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Research Group Spermatophytes

Laboratory of Wood Technology

Age-related anatomical aspects of some temperate and tropical bamboo culms (Poaceae: Bambusoideae)

Leeftijdsgerelateerde anatomische aspecten van enkele gematigde en tropische bamboehalmen (Poaceae: Bambusoideae)

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1. Literature review

1.1. Evolution and biodiversity of woody bamboo

The bamboos (Poaceae: Bambusoideae) are traditionally described as a heterogeneous group of mostly perennial, rhizomatous forest grasses with broad, pseudopetiolate leaves, chlorenchyma with arm and fusoid cells, often bracteate synflorescences, and trimerous flowers (Clark 1997; Zhang & Clark 2000). The Bambusoideae is a polyphyletic assemblage, belonging to the most ancestral components of the grass family (Clark 1997), but a bambusoid clade consisting of the woody bamboos (Bambuseae) and the herbaceous olyroid bamboos (Olyreae, Parianeae and Buergersiochloeae) is supported as monophyletic (Zhang & Clark 2000). Within this bamboo clade, the woody bamboos (Bambuseae) and the herbaceous bamboos (Olyreae) each form a monophyletic clade (Zhang & Clark 2000). The presence of woody culms, the differentiation between foliage and culm leaves (reflecting the two phases of shoot growth and development), and the gregarious flowering cycles at long intervals are all structural characters that unite the woody bamboos (Zhang & Clark 2000). It is generally accepted that the woody bamboos diverged from a herbaceous ancestor adapted to more open environments (Takhtajan 1969; Clayton & Renvoize 1986; Clark *et al.* 1995; Clark 1997). They evolved to compete for light with trees and other woody vegetation and play unquestionably an important role in forest dynamics. They are usually found growing in clearings and along forest margins, and when there is disturbance, the bamboos are superbly equipped to take advantage (Clark 1997). Gregarious, monocarpic flowering behaviour is considered by Widmer (1997) as being associated with forest dynamics. Simultaneous flowering and the subsequent germination of countless seedlings allow bamboos to compete very effectively with tree saplings in the event of a break in the forest canopy. The very rapid growth rate of culms may also be important in this respect.

Janzen (1976) proposed predator satiation as a hypothesis to explain the cyclical, gregarious flowering. In this scenario so much fruit is produced that the local seed predators cannot eat all of it and the remaining seed can regenerate. But given the relatively short life cycles of these predators in comparison to the very long cycles of 50, 60, 80 or over 100 years for some bamboos, it is difficult to explain the selective pressure. Therefore Clark (1997) speculates that energy partitioning during the life cycle of plants competing with trees is a more plausible factor in the evolution of this unusual flowering behaviour. Energy requirements for fast vegetative growth and large size are probably high, so delay in flowering might be a reasonable adaptation. Still a lot of work has to be done to understand this complex flowering behaviour.

Introduction

It is suggested by Clark (1997) that the earliest bamboos were tropical, lowland, and Gondwanan (southern hemisphere) in their early diversification, during which time they split into two major lineages, one herbaceous and the other woody. She speculates that the earliest woody bamboos occupied tropical regions with a single lineage that radiated into the northern temperate zones and into tropical montane habitats.

Bamboos are distributed from 46° North to 47° South latitude and their altitudinal range is from sea level to over 4000 m in equatorial highlands (Soderstrom *et al.* 1988). Bamboos are found in the largest abundance and variety on the southern and south-eastern borders of Asia, from India through China and Japan to Korea. In the vast Eurasian continent north, west, and northwest of Tibet and China, however, no native bamboo has been found (McClure 1966). The natural distribution of bamboo in the world has been modified mainly by human intervention.

The lowest diversity of woody bamboo is found in continental Africa where five species naturally occur (Brystriakova *et al*. 2003a). The greatest potential bamboo richness is in East Africa (Fig. 1-1a). The low diversity of bamboo species in mainland Africa, compared with Asia, Madagascar and the Americas, may relate to past climatic variation on the continent. The ancestral woody bamboos are thought to have arisen in the wet Gondwanaland (Clark 1997). It is possible that after the break-up of Gondwanaland the African genera isolated (Clayton & Renvoize 1999) and that climate and vegetation patterns in Africa have provided limited opportunities for their expansion and radiation within forest habitats, in contrast to genera on other continents. Despite their lack of diversity in Africa, bamboos play an important role in ecology and biodiversity conservation. In many places, especially at high altitude, African bamboo species form vast pure stands, which provide important shelter and resources for some key species of conservation interest, for example for the eastern mountain gorilla (*Gorilla beringei beringei*) (Brystriakova *et al*. 2003a).

Madagascar is considered to have 33 species of woody bamboo and is therefore strikingly more rich in species than continental Africa. Thirty-two of these species are endemic and a single species, *Bambusa vulgaris*, is pantropical in distribution. As this last species is mainly found near villages and along rivers, it is possible that it is introduced. The richness and endemism of the woody bamboo flora of Madagascar reflects the island's long (c. 140 million years) isolation from other land masses and the resulting unique evolutionary pathways that have led to extremely high levels of endemism in both flora and fauna (Brystriakova *et al*. 2003a).

The Americas are collectively much richer in bamboo species than either continental Africa or Madagascar, but have lower diversity than the Asia-Pacific region (Fig. 1-1a). There are approximately 430 species in the Americas of which more than 40 % belongs to a single genus, *Chusquea* (Brystriakova *et al*. 2003a). The greatest diversity is in South America with the highest bamboo diversity in Brazil, the northern and central Andes and Mexico. The Unites States has only a single woody bamboo species (*Arundinaria gigantea*). Throughout South America, woody bamboos are an important part of forested ecosystems. This dense vegetation is often an important refuge for wildlife species from surrounding native hardwood forests that are being destroyed (Judziewicz *et al*. 1999). Bamboo stands provide habitat and food for a wide range of mammals, birds, amphibians and invertebrates.

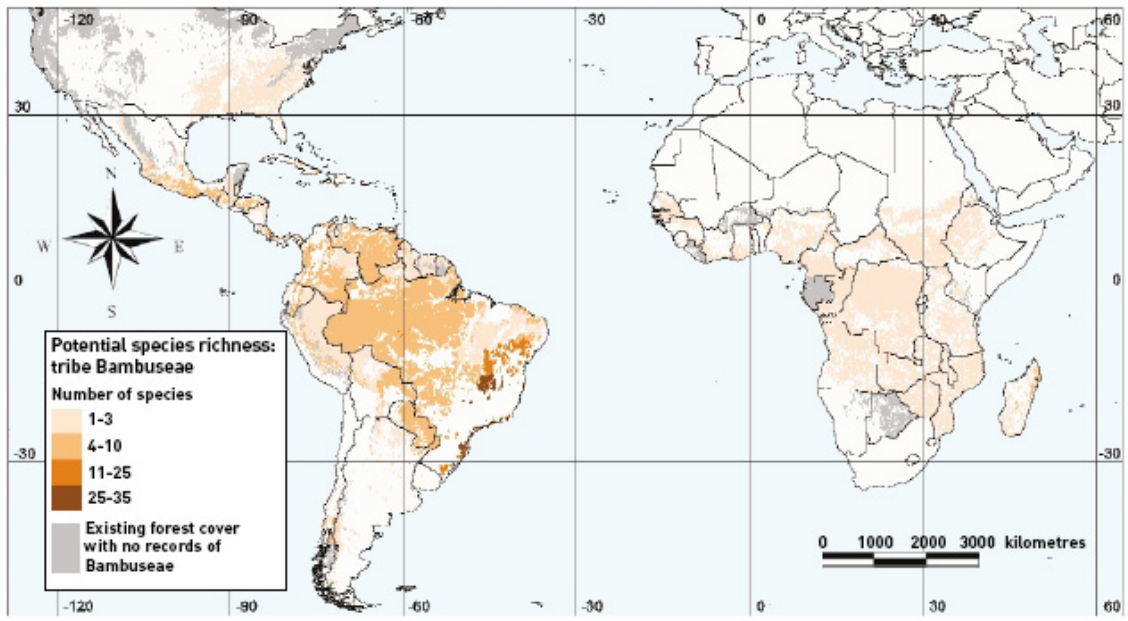


Figure 1-1. (a) Map of potential bamboo species richness in Africa and the Americas, derived by integrating 370 individual species maps. (b) Map of potential bamboo species richness in the Asia-Pacific region, derived by integrating 998 individual species maps. (Brystriakova *et al*. 2003b)

In the Asia-Pacific region nearly 1000 bamboo species (of 60 genera) occur naturally within remaining forests. The largest national complement of species was for China, which had 626 species, followed by India (102 species) and Japan (84 species) (Brystriakova *et al*. 2003b). The highest overall diversity of Asian bamboo is seen in southern China (Fig. 1-1b). It has often been assumed that areas that have high current diversity represent ancestral homes of woody bamboo. However, bamboos may not have existed in these areas for any great length of time. The current global distribution of woody bamboos, with a second important, though less rich, centre of bamboo diversity in South America suggests that early ancestors of woody bamboos evolved in Gondwanaland in the southern hemisphere in post-Cretaceous times. High biodiversity in southern China may only reflect relatively recent diversification there, unless woody bamboos have evolved more than once in separate, unrelated lineages (Brystriakova *et al*. 2003b). Bamboos are an ancient group of forest plants, intrinsically vulnerable to deforestation. They are often associated with threatened plants, and there are many specialized animal species that depend upon them. The best known of these in Asia is the giant panda (*Ailuropoda melanoleuca*). The smallest known bat (*Tylonycteris pachypus*) roots between nodes of bamboo (*Gigantochloa scortechinii*) which it enters through holes created by beetles. More than 15 Asian birds live almost exclusively in bamboo (Brystriakova *et al*. 2003b).

1.2. Morphology of bamboo plants

The following survey of the morphology of bamboo plants is based on McClure (1966).

The vegetative structure of bamboo plants is rather straightforward. The basic frame consists of a ramifying system of segmented vegetative axes, which may be differentiated as rhizomes, culms and culm branches consisting of a series of nodes and internodes. It is clothed (at least during the period of its active growth) by enveloping sheaths that face alternate sides of the axis at the successive nodes (distichous phyllotaxis). Variation in the form and other features of the nodes and internodes (particularly those of the culm) provide useful characters for the differentiation of taxa.

The culms develop from a subterranean rhizome. Individual culms and rhizomes have a relatively limited life span in comparison with the age of the bamboo plant. Old rhizomes and culms are constantly being replaced by new ones. The root system consists of a large amount of adventitious roots, which are not segmented.

1.2.1. The rhizome

The rhizome of bamboos is in charge of the distribution, the anchoring and food stock of the plant. Every year the rhizome forms new culms and immediately distal to the nodes, adventitious roots for the uptake of water and nutrients are formed.

The rhizome may develop into two main different forms: the pachymorph (sympodial) rhizome system and the leptomorph (monopodial) rhizome system (Fig. 1-2) although some subtypes also exist.

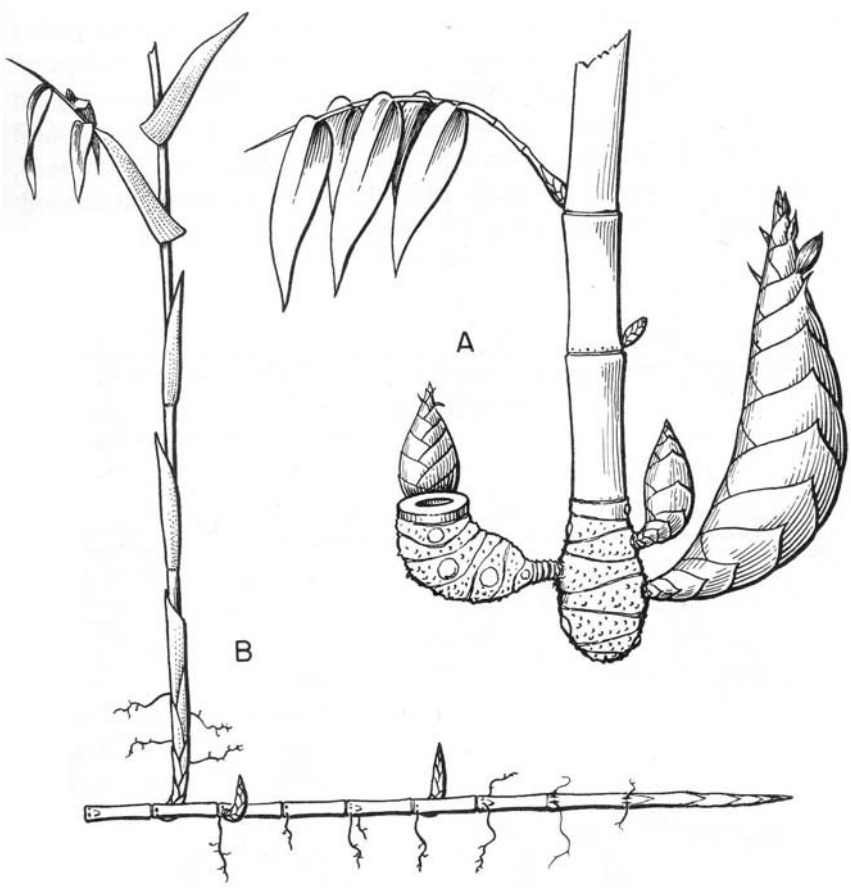


Figure 1-2. The two different main rhizome types of bamboo. (A) pachymorph rhizome system (*Bambusa beecheyana*) (B) leptomorph rhizome system (*Arundinaria amabilis*). (McClure 1966: 21: fig. 8)

Species of *Gigantochloa* and *Dendrocalamus* are examples of a pachymorph rhizome system, which is developing in a horizontal direction for a short distance, after which it turns upward to form a culm. Species of *Phyllostachys* are examples of a leptomorph rhizome system. This rhizome is described as growing widely in a horizontal direction, with more or less widely spaced culms arising from lateral buds at the nodes.

