with potassium permanganate indicating higher lignin content.

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## Discussion

The microscopical observations and numerical results revealed a great variability in cell diameter and cell wall thickness of both early and late maturing fibres. During the first year of culm growth the early maturing fibres fully develop with a thick cell wall and a small cell lumen whereas the cell wall of late maturing fibres show an increasing trend in cell wall thickness. The weaker correlation between fibre diameter and fibre wall thickness in late maturing fibres than in early maturing fibres can be due to the fact that late maturing fibres retain the potential for continued wall thickening when they still have a cell lumen. In early maturing fibres, 46% of the variation in cell wall thickness can be accounted for by the variation in the cell diameter. As they undergo wall thickening earlier and fully mature during the first year of culm growth, leaving almost no cell lumen, larger fibres will have a thicker cell wall. During later years cell wall thickening does not significantly increase in both early and late maturing fibres. This is probably due to the high variability between the cell wall thickness of fibres within one bamboo culm and even within one fibre cap. Nevertheless, the highest mean values for fibre wall thickness were recorded in culms of 44 months old or older. These results suggest that some kind of late

cell wall maturing can take place and can explain that for practical uses culms of 3 years and older are being used. So, cell wall thickening at higher age is possible within one culm although it is not significant for a population of culms.

These results contradict the general accepted idea that bamboo fibres have a prolonged maturation with an increasing cell wall thickness over several years (Liese and Weiner 1996, 1997; Alvin and Murphy 1988; Murphy and Alvin 1997a, b). Liese and Weiner (1996) published the mean fibre wall thickness of vascular bundles at the fourth internode from *P. viridiglaucescens* (Table 1 in Liese and Weiner 1996) from which they ascertain the increase in fibre wall thickness. Table 4 compares the mean values from measurements in this study from the sixth internode of *P. viridiglaucescens* with their measurements. The table does not support the conclusion of Liese and Weiner of an increasing fibre wall thickness with ageing. It has to be noted that comparing mean cell wall thicknesses the high variability of the wall thicknesses within one culm and between different culms is not taken into account. It is possible that older culms have lower mean fibre wall thicknesses than younger culms (Tables 3, 4) although they have higher maximum wall thickness. The fact that the variability, which we have demonstrated to be very important, was taken into account for the new data set (nested design ANOVA's) could partly explain the different conclusions. The results support and explain the outcomes of Van Acker et al. (2000) who could not derive an impact pattern of age on density of some *Phyllostachys* spp. Late maturing fibres situated in free fibre strands (*G. levis* and *D. asper*) keep their potential for continued cell wall thickening longer than late maturing fibres in vascular fibre caps (*Phyllostachys* spp.) as they still have a larger cell lumen. This is in accordance with the study of Murphy and Alvin (1997b) who observed that many fibres of free fibre strands of *G. scortechinii* after 3 years still remained thin-walled. Lybeer and Koch (2005) demonstrated that lignification takes place earlier in late maturing fibres of *P. viridiglaucescens* than in late maturing fibres of *G. levis*. They postulated that the cause of this

**Table 4** Comparison between measurements of fibre wall thickness of *P. viridiglaucescens* made by Liese and Weiner (1996) at the fourth internode and measurements made at the sixth internode from material from Prafrance used in this study

Culm age (months)	Mean fibre wall thickness of fibre sheath 4-5-6 in Table 1 in Liese and Weiner (1996)		Culm age (months)	Mean fibre wall thickness from material from Prafrance sampled for this study	
	Culm 1 ( $\mu\text{m}$ )	Culm 2 ( $\mu\text{m}$ )		Culm 1 ( $\mu\text{m}$ )	Culm 2 ( $\mu\text{m}$ )
3	2.76	2.20			
12	6.56	5.06	8	5.56	6.36
24	7.26	5.73	20	4.75	8.26
36	4.76	5.00	32	4.96	6.51
48	6.96	6.66	44	7.97	4.67
60	4.60	4.06	56	7.81	9.30
72	3.03	3.30	68	7.49	7.87
84	6.83	4.90	80	8.09	7.62
96	7.73	7.23	92	6.74	7.46
108	4.16	5.86	104	8.16	8.20
120	5.96	6.80			
132	8.40	7.40			
144	8.33	6.20			

difference in lignification might be related to the stable tropical climate the bamboo species *G. levis* grow.