1.2.2. The culm

Bamboo culms are either lateral branches or apical differentiations of the rhizome, depending on whether the rhizome proper is leptomorph or pachymorph. In habit, they vary from strictly erect, erect with pendulous tips, or ascending, through broadly arched to clambering and from nearly straight to strongly zigzagging. Given uniform environmental conditions, each habit character is fairly consistent within a given taxon, such as species or subspecies. Bamboo shoots expand in one growing period through a particular process of cell division and cell elongation. Culms have reached their final culm wall thickness when appearing aboveground. They grow very fast,

obtaining their full length in only a few months (Fig. 1-3). The height differs from low ground covering species of 20-50 cm to very high bamboo culms of 30 m or even higher.

A bamboo culm consists of hollow internodes and full nodes. Some species have full internodes, e.g. *Chusquea* species and *Dendrocalamus strictus*. The culm wall is thick or thin, depending on the species. The culm is mostly cylindrical, but often the side where the buds are situated is flattened. The genus *Phyllostachys* for example has a groove, the *sulcus* along the internodal zone.

Figure 1-3. *Phyllostachys viridiglaucescens* stems illustrating the very rapid growth of bamboo culms.

The node is a more or less clearly defined zone around the attachments of the leaves. The nodal ridge connects the leaf structure with the nodal region. The upper part of the nodal region is often limited by a bulge formed by the intercalary meristem, the supranodal ridge (Fig. 1-4).

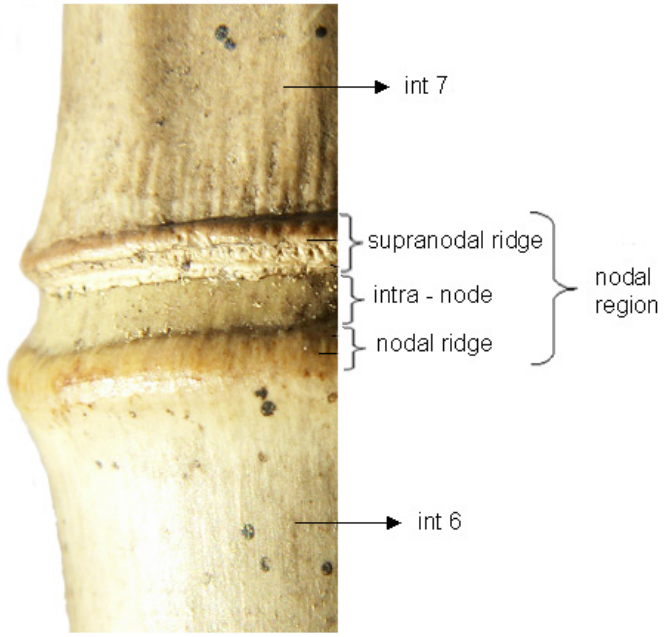


Figure 1-4. Illustration of a nodal region between internode 6 and 7 (numbered from the ground level) of a *Phyllostachys nigra* culm.

1.2.3. The vegetative leaves

A bamboo culm is characterized by two types of leaves, the culm sheaths and the foliage leaves (Fig. 1-5). Every node of every segmented vegetative axis of a bamboo plant bears a sheathing organ, which embraces the developing internode(s). Foliage leaves differ from the culm sheaths in being pseudopetiolate. The culm sheaths protect the plant parts that need mechanical protection. As soon as the growth has ended, they loose their function, dry and fall of or stay on the plant, depending on the species. The function of the foliage leaves is photosynthesis with the production of sugars necessary for the growth.

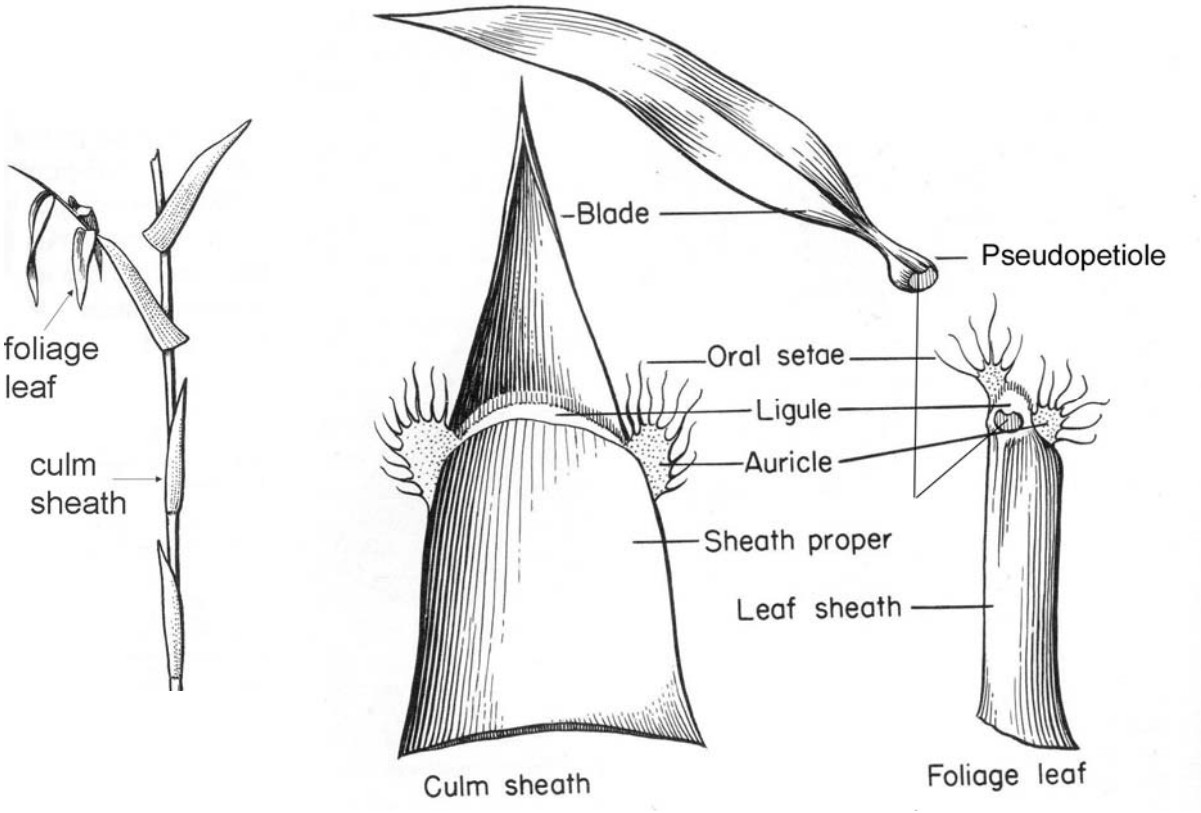


Figure 1-5. Illustration of a culm sheath and a foliage leaf showing the several component parts. (Adapted from McClure 1966: 64: fig. 35)

1.3. Anatomy of bamboo plants

Liese (1998) published a comprehensive survey of the anatomy of bamboo culms, which is used as a basis for the following overview.

1.3.1. Anatomy of the rhizome

Before a new culm is able to assimilate carbohydrates, its growth depends on the accumulated substances from older culms, which is either stored in the rhizome or transported directly from the older culms through the rhizome to the growing shoot. When new culms develop, new rhizomes will develop. These functions are reflected in the structure of the rhizome (Hsieh *et al.* 1986; Raechel & Curtis 1990; Ding *et al.* 1993, 1996, 1997).

The rhizome consists of internodes and nodes. Immediately distal to the nodes adventitious roots develop with root hairs as outgrowth of the rhizodermis for the uptake of water and mineral elements. However, the adventitious roots develop not only from the rhizome, but also from the culm base.

Generally, the rhizome has a smaller diameter and shorter internodes than the culm. The anatomical structure is basically similar to the culm. Conspicuous differences, however, exist regarding the thick cortex, random orientation of vascular bundles, poorly developed fibre strands, absence of a pith ring, and only a small pith cavity. Based on the arrangement of vascular bundles beneath the cortex and the development of air canals in the cortex, monopodial bamboos can be grouped into four types (Raechel & Curtis 1990; Ding *et al.* 1993). The rhizomes consist of, on an average, about 62 % parenchyma cells, 20 % fibres and 18 % conducting tissue. This is significantly different from the culm tissue, which consists of about 52 % parenchyma cells, 40 % fibres and only 8 % conducting tissue. These differences are closely related to the different functions (Hsuing *et al.* 1981).

The culm neck, a part of the culm axes, is very short in monopodial bamboos, but elongated in some sympodial bamboos. Culms develop from lateral buds in monopodial rhizomes but from terminal buds in sympodial bamboo culms. The implication is that the so-called sympodial rhizome is more an elongated culm neck than a rhizome (Ding *et al.* 1997b). Hence, they concluded that only monopodial bamboos have a true rhizome, while sympodial bamboos have a 'pseudorhizome'. The subterranean parts of sympodial bamboo axes are thus to be considered as culm necks rather than as rhizomes. Morphologically, the culm neck consists of a culm stalk that never bears buds nor adventitious roots, and a culm base that does form buds and roots (Fig. 1-6). Anatomically, the culm neck is mostly solid, without a lacuna and with characteristic vascular bundle structure. The xylem consists of only one metaxylem vessel. Protoxylem is either absent or poorly developed. The weakly developed fibre sheaths either surround the vascular bundles or are present only on the phloem side. There are no isolated fibre bundles as in sympodial culms.

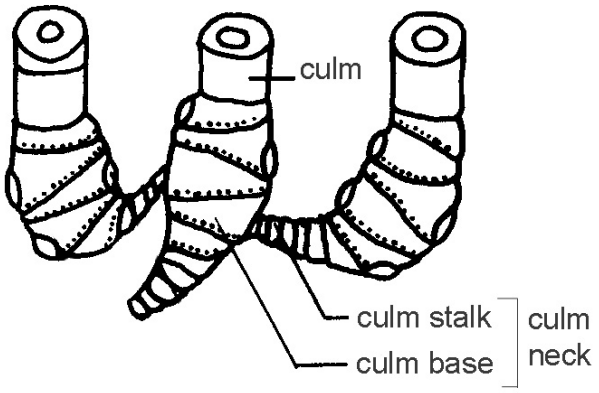


Figure 1-6. Illustration of a sympodial bamboo with a short culm neck consisting of a culm stalk and a culm base. (Adapted from Ding *et al*. 1997b)

1.3.2. Anatomy of the developing shoot

De Windt (2002) studied the shoot apex anatomy of *P. aurea* (Fig. 1-7). The apical meristem is made up of tunica cells and corpus initials. The corpus initial cells divide periclinally and anticlinally and give rise to central cells and peripher meristem. The tunica cells form the epidermis. Where the nodes will be formed, the central cells become intercalary meristem differentiating into the pith cells and the ground parenchyma cells lying in axial direction. The pith cells will die and disappear to form the lacuna. The remaining central cells differentiate into the nodal diaphragm. The vascular tissue and the fibre caps originate from the procambium. Before the culm has reached its full length, cells start to differentiate in the lowest internodes and from the inside towards the outside. The cell walls start to thicken approximately around the time the culm looses its culm sheaths. Crow (2000) studied the anatomy of the developing shoot in *P. viridiglaucescens.* As in the study of De Windt (2000), she observes the intercalary meristem as newly formed because after elongation of the internode the central cells, originally making up the core of the shoot, are positioned at the top of the internode. This is in contrast to Fischer & French (1976) who stated that the intercalary meristem is a residual meristem.

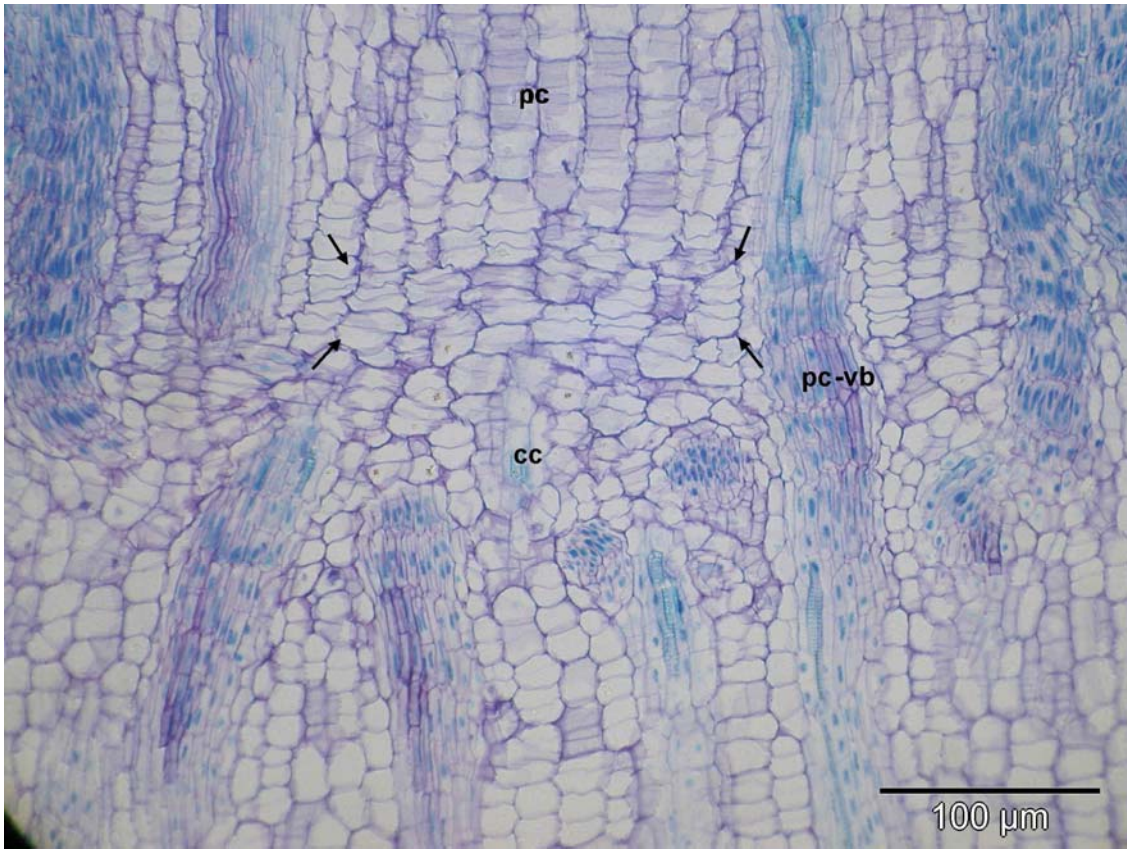


Figure 1-7. Longitudinal section through an upper internode (apex) of a 1-week old *P. aurea* shoot: the arrows indicate the intercalary meristematic cells that form pith cells above (pc). The central cells (cc) will form the nodal diaphragm. Procambium cells (pc-vb) are present and will form the vascular bundle and its supporting tissue. (Adapted from De Windt 2002)

1.3.3. General anatomy of the internode

Bamboo culms develop their tissue faster than any other plant, within only a few months. The internodes have a culm wall surrounding a large cavity, the lacuna. Contrary to dicotyledonous wood no radial cell elements exist, which greatly hinders lateral movements of nutrients or liquids. Wood consists basically of xylem, being the water transport system that gradually looses its function with age. Bamboo tissue on the other hand remains functional throughout the life span of a culm. The nodes provide the transversal interconnection in the culm by their solid cross wall, the diaphragm. In the internodes the cells are strongly oriented axially. In the nodes a more complex structure is found (Ding & Liese 1997; André 1998).

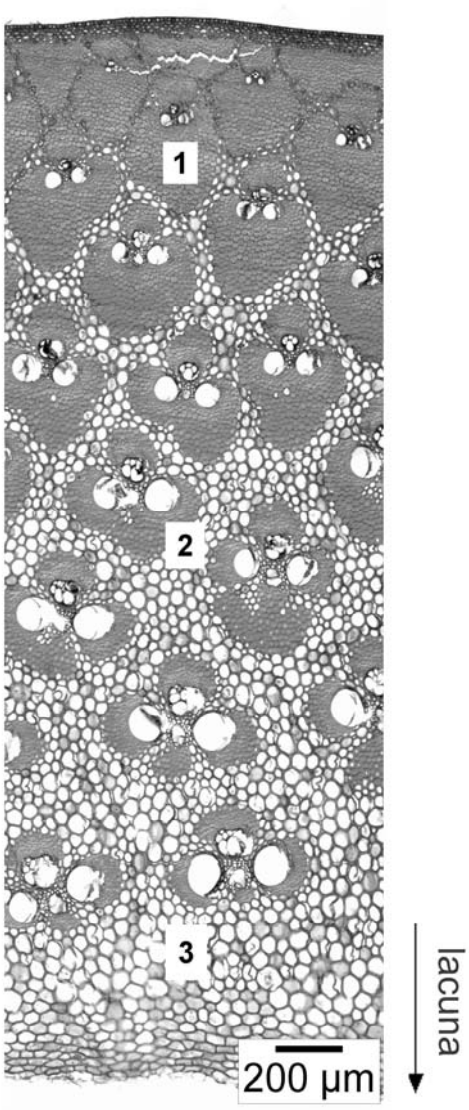


Figure 1-8. Transversal section through internode 6 (numbered from the ground level) of a culm of *Phyllostachys viridiglaucescens*. The vascular bundles are embedded in the parenchymatous ground tissue. 1: outer part of the culm wall, 2: middle part of the culm wall, 3: inner part of the culm wall.

A transversal section of a culm internode shows the parenchymatous ground tissue in which collateral vascular bundles composed of protoxylem and metaxylem vessels, sieve tubes with companion cells and fibres sheaths are embedded (Fig. 1-8). The outermost layer of the culm wall is the epidermis. The epidermal cells are often covered by a cutinized layer of cellulose and pectin with tangential lamellation. A wax coating is formed on top and appears as irregular plates, rods or granules. Beneath the epidermis lies the hypodermis, consisting of several layers of thick-walled sclerenchymatous cells. The compact composition of the cortex and the wax coating prevent loss of water from the culm. It also hinders the penetration of liquid. Particular hindering is observed for the penetration of chemicals during the pulping process and of preservatives during vacuum pressure impregnation.

The parenchyma cells of the ground tissue are small near the outer part of the culm wall and become larger, especially axially, towards the inner part, and get smaller again near the lacuna. They are of two types: vertically elongated cells and short cube-like ones interspersed among the former. He *et al.* (2002) showed a difference in lignification and in hemicellulose distribution between the two parenchyma cell types, concluding that the short cells remain in the stage before the onset of cell elongation. As parenchyma cells are the storage tissue for the plant, starch granules can fill up the cells. Starch is abundant in elongated parenchyma of ground tissue, vascular parenchyma cells, parenchyma cells around the nodes and in the diaphragm.

On average, a culm consists of about 52 % parenchyma, 40 % fibres and 8 % conductive tissue (vessels, sieve tubes and companion cells) (Liese & Mende 1969; Grosser & Liese 1974). Londoño *et al*. (2002) found that culms of *Guadua angustifolia* are formed by 40 % fibres, 51 % parenchyma and 9 % vascular tissue on average. A large variability is present within the vascular bundles of one culm, as well as in transversal section as in longitudinal direction (Fig. 1-9).

Near the periphery of the culm (outer part of the culm wall), the bundles are smaller and numerous with only a few parenchyma cells in between. The three xylem fibre sheaths are often merged into an ellipsoid, making the xylem sheath larger than the phloem sheath. Vessels towards the outer part of the culm wall are smaller than in the middle and inner parts. The percentage of fibre decreases from the outer part of the culm wall to the inner part of the culm wall, while the percentage of parenchyma increases.

In the longitudinal direction, the narrowing of the culm wall results in a reduction of the inner portion that has more parenchyma and less vascular bundles. Thus the upper part of a culm contains as much or more fibres per unit area resulting in a higher density, whereas the basal part has higher parenchyma content. The size of vascular bundles decreases from the base to the top of the culm. As their radial diameter reduces much more than the tangential diameter, the shape of vascular bundles changes from a radially elongated form to a roundish or oval form near the top.

The variation of vascular bundles in longitudinal direction is more significant than in transverse direction. Londoño *et al*. (2002) studied anatomical features as size, form and distribution of the vascular bundles, diameter and percentage of metaxylem, and percentage of fibres along the width and length of the culm wall in *Guadua angustifolia*. They showed variation in relation to the thickness of the culm wall, with a higher concentration and smaller size of the vascular bundles towards the periphery. A relationship between the features evaluated and the culm age could not be found.

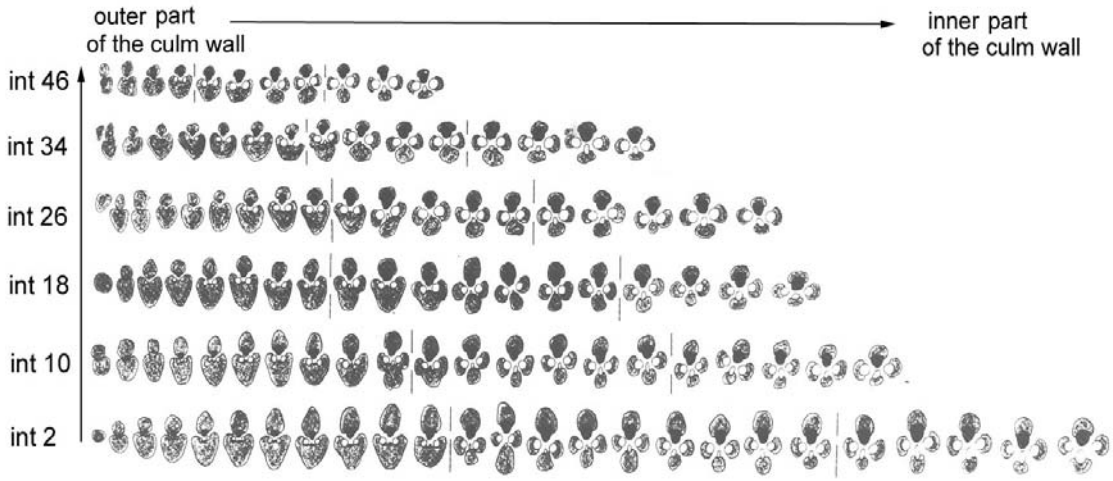


Figure 1-9. Variability in shape of vascular bundles in transverse and longitudinal direction of a *Phyllostachys edulis* culm. (Adapted from Liese 1998: 40: fig. 28)

The protoxylem consists of one or more tracheal elements located between two large metaxylem vessels towards the pith cavity. Often, the protoxylem of monopodial species shows tyloses in the cells, developed from the surrounding parenchyma. The two large vessels of the metaxylem are separated by parenchyma cells, which are generally smaller than the ground parenchyma cells. The phloem consists of large, thin-walled sieve tubes and smaller companion cells originating from the same mother cells. Fibres occur as fibre caps (sheaths) surrounding the conducting elements. Grosser & Liese (1971) analysed the variability of vascular bundles in form and size and grouped them into four basic types. Liese & Grosser (2000) described two additional vascular bundles types. Vascular bundles of type I consists of only one part: the central vascular bundle, with a supporting tissue of four fibre caps on the sides, symmetrically located and nearly of the same size (Fig. 1-10a). This type is present in species with monopodial rhizomes, for example *Phyllostachys* species and *Arundinaria* species. Type II also consists of one part, the central vascular bundle, with a supporting tissue of four fibre caps. The inner fibre cap at the protoxylem is strikingly larger than the two lateral ones (Fig. 1-10b). Vascular bundle type III consists of two parts. Besides the central vascular strand, a free fibre strand is present, located at the inner side of the vascular bundle (Fig. 1-10c). This type is present only in sympodial genera (e.g.

Gigantochloa, *Dendrocalamus Bambusa*). Vascular bundle type IV (Fig. 1-10d) consists of three parts: the central vascular strand with small sclerenchyma sheaths, and two free fibre strands located at the phloem and the protoxylem side. It is present in sympodial genera growing in dense clumps (*Gigantochloa*, *Dendrocalamus, Bambusa*,) and occurs always combined with type III.