Parameswaran and Liese (1976) described the thick-walled bamboo fibres as exhibiting a polylamellate structure with alternating broad and narrow layers. They stated that thinner walled fibres are less polylamellate. In contrast, Murphy and Alvin (1992) found that the number of layers was variable but tended to be higher in fibres adjacent to either vascular elements or ground tissue at the periphery of the fibre bundles. Similarly, Gritsch et al. (2004) concluded that the multilayered structure of the fibre cell walls was mainly formed during the first year of growth by the deposition of new wall layers of variable thickness, resulting in a high degree of heterogeneity in the layering patterns amongst individual fibres. The TEM photographs demonstrated that all fibres have a S<sub>2</sub> wall with different layering patterns as well as different number and thickness of layers within one fibre cap. The different layer thicknesses are most obvious in the broad layers; the narrow layers are more uniform in thickness. The layering structure of the parenchyma cell wall is different from the layering structure of the fibre as they consist of several narrow layers. The variability is present in both cell wall layers of temperate bamboos with type I vascular bundles and tropical bamboos with types III and IV vascular bundles. This study is the first, which revealed that the cell wall ultrastructure of fibres adjacent to the conductive tissues (early maturing fibres) is similar for both vascular bundle types. The late maturing fibres (i.e. fibres adjacent to the parenchyma in type I vascular bundles and fibres of free fibre strands in types III and IV vascular bundles) have a different structure. The late maturing fibres of type I vascular bundles have a thicker cell wall with more layers similar to the early maturing fibres. In contrast, the late maturing fibres of types III and IV vascular bundles have a thinner cell wall with fewer cell wall layers. Only Murphy and Alvin (1997b) studied free fibre strands in *G. scortechinii*. They observed a higher heterogeneity in terms of their diameter and even after 3 years many still remain relatively thin-walled with potential for continued wall thickening. They state that it is possible that during the early stages of tissue differentiation the 'release' of blocks of potential fibres into the ground tissue may allow some of the elements to expand to a larger final diameter. Work is required on the origin and early development of these strands in bamboos with bundle types III and IV.

In all studied species the proportion of parenchymatous tissue in the middle of the culm wall at internode 6 is about 56%. Grosser and Liese (1974) give average values of 54–55% for some culms of *Phyllostachys* spp. and of 50–51% for some culms of *Dendrocalamus* spp. The percentage parenchyma cells measured in the middle of the culm wall at internode 6 corresponds to those mentioned in Grosser and Liese (1974) for a whole culm. He et al. (2002) demonstrated that a significant increase in wall thickness and lignification occurs in parenchyma cells of up to 7 years. In this study, in contrast to He et al. (2002) no significant increase in wall thickness during later years could be demonstrated. Similar as described for the fibres, this is probably due to the high variability in parenchyma wall thickness within one culm. However, the parenchyma cell wall thickness increases significantly during the first year of development. Parenchyma cell wall thickening is like fibre wall thickening due to deposition of cell wall layers of variable thickness.

It is noteworthy that even within one culm the variability is very high. Hence nor the genetic variability, nor site differences can explain the high variability in cell wall thickness and cell diameter.

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## Conclusion

In contrast to the general accepted idea that bamboo fibre and parenchyma cell walls are thickening over several years, this study concludes that there is no significant overall increase in wall thickness of fibre and parenchyma cells of bamboo culms older than 1 year. Misinterpretation of this trend is probably partly due to the high variability within one culm and between culms of the same age. Nevertheless, the highest mean values for fibre wall thickness were recorded in culms of 44 months old or older. These results suggest that some kind of late cell wall maturing can take place and can explain that for practical uses culms of 3 years and older are being used. However, some younger culms also display high values. So, not only culms of 3 years and older could be used, but culms older than 1 year would be appropriate for industrial uses in respect of the parameters studied here.

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