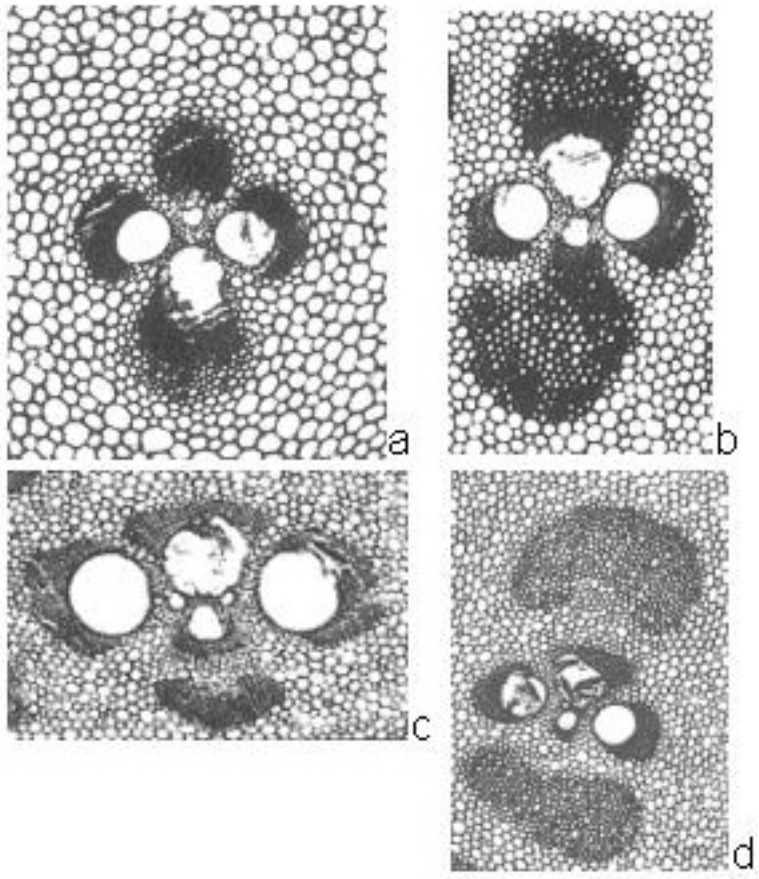


Figure 1-10. (a) Type I vascular bundle of *Phyllostachys edulis*. (b) Type II vascular bundle of *Cephalostachyum pergracile*. (c) Type III vascular bundle of *Oxytenanthera albociliata*. (d) Type IV vascular bundle of *Bambusa polymorpha*. (Adapted from Liese 1998: 29-31: fig. 18-21)

Type V corresponds to type III and IV, but the fibre strand at the inner side of the central vascular strand opposite the protoxylem appears subdivided, consisting of two or more separate units (Fig. 1-11a). This type occurs more sporadic than regular, but no specific taxa have been assigned to it. Vascular bundle type VI occurs in climbing bamboos (e.g. *Dinochloa* spp.) and consists of a central vascular strand with a large often elongated-oval phloem area, two metaxylem vessels of extremely large diameter, and an only small protoxylem space. The sclerenchyma is mostly limited to very small sheaths of few cells wide (Fig. 1-11b).

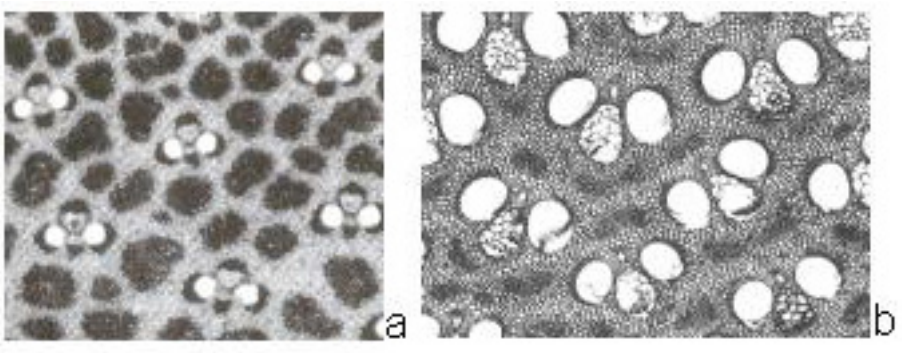


Figure 1-11. (a) Type V vascular bundle of *Bambusa forbesii*. (b) Type VI vascular bundle of *Dinochloa scandens*. (Adapted from Liese 1998: 32-33: fig. 22-23)

At the inner side of the culm wall, cellular layers surround the pith cavity, becoming more pronounced as the culm ages. This pith ring is a non-vascular tissue composed of layers of parenchyma cells, which are often heavily thickened and lignified.

Bamboo culms and rhizomes respond to wounds in order to protect the surrounding tissues against damaging influences through the wound surfaces. The defense arsenal consists of a number of cellular reactions such as closure of sieve tubes by callose, formation of slime and tyloses, phenolics, suberised cell walls, wall lignification and also septa development in fibres. There is also accumulation and mobilization of starch around the wound, development of additional layers of the cell wall in parenchyma cells and fibres, and the formation of a suberin layer in vascular parenchyma cells (Weiner & Liese 1997).

1.3.4. Maturation and fine structure of bamboo fibres and parenchyma cells

Cell wall thickening

Structural modification during the maturation phase and the following years relate to fibres and parenchyma cells. The results obtained by Alvin & Murphy (1988), Majima *et al.* (1991), Liese & Weiner (1996, 1997) and Murphy & Alvin (1997a, b) have demonstrated that the maturation process of fibres proceeds quite differently over the transverse section of a culm wall. The process starts at the outer part of the culm wall and proceeds toward the centre. Furthermore, it is influenced by the position of the fibres within the vascular bundle. Cell wall thickening of bundle sheath fibres starts from the inner vascular side and proceeds to the outer parenchyma side.

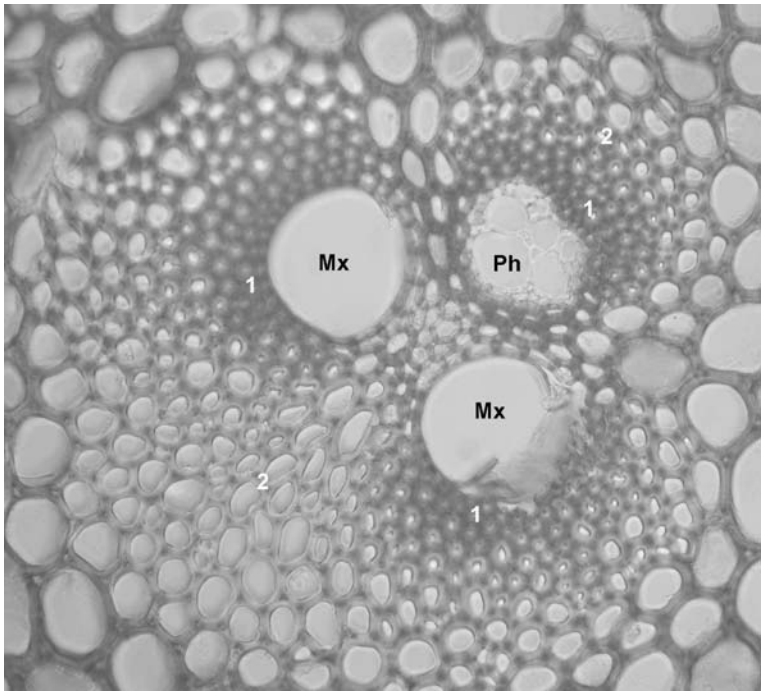


Figure 1-12. Transversal section of a vascular bundle in the middle part of the culm wall (internode 6) of a 6-month old *P. viridiglaucescens* culm. 1: early maturing fibres, 2: late maturing fibres, Mx: metaxylem, Ph: phloem.

Murphy & Alvin (1997a) describe two fibre types distinguishable during bamboo culm development. The early maturing fibres occur around the periphery of the culm wall and adjacent or close to the vascular tissue and thicken to almost their maximum during the first year of culm growth. The second type, the late maturing fibres, keep their potential for continued wall thickening even in 3-year old culms and occur adjacent or close to the parenchyma. In figure 1-12 it is shown that the early maturing fibres have already a thick cell wall comparing to the late maturing fibres, which are characterized by a thinner cell wall in a 6-month old culm. Liese & Weiner (1996, 1997) investigated in detail culms of *Phyllostachys viridiglaucescens* aged up to 12 years. During the first month of the growing period the fibre wall thickness at the 20th internode was 1.5 - 1.7 µm but most fibres were still unlignified. At 3 months they were fully elongated, had three lignified layers and a fibre wall thickness of 2 - 3 µm. The wall thickness grew from 5 µm after 1 year, over ± 6 µm after 3 years to 8 µm after 12 years containing eight layers. They concluded that the increase in wall thickness was caused by the deposition of additional layers. This ability of prolonged cell wall thickening of fibres and parenchyma cells may provide an excellent mechanism for further strengthening the culm as it ages, and might be to an extent functionally comparable with the development of secondary thickening in dicotyledonous plants (Murphy & Alvin 1997a). Liese & Weiner (1996) and Murphy & Alvin (1997a) showed that bamboo fibre and parenchyma cells retain their living protoplast and have the ability to maintain viability during cell wall lignification, which is necessary to have a continued cell wall thickening. The heterogeneity in fibre maturation with both location (early and late maturing fibres) in the culm and with time may have its explanation in the very rapid culm height growth in bamboos (Murphy & Alvin 1997a). Provision of a fully developed and strengthened culm during the early growth phase would impose a very large resource demand at the likely expense of a reduced growth rate, resulting in a reduced height. Instead, the most critical elements of the culm for provision of mechanical support (outer part of the culm wall) and the protection of the vascular tissues are selectively developed at the earliest opportunity.

Lignification

The incorporation of the three-dimensional phenolic polymer lignin into the space between cell wall microfibrils is termed lignification. Lignin decreases the permeability and degradability of walls and is important in determining the mechanical behaviour of predominantly nonliving tissues such as wood (Donaldson 2001). Lignins are complex aromatic heteropolymers derived mainly from three hydroxycinnamyl alcohol monomers differing in their degree of methoxylation, *p*-coumaryl, coniferyl, and sinapyl alcohols. These monolignols produce, respectively *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid units when incorporated into the lignin polymer. The amount and composition of lignins vary among taxa, cell types, and individual cell wall layers and are influenced by developmental and environmental cues. Although exceptions exist, dicotyledonous angiosperm lignins consist principally of G and S units and traces of H units, whereas gymnosperm lignins are composed mostly of G units with low levels of H units. Lignins from grasses incorporate G and S units, and more H units. Because lignification occurs in the cell corner and middle lamella at an early stage and in the secondary wall at the late stage, the monolignols deposit to different extents in different morphological regions of the

developing cell wall. In woody tissues the middle lamella and the primary wall are stronger lignified than the secondary wall. However, because the secondary wall occupies a larger portion of the wall, the secondary wall has the highest lignin content. Secondary walls of vessels generally have higher lignin content than those of fibres. Environmental conditions also influence lignin amount and composition: secondary walls of angiosperm tension wood are characterized by the presence of an unlignified gelatinous layer. By contrast, the secondary wall layer of gymnosperm compression wood is characterized by a highly lignified ring. In grasses *p*-coumaric and ferulic acids are widely distributed along with polylignol moieties. The composition and content of these phenolic acids depend on the morphological location and differentiation stage. (Fengel & Wegener 1983; Terashima *et al.* 1993; Boerjan *et al.* 2003).

The lignification of *Phyllostachys heterocycla* was studied by Itoh (1990), revealing full lignification of the component cells within one growing season. The lignin content determined on a dry weight basis by the sulphuric method in culms aged 2 to 4 years determined at internodes 10, 20 and 30 only varied from 23.2 to 26.4 %. Abd. Latif *et al.* (1996) showed that the lignin increased from 20.6 % in the half-year old culm of *Bambusa heterostachya* to 22.6 % in the two-year old culm, decreased to 20.8 % in the three-year old culm but increased again to 21.3 % in the four-year old culm. They state that this is due to the fact that the full lignification of the bamboo is completed within one growing season with no further significant ageing effects. Murphy & Alvin (1997b) showed that *Phyllostachys viridiglaucescens* bamboo culms were only half lignified at year 1 (approx. 13 %) compared to 3-year old culms (over 26 %) having lignin content comparable to wood. Lin *et al.* (2002) showed that fibre walls are rich in guaiacyl lignin in the early stage of lignification, and lignin rich in syringyl units is deposited in the later stage. The lignification process may last even up to 7 years.

An important feature of a lignified fibre is its liveliness. Whereas the fibres in hardwood normally die after cell wall differentiation with the simultaneous degeneration of their cytoplasm, bamboo fibres retain their cytoplasmatic activity long after cell wall lignification (Murphy & Alvin 1997a). He *et al.* (2000) studied the ultrastructure of the secondary wall formation and the related cellular organelles during the maturation of a bamboo stem. They divide the development of bamboo fibre into four stages: cell differentiation, cell elongation (primary wall formation), cell wall thickening and lignification (secondary wall formation). At the stage of the wall thickening, the number of organelles increased, especially the endoplasmic reticulum, Golgi bodies and mitochondria. With the progress of wall thickening and lignification, plasmolysis was observed, there was thinning of cytoplasm and decreasing of the number of organelles, with concurrent appearance of abundant $KMnO_4$ stained lomasomes. They considered these facts in cells of late fibre development to be evidence of programmed cell death in bamboo fibre. However, Gritsch & Murphy (2005) mention that a full examination of protoplast contents is not possible due to the poor fixation of specimens. Crow (2000) tested several fixation protocols using *Phyllostachys viridiglaucescens* and also found that ultrastructure preservation of bamboo tissues tended to be erratic. Only the youngest tissues are reasonable well preserved. Older tissues show a poor preservation of membranes and, consequently of organelles. Probably, the samples studied by He *et al.* (2000) were not mature and furthermore, bamboo fibre cell walls are known to continue thickening even in very old culms and so they clearly retain protoplasts (Liese & Weiner 1996; Murphy & Alvin 1997a).

The fibres appear to undergo progressive septation (Murphy & Alvin 1997a). Contrary to dicotyledons, the septate fibres show secondary wall apposition and become multilayered (Parameswaran & Liese 1977).

Ultrastructure

A plant cell wall is composed of several layers. The middle lamella consists largely of highly hydrated, pectinaceous substances and acts as an intercellular glue to bind the walls of adjacent cells together. The primary cell wall is the first layer of the cell wall. It develops on either side of the middle lamella and largely determines cell shape and size during plant growth and development. It is composed of a continuous interconnected system of aggregated cellulosic microfibrils that result from the simultaneous polymerization of cellulose molecules. Differences in the orientation and other mechanical properties of cellulosic fibrils within cell walls are primarily responsible for the high specific strength and resistance to tensile forces. The microfibrils are embedded in a less ordered matrix rich in hemicellulosic materials and pectic polysaccharides. The middle lamella and primary walls of two adjacent cells combine to constitute the compound middle lamella. The compound middle lamella is composed primarily of a complex organic molecule called lignin, along with some pectines, cellulose, hemicellulose and other minor constituents.

Some cells, particularly those with strengthening and supporting functions, continue to add wall material inside the primary wall during cell expansion, before the cell has reached its final size. This additional wall material is called the secondary wall and is represented by the further deposition of laminated cellulose upon the primary wall. The proportion of cellulose in secondary walls is typically higher than in primary walls. In contrast to the random pattern of microfibril orientation in the primary wall, microfibrils in the secondary wall are arranged in an ordered, parallel manner. The crossed structure of the microfibrils prevents the formation of splits and gaps in the framework of the wall. Cellulosic microfibrillar components make up over 50 % of secondary walls, a higher percentage than hemicelluloses and pectines. These wall materials are embedded in a matrix of the amorphous molecule lignin (Esau 1965; Terashima 1993; Dickinson 2000).

Detailed investigations by Tono & Ono (1962) and Parameswaran & Liese (1975, 1976, 1980), as well as contributions by Fujii (1985) and Murphy & Alvin (1992) have revealed the numerous layers that constitute the bamboo cell wall. Parameswaran & Liese (1975) described parenchyma cells in *Phyllostachys edulis* to exhibit a polylamellate structure consisting of about 15 successive layers arranged in an alternating manner. The wider layers are characterized by the orientation of cellulose fibrils perpendicular to the cell axis, and the narrow ones by fibrils oriented parallel to the cell axis. Parameswaran & Liese (1976) showed that the mature fibre wall is characterized by a regular alternation of wide and narrow layers (Fig. 1-13).

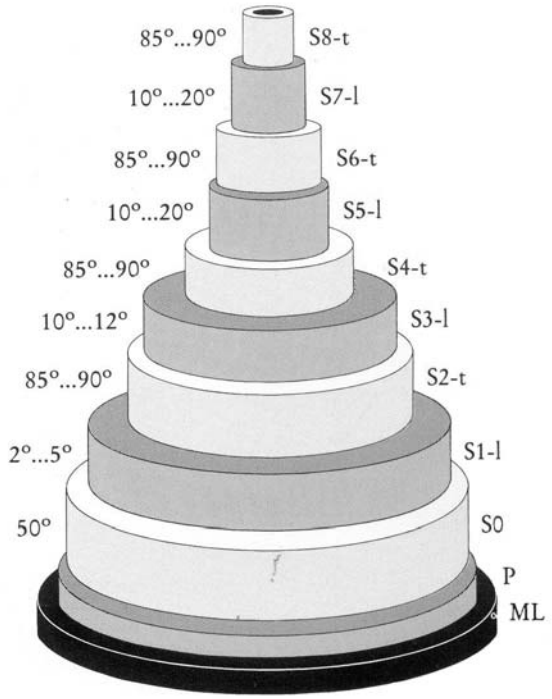


Figure 1-13. Model of the polylamellate structure of a thick walled bamboo fibre (figures on the left indicate fibril angle, letters on the right the terminology of the wall layers. (Liese 1998: 68: fig. 51)

Near the middle lamella, the first layer of the secondary wall shows microfibrils oriented at an angle of 50° to the cell axis. In the wide layers the microfibrils are oriented at an angle of 2-5°, which increases up to 10-20° towards the inner part of the wall. The narrow layers show mostly fibrils oriented at an angle of 85-90°, which remains constant over the whole width of the wall. Characteristically, the fibrils show a slight depression before merging into the fibrils of the next broad lamellar zone. The innermost layers near the lumen are usually thinner than the others. The layers at the lumen boundary have no resemblance in their fibrillar texture to the tertiary wall typical of wood fibres.

Crow & Murphy (2000) observed the orientation of microfibrils in the inner walls of differentiating and maturing fibres and parenchyma cells under the FESEM. Orientation changes were similar in both cell types. During very early primary wall development, deposition of microfibrils was in more or less axial alignment, which was later superseded by microfibrils in transverse orientation. An abrupt shift to a sloped orientation occurred during late primary wall synthesis. Microfibrils of the first secondary wall layer were in axial alignment or steeply sloped. In subsequent secondary wall deposition there was an alternation between a transverse and a sloped or axial alignment in maturing fibres and parenchyma cells. This alternation of microfibril orientation is responsible for the formation of multi-layered secondary cell walls. Suzuki & Itoh (2001) characterized the

primary wall of *Phyllostachys aurea* by having narrow spacing between the cellulose microfibrils in fibres, but not in parenchyma cells. The secondary wall largely consisted of dense cellulose microfibrils also with narrow spacing. They suppose that the deposition of lignin in the secondary wall proceeds in the pores between the cellulose microfibrils during maturation and they hypothesize that the smaller pore size in *Phyllostachys* than in *Pinus* or *Eucalyptus* may be one of the reasons for the deposition of less lignin in bamboo fibres than in tree species. However, because the total Klason lignin of bamboo is not lower than in tree species, a large quantity of lignin in bamboo culms should be accumulated in the larger pores of the middle lamella.

The number of wall layers varies in different fibres, and is the highest in fibres either adjacent to vascular bundle elements or at the periphery of fibre bundle close to the ground tissue. This can be partly attributed to the position of the vascular bundle, the location of the fibres within the vascular fibre cap, and the maturity state of the fibres (Murphy & Alvin 1992; Liese & Weiner 1996). Results on the number of layers in the fibre cell wall have been reported up to a maximum of 18. Gritsch *et al.* (2004b) concluded that the multilayered structure of the fibre cell walls was formed mainly 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 layering was not found to be specifically related to the thickness of the cell wall. The number is highest in the fibres either adjacent to vascular bundle elements or at the periphery of fibre bundles close to the ground tissue. The polylamellate structure may be associated with the ability of cell walls to resist the development of compression creases (kinks) under compressive loads during culm bending (Murphy & Alvin 1992).

In a similar way, thickening of parenchyma cell walls was noted in older culms (Alvin & Murphy 1988) up to 3 years. Cells with thin walls may represent still immature fibres, but often they are parenchyma cells, recognizable by their square ends in the longitudinal section (Murphy & Alvin 1992). Bath (2003) found that for two Indian bamboos cell wall thickening of fibres was accomplished leading to a polylamellate cell wall structure. In ground parenchyma, although the wall thickening was evident, layering was not distinct.

The fact that the cell wall structure of bamboo fibres and parenchyma cells comprises several layers in the secondary wall has implications for the nomenclature of the secondary wall in bamboo. In this work the term '*secondary wall* ' will be used when all cell wall layers (except the compound middle lamella) are indicated. When a specific layer of the secondary wall is meant, the term L_x will be used (Fig. 1-14).

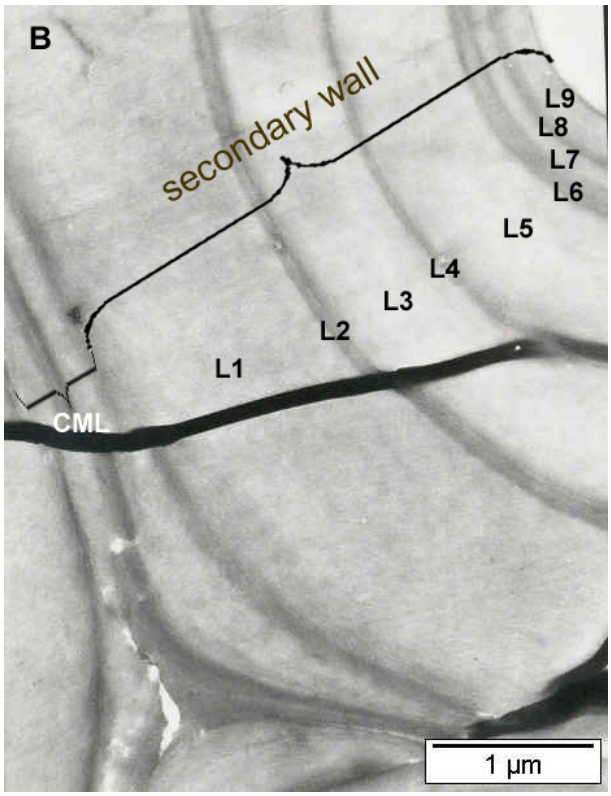


Figure 1-14. Transversal section of a fibre from the inner side of a xylem fibre cap of a 3-year old *P. viridiglaucescens* culm (internode 6 – middle part of the culm wall). CML:compound middle lamella. L_1-L_9: layers of the secondary cell wall.

Murphy *et al.* (1991) studied the development of soft rot decay in the bamboo *Sinobambusa tootsik*. They only found soft rot attack in the cell walls of fibres; parenchyma and vessels remained unattacked. Decay was greatest in the walls of late maturing fibres in which the wall contained zones of low lignin content. Murphy *et al.* (1997b) also found that young culms are more susceptible than mature material. They concluded that bamboo was no more susceptible to decay by white or brown rot fungi than *Betula bendula* and *Pinus sylvestris*. Murphy *et al.* (1997a) also observed by studying soft rot-decayed material that cell wall microfibrils were laid down in sheets from the lumen, and obtained evidence for a helicoidal microfibrillar orientation in parenchyma cell walls. Fibre cells were characterized by thick layers that probably have a single, broadly axial microfibrillar orientation and thin layers having a broadly transverse microfibrillar orientation.

Observations made by Gritsch & Murphy (2005) indicated that the primary wall of the bamboo *Dendrocalamus asper* is formed by the deposition of two distinct layers during the elongation of the internode and that secondary wall synthesis may begin before the complete cessation of internode and fibre elongation. They suggest that this may act to cause the shutdown and eventual cessation of cell elongation.

1.3.5. Anatomy of the node

The anatomical structure of bamboo culms has gained considerable interest because of their economic importance and their multiple uses in daily life. In contrast to the anatomy of the internodes, the composition and structural details of the nodes have been poorly analysed so far (Zee 1974; Liese & Ding 1994; Ding & Liese 1995, 1997; André 1998). Nevertheless, the nodes have special significance for the culm function. Owing to the lack of radial conduction cells, they enable the necessary communication for cross-transport of water and nutrients.

Furthermore, the nodal structure is important for liquid movement during drying and preservation as well as for the physical and mechanical properties of the culm.

Ding & Liese (1997) studied the structure of the nodal region of several bamboo species. From serial sections they reconstructed the three-dimensional structure of the vascular system (Fig. 1-15). Most of the main axial vascular bundles pass directly from an internode through the node into the next internode. In the peripheral zone of the culm, they bend slightly outwards while branching partly into the culm sheath. In the inner zone, they are connected with those in the diaphragm. In the node, the bundles become larger in diameter and vascular anastomoses develop intensively.

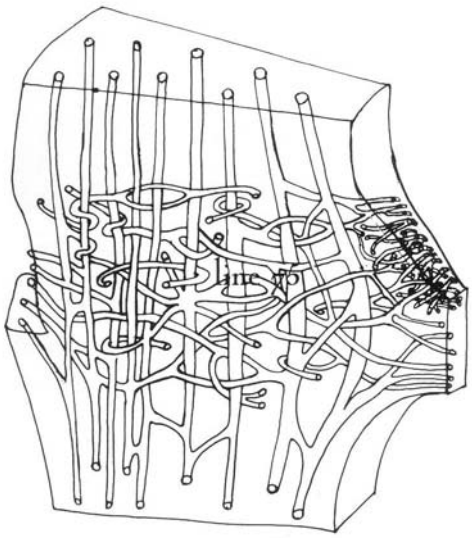


Figure 1-15. Illustration of vascular anastomoses within the nodal region. (Liese 1998: 93: fig. 75)

In the diaphragm, the typical vascular bundle structure of bamboo disappears. The xylem consists of only one metaxylem vessel. At the branching of vascular bundles, smaller cells develop abundantly with an intensive reticulate pitting. The vascularization of the bamboo node was demonstrated by André (1998) by means of a microcasting method in which bamboo segments were immersed with silicon elastomer components. After the elastomer became an inert material, the bamboo tissue was destroyed leaving a cast of the bundle ramifications.

The ground tissue of the diaphragm consists of short parenchyma cells interspersed with sclerified cells. At the branching point of vascular bundles, the phloem shows special elements consisting of numerous filiform cells. Similar phloem anastomoses have been described in the nodes of the monocotyledon *Dioscorea* and were called *'Phloem Beckenzellen'* (Behnke 1990).

1.4. Anatomical properties relating to the strength of bamboo

1.4.1. Chemical properties of bamboo

Fengel & Shao (1984, 1985) determined as chemical composition for *Phyllostachys makinoi* 25.5 % lignin, 45.3 % alpha-cellulose, 24.3 % polyoses and 2.6 % extractives. The main polyose is arabinoxylan and the bamboo lignin is rich in syringyl units. Lin *et al.* (2002) showed that fibre walls are rich in guaiacyl lignin in the early stage of lignification, and lignin rich in syringyl units is deposited in the later stage. As a unique feature, bamboo lignin also contains 5-10 % of *p*-coumaric acid ester (Higuchi 1987), located at the γ-positions of grass lignin. Azuma *et al.* (1996) showed that lignin and phenolic acids (mainly composed of *p*-coumaric and ferulic acid) are lower in immature parts of a shoot in comparison to lower mature parts of the same shoot. They showed that the esterified *p*-coumaric acid content was closely related to the lignin content, whereas ferulic acid was rich in upper immature portions of the culm shoot.

The starch content of a culm is of considerable importance in terms of utilization. Starch accounts for the susceptibility of harvested culms to beetle attack and accelerates fungal deterioration. The age of the culm influences the starch content. Alvin & Murphy (1988) investigated culms up to three years and found virtually no starch during the first year of growth but a lot in older culms. Abd. Latif *et al.* (1994) found that starch content was significantly correlated with age. Seki & Aoyama (1995) demonstrated that the starch content varied significantly during the year. The content decreased markedly during the period of rapid growth of young culms. Subsequently, starch was accumulated steadily in the culms during autumn and winter, and then the content peaked towards the next sprouting season. They found that the fluctuation pattern of starch in the young culms was similar to that of the aged ones, except that the former contained only small amounts of starch. Research on *P. viridiglaucescens* (Weiner & Liese 1996, 1997) has shown the lack of starch granules in young, growing culms. Liese & Abd. Latif (2000) showed that starch content differs significantly between species, site, age, culm height and harvesting month.

Bamboo consists for 0.8 % up to 9.7 % of inorganic components with a higher amount in the nodal region than in the internodes. Silica is a major constituent of the cortex region with values between 1.5 % and 6.4 % and is mainly localized in the epidermal cells. The culm tissue itself contains hardly any silica and the nodes contain only small amounts. The presence of silica affects the cutting and pulping properties of bamboo. Some sympodial bamboo taxa from tropical climates contains a siliceous deposit in the lacuna, called tabashir (used in traditional medicine, properties as a catalyst). Although most silica appears situated in the cortex region, more knowledge about its location would be useful for processing technologies. The selection of species with a lower amount of silica is significant for the manufacture of products as furniture, structural components and skewers (Hamdan & Abd. Latif 1992). Schmitt *et al.* (2002) localized silica polymers as extracellular deposits within the wall of epidermal cells of bamboo culms.

Young internodes showed distinct silica deposition in epidermal wall regions underneath the cuticle. In the outer wall regions silica granular deposits were found in increasing amounts during maturation but were only present in inner wall regions in late development stages. Gritsch *et al.* (2004a) observed in *Guadua angustifolia* silica bodies embedded amongst fibres in the periphery of the vascular bundles and in the intercellular spaces between parenchyma cells in the ground tissue.

The nodal portion of a culm has a lower holocellulose content but a higher content of extractives, pentosans, lignin and ash than the internodal portion (Bambang 1996).

1.4.2. Physical-mechanical properties of bamboo

The anatomical structure of bamboo culms with a considerable higher percentage of fibre cells in the outer third of the culm wall, a higher percentage of parenchyma in the inner third, a higher amount of parenchyma at the base of the culm and of fibres at the top of the culm, all influences the mechanical properties.

Higher moisture content, resulting in a decrease in strength of bamboo (Prawirohatmodjo 1990) is found at the base of a culm (Liese 1980; Siti & Abd. Latif 1992; Sattar *et al*. 1994; Abd. Latif *et al.* 1996; Kabir *et al.* 1996) and at the innermost layers due to the higher amount of parenchyma. This is in agreement with Janssen (1981) who suggested that the parenchyma cells form the weakest part of the culm. The high amount of parenchyma in bamboo is also reflected in considerable amounts of dust when processed. Kabir *et al.* (1996) found that the node exhibited lower moisture content, which he related to the reduced quantity of parenchyma in which water is stored. In contrast, Bambang (1996) found that the node has higher water content than the internode. Several authors (Abd. Latif *et al.* 1993; Espiloy 1994; Abd. Latif *et al.* 1996) found a negative correlation of moisture content and shrinkage with age.

The strength of a material is related to its density. The density of bamboo varies approximately from 0.5 to 0.9 g/cm³ but can differ considerably within the culm (increase with the height of the culm (Siti & Abd. Latif 1992; Jamaludin *et al.* 1995; Kabir *et al.* 1996; Subyakto 1996) and between species (Janssen 1991; Jamaludin *et al.* 1995). Cell wall thickening during maturation of the culm from 1 to 3 years leads to an increase in basic density of the culm material (Jamaludin *et al.* 1992; Siti & Abd. Latif 1992; Abd. Latif 1993; Espiloy 1994; Sattar *et al*. 1994). The increase in density is dramatic during the first two years but becomes more gradual during the third year and stabilized thereafter (Bath 2003). The culm node shows a higher density than the internode (Kabir *et al.* 1996).

The stage of fibre maturation and the density of vascular bundles both influence shrinkage. If extreme shrinkage occurs due to moisture loss, the outer culm wall regions, which contain a larger concentration of the strong fibrovascular bundles, set the weaker inner culm wall region in tension, due to the presence of larger concentrations of parenchyma cells. This causes the inner culm wall region to develop cracks. Both the radial and tangential shrinkage decrease with the height of the culm since the top portion has a higher number of vascular bundles and lower initial moisture content. Older bamboos were also found to shrink less than younger ones (Siti & Abd. Latif 1992; Abd. Latif *et al.* 1995). Lee *et al.* (1994) suggested usage of at least 2-year old culms in order to avoid less lignified fibres and parenchyma walls. Culms of higher age are preferred for bamboo furniture since they show less shrinkage and splitting. Immature culms are prone to splitting, shrinkage, breakage and biological attack. The internode of the culm has a higher volumetric shrinkage than the node due to the higher initial moisture content (Kabir *et al.* 1996).

Janssen (1990) states that compared with wood, shear strength in split bamboo is stronger than in wood due to the axial arrangement of fibre cells and the absence of rays, which create weak spots. The axial alignment of fibre cells leads to a material, which shows its best mechanical strength parallel to the grain (Arce 1994). Shear strength and modulus of elasticity have been shown to increase significantly towards the top of the culm (Abd. Latif *et al.* 1990). There is also an increase in compression and bending strength towards the top of the culm, all corresponding to a proportional increase in fibrovascular bundles (Limaye 1952; Grosser & Liese 1974; Espiloy 1987; Subyakto 1996). Zhang *et al.* (1994) found that tensile strength decreased gradually with increased fibre length and increased dramatically with fibre cell diameter. Fibre length shows a positive correlation with the modulus of elasticity (MOE) and the compression strength. An increase in cell wall thickness has been shown to correlate with an increase in compression strength and in modulus of elasticity (MOE), but negatively with the modulus of rupture (MOR) (Abd. Latif *et al.* 1992; Abd. Latif 1993). The nodes have been shown to be weak in tensile strength compared with the internodes (Hsuing *et al.* 1981; Kabir *et al* 1996), probably because the fibre cells interconnect and enter the diaphragm at these points, creating a discontinuity of fibre alignment (Arce 1994). Liese & Ding (1994) suggest a reduction in mechanical elasticity due to shorter, wider fibre cells, which are sometimes forked in the nodal region. Nodes show higher specific gravity and lower volume shrinkage than internodes (Kabir *et al.* 1996).

Janssen (1991) compiled his knowledge on mechanical properties showing increased strength with age, with decreasing culm diameter, with increasing height in the culm, with lower moisture content (below the fibre saturation of approximately 30 %), with higher ratio outer to inner layer, with higher ratio of vascular bundles to parenchyma and with increased fibre length. The ratio between the ultimate compression strength and the mass per volume is slightly higher for dry bamboo (0.094) as compared with 0.084 for dry wood. According to Janssen (1990) this might be due to the higher cellulosic content of 55 percent of bamboo compared with 50 percent in wood.

Fibres are characterized by their slender form. Their length influences the strength of the culm and its pulping properties. Fibres of bamboo are longer than those of hardwood (approximately 1 mm), but shorter than softwood tracheids (3 - 4 mm). Often, shorter and smaller fibres occur at the peripheral layer, their length increasing to a maximum in the outer third of the culm wall and decreasing again towards the inner wall. The length differences across the culm wall amount to about 20 - 50 %. Longitudinal fibre length variations of more than 100 % exist within one internode (Liese & Grosser 1972). The shortest fibres are always located near the nodes and the longest are to be found in the middle of the internode (Liese & Grosser 1972; Wu & Hsieh 1994). The mean fibre length of an internode is correlated with the internode length. Both longer fibres and the longest internodes are found in the middle region of a culm. The fibres are generally thicker in the bottom portion than at the top. The fibre length-to-width ratio varies across the culm wall from 70:1 to 150:1. The fibre length is strongly correlated with the fibre diameter (10 - 40 µm), the cell wall thickness and the internode diameter. The Runkel ratio or the ratio between 2x cell wall thickness (4 - 10 µm) and the lumen diameter (2 - 20 µm) ranges from 1 to 4. These values are influenced by fibre maturation, which leads to an increase in wall thickness. The smaller fibre wall thickness of immature culms gives a lower Runkel ratio that makes them unsuitable for pulping. Abd. Latif & Liese (2001) could correlate vascular bundle distribution and size, metaxylem diameter, percentages of metaxylem vessel and fibre sheaths, fibre length and lumen diameter, fibre wall thickness and Runkel ratio with age and culm height.

The fractural behaviour of a culm is different from that of wood; no spontaneous fracture occurs through the whole culm, the cracks are deflected in the direction of the fibres. Parameswaran & Liese (1976) showed that the separation tended to occur in the weakest region of the cell wall, which corresponded to the narrow layers. The wide layers, with which the narrow layers alternate, remained largely intact. It seems likely that a combination of gently sloped/transverse microfibril orientation may be responsible for an observed weakness in this area of the wall. In bamboo there is an undeniable correlation between the position of fibre cells with highly multi-layered walls, and areas of the culm wall which require maximum reinforcement (peripheral culm zone) (Parameswaran & Liese 1980). Several workers have theorised that the multi-layered wall structure of mature fibre cells in bamboo may be partly responsible for a high tensile strength, especially in outer culm wall regions were wall layering is highest (Liese 1980; Xingjuan & Dingguo 1990). Murphy & Alvin (1992) suggest that in that case of longitudinal compressive stresses resulting from bending, changes in microfibril angle at each layer in walls of fibre cells, may inhibit the formation of kinks across the wall. They suggest the distribution of fibre cells with more highly multi-layered walls at the periphery of vascular bundles as being mechanical strategic.

1.5. Uses of bamboo

In many countries bamboo is a basic raw material with numerous traditional uses. In many parts of the tropical world the rural people are dependent on bamboo for their shelter and for every-day utilities. They are used for construction to irrigation systems, from musical instruments to food and fuel. During the last decade, increased knowledge and research about bamboo has had a tremendous economic impact and has given rise to many new industries and products. The use of bamboo has expanded including its transformation into various structural composite panels. There is also general interest and expertise in using it for producing pulp and paper. Bamboo silviculture is an option for conservation and protection of the tropical forests while creating enduring supplies for the wood and cellulose industries.

African bamboos have not yet been exploited in pulp and paper production, or in any large-scale bamboo industry. The economic value of trade in bamboo products in Africa is negligible. Although there is little cultivation of bamboo in Africa, many products are domestically and can be very significant in both household and local economies. Key bamboo uses include small-scale construction, handicrafts, water pipes, farm props for banana plantations, furniture, and other minor cottage industry products like basketry and toothpicks. In some parts of Africa, bamboo is also a source of food and drink. In Tanzania and Uganda, young shoots and seeds of *Oxytenenthera abyssinica* are consumed as food. The principal use of this species in Tanzania, however, is in the production of bamboo wine, also known as *ulanzi*. The role of bamboo in conserving soil and protecting watersheds is also substantial in Africa (Brystriakova *et al*. 2003a).

Bamboos are extremely important to local communities in Madagascar. Nearly half of all households use bamboo domestically: for construction (walls, roofs) and for items ranging from handicrafts to musical instruments. Bamboo is also used for flooring and in irrigation systems. Water containers, fishing traps and poles, baskets and storage containers are all made from a number of bamboo species. Much of the bamboo used domestically comes from secondary forests, and there are some plantations in and around villages (Brystriakova *et al*. 2003a).

The exploitation of native bamboo in Latin America is limited to the use of local species found nearby. The most useful species in Latin America are found in the genus *Guadua*. Bamboo (mostly *Guadua angustifolia*) has a long history of use in construction in the Americas. Bamboos are used as a food source both for humans and for livestock (Brystriakova *et al*. 2003a).

Bamboos play an important role in local economies and are growing in national and international commercial importance in the Asia-Pacific region. They are multipurpose crops, with more than 1500 documented uses. The most important traditional uses include housing, food and material for handicrafts. Modern manufacturing techniques allow the use of bamboo in timber-based industries, to provide bamboo flooring, board products, laminates and furniture. Bamboo is becoming a substitute for wood in pulp and paper production. Bamboo shoots are now an important food crop on the international market as well as locally and nationally. China is by far the leading exporter of bamboo shoot products. Bamboo furniture is an expanding business in many countries e.g. the Philippines (Brystriakova *et al*. 2003b).

2. Aims and outline of this thesis

In the “Bamboo for Europe” project (FAIR-CT96-1747, 1997-2000) raw bamboo material was supplied to industries to use it in their existing processing system. Several trials conducted to produce bamboo particleboard and medium density fibreboard (MDF) show that bamboo can be used as additional or alternative raw material for the wood processing industry and especially for the panel industry in Europe (Van Acker *et al*. 2000). As in wood, anatomical structures determine the quality of the product. The physical and chemical properties of bamboo culms are correlated with the anatomical structures as is demonstrated in different studies by e.g. Janssen (1981), Liese (1987), Widjaja & Risyad (1987), Abd. Latif *et al*. (1990, 1992), Sattar *et al*. (1994), Espiloy (1994). In contrast to trees, bamboo culms grow very fast during a few months. The fibre and parenchyma cells retain their living protoplasts and connections (plasmodesmata and pits) with neighbouring cells. This cell activity associated with storage and mobilization of carbohydrates makes structural modification possible (Liese & Weiner 1997; Murphy & Alvin 1997a; Liese 1998). Anatomical changes are often considered to take place during a maturation process of 1-2 years and during the years following. The mentioned modifications are lignification (Fujii 1985; Kawase *et al*. 1986; Yoshizawa *et al*. 1991) and cell wall thickening by deposition of additional layers (Alvin & Murphy 1988; Liese & Weiner 1996; Liese & Weiner 1997; Liese 1998) of the fibre and parenchyma cells.

In contrast to these authors, 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. Similarly, Itoh (1990) and Abd. Latif *et al.* (1996) found that lignification is completed at the end of one growing season, while other authors (Fujii 1985; Kawase *et al*. 1986; Yoshizawa *et al*. 1991) found progressive lignification of the fibres. These discussed structural modifications are very important for the strength of the culm (Murphy & Alvin 1992). They have a major impact on the applicability of different bamboo species and on the suitable age for harvest in relation to end use.

The present study concentrates upon these age-related changes in bamboo culms in consideration of industrial use. Aspects of lignification and cell wall thickening for which contradicting results are reported in the literature of internodal and nodal fibre and parenchyma cells are investigated. Furthermore, silica distribution and content was studied because more knowledge on the location of the silica cells would be useful for the application (Van Acker *et al*. 2000).

The aims of this study are:

- getting further insight into the lignification process of fibre and parenchyma cells of different bamboo species. Lignification has a major impact on harvesting age, strength and shrinkage, all determinative factors for use in the wood processing industry. Special attention was attributed to the deposition sequence and distribution of lignin structural units in different anatomical regions of culms during ageing.
- clarifying the conflicting results from previous studies on cell wall thickening in bamboo culms. Because cell wall thickening increases the density, it will have an effect on strength. It focuses on both fibre and parenchyma wall thickness of both temperate and tropical bamboo culms of different ages in the light of their suitability for the wood industry.
- investigating the lignification and cell wall thickening in developing and maturing bamboo nodes because the anatomical structure and mechanical properties of the culm internode are far better documented than the culm node.
- studying the silica distribution and content in both temperate and tropical bamboo species because a selection of species with lower content silica is significant for the production of e.g. furniture (Hamdan & Abd. Latif 1992).

Each chapter of the present thesis is intended to be an autonomous part of work. Therefore, each chapter can be read separately from the rest. On the other hand, some overlap is present, especially in the introductions. Chapters are arranged according to their content: first, a general introduction (chapter 1); next a chapter devoted to the lignification (chapter 2), followed by a study on cell wall thickening (chapter 3). After that, lignification and cell wall thickening in the node is considered (chapter 4) and finally, the silica in woody bamboo culms is discussed (chapter 5). Chapter 6 lists the general conclusions and future perspectives. At the end of chapters 3 and 4 a discussion on the preceding topics in relation to the chapter is given.

Chapter 1 provides a general introduction to the thesis. In the literature review, an introduction to the morphology and anatomy of the bamboo plant is presented sometimes completed with own observations. At the end of this chapter, the materials and methods used for this work are given.

In chapter 2 lignin distribution and lignification during ageing is studied in detail by means of UV-microspectrophotometry and TEM. The first part of the chapter deals with the bamboo species *Phyllostachys viridiglaucescens*. The second part discusses the lignin distribution in the tropical species *Gigantochloa levis*.

A more complete picture on the cell wall thickness in temperate and tropical bamboo species is given in chapter 3. It deals with the large variation in cell wall thickness and in cell wall layering even within one bamboo culm. This chapter aims at clarifying the controversial results found in the literature of the cell wall thickening during ageing of bamboo culms by applying light microscopy and transmission electron microscopy in combination with image analysis.

In chapters 2 and 3, attention was paid to the anatomy and structural modification of the internode. The next chapter (chapter 4) emphasizes cell wall thickening and lignification in the nodal structure of *Phyllostachys nigra* and *P. viridiglaucescens*. The same techniques, UV-microspectrophotometry, light microscopy and image analysis were used.

Besides cell wall thickening and lignification, the silica content and distribution in the culm wall was investigated (chapter 5). The content was measured using the molybdenum blue method and the distribution was visualized using SEM-EDX mapping.

In chapter 6 the general conclusions of this work and perspectives for future research are formulated.

3. Materials and Methods

3.1. Species used in this study

The generic name of the genus *Phyllostachys* is derived from the Greek words *phyllon* meaning leaf and *stachys*, a spike, alluding the leafy pseudospikelets of the inflorescence. However, the vegetative parts are used to taxonomically distinguish the genus *Phyllostachys* from other bamboos. These include (taken from the mid-culm region) more or less prominent nodes, sulcate (or flattened) internodes on the branch bearing side and typically two strong branches per node (Ohrnberger 1996). Taxonomic boundaries of the genus are well established and have undergone few changes. It is currently one of the largest genera of bamboo, with 75 species, all of which are utilized to a larger or lesser extent as raw material for domestic, commercial and industrial purposes in the temperate zone of Asia (Renvoize & Hodkinson 1997).

The centre of diversity of the genus is eastern China and species occur wild in subtropical and temperate regions of eastern Asia. Its natural distribution has probably become obscure through years of cultivation. Many *Phyllostachys* species are adapted to very low temperatures and can usually tolerate about –12 °C without any serious damage (Ohrnberger 1996). Within the bamboos *Phyllostachys* species are the most northerly distributed giant bamboos.

The generic name of the genus *Dendrocalamus* Nees is derived from the Greek words *dendron* (tree) and *kalamos* (reed): tree like reeds. The genus includes *D. giganteus* being probably the tallest grass of the world. The genus has about 35 species occurring in the paleotropics (Indomalesian, Indian, Indo-Chinese, and Malesian) (Watson & Dallwitz 1992).

The generic name of the genus *Gigantochloa* Kurtz ex Munro is derived from the Greek words *gigas* (giant) and *chloa* (grass). The genus is has about 20 species occurring in Asia mainly in forests (Watson & Dallwitz 1992).

The following descriptions of the species used in this study are based on Zhu (1994).

3.1.1. *Phyllostachys viridiglaucescens* (Carrière) A. et C. Rivière

Height 4-9 m, diameter 2-4 cm. Young culm dark green, thickly pruinose with no apparent longitudinal veins on internodes. Culm sheath light purplish brown with visible erect setae and tiny spots, groups of spots on its upper part. Sheath auricles fully developed, long narrow falciform, brownish purple, or light green with a purplish hue, covered with dense, long and light green cilia. Sheath ligule highly convex, brownish purple. Sheath blade ribbon like, with the upper half rugose.

Figure 1-16. Illustration of a *P. viridiglaucescens* plant.

3.1.2. *Phyllostachys nigra* (Loddiges ex Lindley) Munro

Height 4-10 m, diameter 2-5 cm. Young culm green thickly pruinose and covered with setae. Black specks gradually appearing in autumn and winter turning the whole culm dark purple. Culm sheath light (red) brown, spotless, thickly covered with brown hair; sheath auricles developed, dark purple. Sheath blade green, triangular, somewhat rugose.

Figure 1-17. Illustration of some *P. nigra* culms

3.1.3. *Gigantochloa levis* (Blanco) Merril

Height 12-15 m, diameter 8-13 cm, internode 25-45 cm. Young culm densely covered with brown or white tomenta turning whitish grey when full grown. Culm sheath deciduous, thickly coriaceous, densely covered with brown setae; sheath auricles oblong, almost of equal size, with curved brown cilia on margin; sheath ligule developed, 6-15 mm high, its top deeply incised. Sheath blade ovally triangular, its base contracted, its width being one half of the sheath top; most of branches fascicled, main branch inconspicuous; foliage leaves 12-25 cm long, 1.8-3 cm wide. Shoot edible, culm used for weaving.

Figure 1-18. Illustration of a *G. levis* shoot

3.1.4. *Dendrocalamus asper* (Schultes f.) Backer ex K. Heyne

Height 20 m, diameter 6-20 cm, internode 30-50 cm. Internodes at culm base very short, with aerial roots; young culm densely covered with brownish pubescence which gradually turns white; a ring of greyish brown tomenta around joint and below it. Culm sheath deciduous, coriaceous, initially covered with brownish tomenta and brown adnate setae; sheath ligule narrow, 20 mm high, 7 mm wide, wavy, with a ciliate tip. Sheath blade ovally lanceolate; 5-9 foliage leaves on each twig; leaves 20-30 cm long, 3-5 cm wide. A major shoot bamboo species, also ornamental species.

Figure 1-19. Illustration of a *D. asper* shoot

3.2. Bamboo samples

Both temperate (*Phyllostachys* spp.) as well as tropical (*Dendrocalamus asper* and *Gigantochloa levis*) species were used in this study. To investigate the effect of ageing, it was important to sample culms of known age.

In the National Botanical Garden, Meise and in the University Botanical Garden, Ghent University shoots of *P. viridiglaucescens* and *P. nigra* respectively were marked and sampled during the first year of development and during later years. Culms of *P. viridiglaucescens* from which the exact age was not know were also sampled in the National Botanical Garden, Meise. The culms were divided into culms of 1-year old and older than 1 year (approximately 3-4 years).

Samples of culms from different ages of *Phyllostachys viridiglaucescens*, *P. nigra* and *P. viridis* were taken in the Bambuseraie in Prafrance, France. All plant samples had been marked with the year of emergence giving precise ageing.

The tropical bamboo species *Gigantochloa levis* and *Dendrocalamus asper* were sampled at the 4-year old plantation in Real Quezon in the Philippines. These culms had been marked with the year of emergence giving precise ageing.

An overview of the samples is given in appendix I.

3.3. Methods used in this study

Appendix I gives an overview of which sample is used for which aspect of this research. Tables with internode length, internode diameter and internode wall thickness of the used culms are presented. The methods used in this study are described in each chapter separately. However, some methods need more explanation.

UV – microspectrophotometry

Ultraviolet absorption is a well-known and widely used tool for lignin identification, qualitative and quantitative determination, as well as characterization of changes in lignin structure and properties. The distinct absorption of lignin in the ultraviolet range is based on its aromatic character, i.e. the sum of phenylpropane units, and of several chromophoric structural elements. The typical lignin spectrum comprises a maximum at about 280 nm followed by a slope to lower wavelengths, with a more pronounced shoulder in the region of 230 nm. A second typical extinction maximum with a high absorptivity value appears in the range between 200 and 208 nm (Fengel & Wegener 1983). No other major component of the mature wood cell displays ultraviolet absorption properties in the same spectral region and the intensity of absorption may therefore be related to the concentration of lignin across the cell wall (Scott *et al*. 1969).

In UV-microspectrophotometry, micrographs are taken with monochromatic ultraviolet light and subsequently measured with a microdensitometer. By measuring light absorption over a range of wavelengths, the UV-spectrum of a section can be obtained. UV-spectra differ on the type of lignin. Guaiacyl, syringyl and *p*-hydroxyphenyl units have different absorption maxima in their UV-spectra. According to Fergus & Goring (1970a, b) UV-absorbance maxima for guaiacyl and syringyl model compounds occur at 280 and 270 nm, respectively. In contrast to softwood lignins with a maximum at about 280 nm (strongly absorbing guaiacyl lignin), hardwood lignins show a shift of this maximum toward shorter wavelengths in the range of 270-277 nm (syringyl-guaiacyl lignin in varying ratios) (Fergus & Goring 1970a, b). This fact contributes to the higher symmetry of the phenylpropane units in hardwood lignins, caused by the higher amount of syringyl units (Musha & Goring 1975; Fengel & Wegener 1983). The UV absorbance of a specific anatomical region depends both on the concentration of the various structural units of lignin, and the extinction coefficient of each structural unit. The extinction coefficient of the G (guaiacyl) unit at 280 nm is 3.5 times that of the S (syringyl) unit (Fergus & Goring 1970a), and the extinction coefficient of the H (*p*-hydroxyphenyl) unit is lower than that of the G unit, but higher than that of the S unit (Faix & Schweers 1974). The absorbance values can amount higher than 1, because the extinction = ratio of transmission (cell lumen) and absorption (cell wall) is not calibrated to 100 percent (open scale).

Grass lignin also contains *p*-coumaric and ferulic acid esters, which gives a broad shoulder between 310-320 nm in the UV-spectrum (Higuchi 1987). He & Terashima (1991) worked out from model compounds that with the increase in the ratio *p*-coumaric acid ester to ferulic acid esters an absorption shift from 324 nm toward shorter wavelength is present. The absorption maximum reached 314 nm when the ratio *p*-coumaric acid ester to ferulic acid esters is equal or higher than 1.14.

UV-microspectrophotometry can also be used to detect aromatic compounds associated with the woody tissue. These compounds show much higher absorbance values than cell wall associated lignins. Furthermore, their absorbance maxima reveal a bathochromic shift to longer wavelength range of 310 nm. This spectral behaviour can be interpreted by the occurrence of carbonyl groups and conjugated double bonds (Koch & Kleist 2001, Koch *et al*. 2003).

SEM-EDX

Under a scanning electron microscope equipped with an x-ray detector, the sample is subjected to irradiation by a focused electron beam, which results in back-scattered electrons for surface imaging and in x-rays. The energy of the x-rays depends on the chemical composition of the sample and is received in the energy dispersive detector. This type of detector allows a user to analyse a samples molecular composition.

CHAPTER II

LIGNIFICATION IN FIBRE AND PARENCHYMA CELL WALLS OF BAMBOO INTERNODES

CHAPTER II

LIGNIFICATION IN FIBRE AND PARENCHYMA CELL WALLS OF BAMBOO INTERNODES

1. Introduction

The incorporation of the three-dimensional phenolic polymer lignin into the space between cell wall microfibrils is termed lignification. Lignin decreases the permeability and degradability of walls and is important in determining the mechanical behavior of predominantly nonliving tissues such as wood (Donaldson 2001).

The lignification of *Phyllostachys heterocycla* was studied by Itoh (1990), revealing full lignification of the cells within one growing season. Abd. Latif *et al.* (1996) also found that lignification of bamboo is completed within one growing season with no further significant ageing effects. However, Murphy & Alvin (1997a) showed that *Phyllostachys viridiglaucescens* bamboo culms were only half lignified at year 1 compared to 3-year old culms. Similarly, Lin *et al.* (2002) showed that the lignification process may last even up to 7 years. These contradicting results were the basis for further detailed investigation of the lignification and lignin distribution in bamboo internodes.

The first part of this chapter describes the lignification in internodes of the temperate bamboo species *Phyllostachys viridiglaucescens*. It follows the lignification from a very young culm to older culms with emphasis on the lignin distribution and structural variation in different anatomical regions of the bamboo tissue. In the second part, only older culms of the tropical bamboo species *Gigantochloa levis* are investigated as no very young material could be sampled. In both studies cellular UV-microspectrophotometry was used to measure the relative lignin content semiquantitatively and to obtain insight into the lignin structural units. In the second part TEM photographs are included. TEM photographs of *P. viridiglaucescens* are shown in Chapter III.

2. Lignification during ageing of the temperate bamboo species *Phyllostachys viridiglaucescens* (Carr.) Riv. & Riv.[1]

Summary

The lignification of *Phyllostachys viridiglaucescens* (Carr.) Riv. & Riv. during ageing was studied topochemically by means of UV-microspectrophotometry. The study revealed that *p*-coumaric and ferulic acids are widely distributed in *P. viridiglaucescens* and that their content is dependent on the anatomical location and the differentiation phase. The lignin in the epidermis cell wall is deposited early in the development and does not increase with age. This is in contrast with the fibres and the ground parenchyma cells where an increasing trend in lignification during the first year is shown. The early maturing fibres of the vascular bundles reveal a maximum absorbance value at 280 nm (guaiacyl peak) whereas the late maturing fibres display a shoulder at 310-320 nm in young culms and a guaiacyl peak in older culms. The secondary fibre wall has a lamellar structure with an increasing lignin content from the centre towards the compound middle lamella. The compound middle lamellae show higher absorbance values and are richer in *p*-coumaric and ferulic acid esters in comparison to the secondary wall layers. The vessel walls have a low lignin content.

[1] Adapted from:

Bieke Lybeer & Gerald Koch. 2005a. A topochemical and semiquantitative study of the lignification during ageing of bamboo culms (*Phyllostachys viridiglaucescens*). IAWA Journal 26 (1): 99-109.

2.1. Introduction

Lignin is a major component of the cell wall of fibres, parenchyma cells, and vessels in woody bamboo species. In studies on the course of lignification during growth, Itoh (1990) concluded that lignification increased progressively in both fibres and parenchyma cells and was completed in one growing season. In contrast, Murphy & Alvin (1997a) and Lin *et al*. (2002) found an increasing lignin content during maturation in later years. The distribution and the structural variation of the lignin in different anatomical regions of bamboo tissue are still open to further investigation.

Cellular UV-microspectrophotometry is a reliable technique to measure the relative lignin content semiquantitatively and to obtain insight into the lignin structural units. Koch & Kleist (2001) studied the fibre secondary walls of *Phyllostachys edulis* performing point measurements and scanning UV-microspectrophotometry and could clearly demonstrate the lamellar structure with an increasing lignin content from the lumen side towards the middle lamella. They observed a typical UV-spectrum for bamboo fibres with a guaiacyl peak at 280 nm and a shoulder between 310-315 nm, which can be linked to the presence of *p*-coumaric acid esters.

This paper describes a study of the lignification of various age classes in bamboo. It deals with the deposition sequence and distribution of lignin structural units in different anatomical regions of culms of *Phyllostachys viridiglaucescens* (Carr.) Riv. & Riv.

2.2. Materials and Methods

2.2.1. Bamboo samples

Bamboo culms of the species *Phyllostachys viridiglaucescens* from 1-, 3-, 6-, 9 -, 12- and more than 12-months (approximately 3-4 years) old were harvested in the National Botanical Garden of Belgium (Meise). Older culms (8-, 32-, 56- and 104-months old) were sampled at the Bambuseraie in Prafrance (France). Blocks of about 1-2 cm along the grain were cut from the middle part of the 6th internode (numbered from the ground level) and preserved in a mixture of 50 % alcohol, 10 % glycerine and 40 % water.

2.2.2. Preparation

Small blocks (1 x 1 x 5 mm^3) were cut from the sampled material. The specimens were dehydrated in a graded series of acetone and impregnated with Spurr's resin (Spurr 1969) through a series of propylenoxide/spurr resin mixture, followed by immersion in pure resin.

Transverse sections of 1 µm in thickness were cut with a Reichert Ultracut ultramicrotome using a diamond knife. The sections were transferred to quartz microscope slides, immersed in a drop of non-UV absorbing glycerine and covered with a quartz cover slip. The immersion solution consisted of a glycerine/water mixture n_D = 1.46. The sections were observed using the immersion ultrafluar objective 32:1.

2.2.3. UV-microspectrophotometry

Examination of the sections was carried out using a ZEISS UMSP 80 microspectrophotometer equipped with a scanning stage enabling the determination of image profiles at defined wavelengths. The specimens were investigated by point measurements with a spot size of 1 μm^2 between 240 and 400 nm wavelength using the programme LAMWIN® (Zeiss). Epidermis and hypodermis cell walls, cell wall layers in the middle of the fibre and parenchyma secondary wall $(L_x)^{\circ}$ and compound middle lamellae (CML) were measured at the outer, middle and inner part of the culm wall (mean thickness of the culm wall for the samples harvested in Belgium and France was 4,6 mm and 6,5 mm, respectively). In a vascular bundle, inner early maturing fibres adjacent to the vascular tissue and outer late maturing fibres adjacent to the parenchyma at the xylem and phloem side were measured. For each sampled culm, one measurement for each position in the culm wall and for each position within a vascular bundle was performed.

Image scan profiles at a constant wavelength of 280 nm were generated using the scan programme APAMOS® (Zeiss). This programme digitizes rectangular fields of the tissue with a geometrical resolution of 0.25 μm^2 and a photometrical resolution of 4096 grey scale levels, which are converted in 14 basic colours to visualise the absorbance intensities.

$^{\circ}$ In this work the term '*secondary wall*' is used when all cell wall layers (except the compound middle lamella) are indicated. When a specific layer is meant, the term L_x is used (See Introduction p. 23-24).

2.3. Results

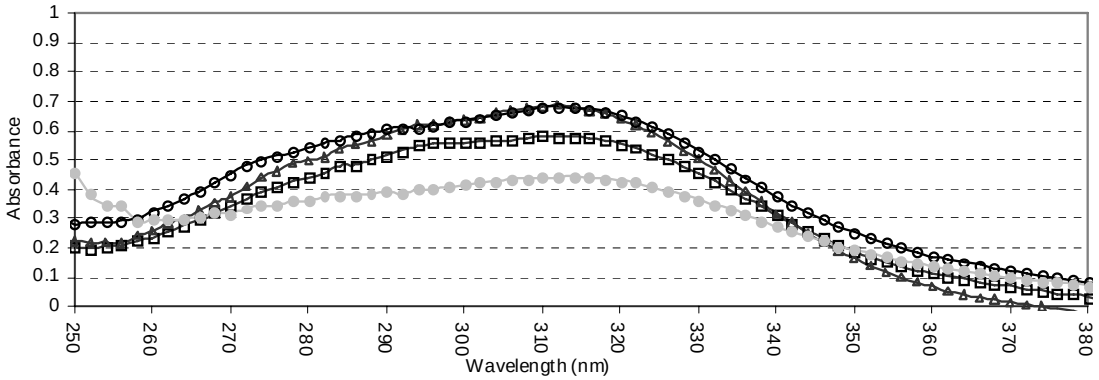
2.3.1. UV-absorbance spectra of individual cell wall layers of the tissue

The spectra of the epidermis cell walls show a clear broad shoulder with absorbance maxima between 310 and 320 nm (Fig. 2-1a). This shoulder is typical for grasses and can be linked to the presence of *p*-coumaroylation as demonstrated by Nakamura & Higuchi (1976) and Higuchi (1987). There is no difference in absorbance values between the samples. The spectra of the hypodermis cell walls show similar absorbance behaviour as compared to the epidermis cell walls.

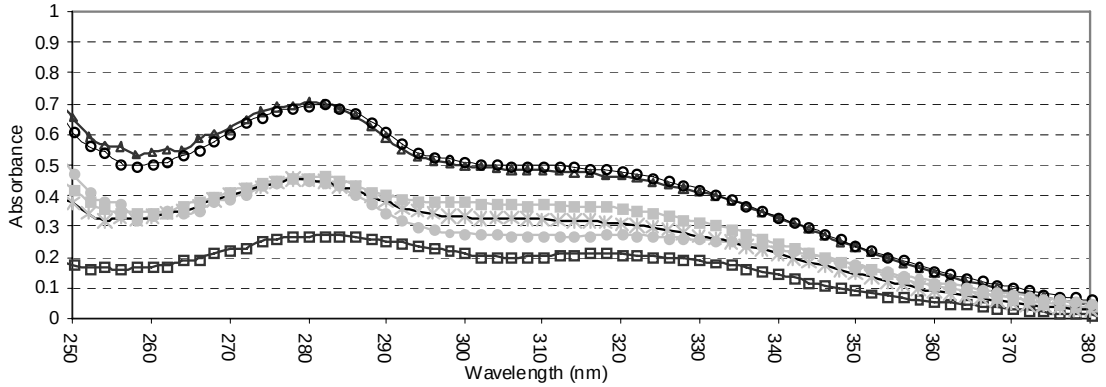
The spectra curves also have an absorbance peak at 280-282 nm, which indicates the presence of the strong absorbing guaiacyl lignin (Fergus & Goring 1970b; Musha & Goring 1975). The UV absorbance of a specific anatomical region depends both on the concentration of the various structural units of lignin, and the extinction coefficient of each structural unit. As the spectra curves are similar, they can be interpreted as reflecting the lignin content. A lower lignin content can be observed only in the cell walls of the one-month old sample. The absorbance at 280 and 312 nm is significantly higher in the compound middle lamellae (CML) than in the layers of the secondary wall (paired t-test, 1-tailed; $P_{280\ nm}=0.047$; $P_{312\ nm}=0.0015$) (Fig. 2-1b and 2-1c). The CML of the youngest sample (1-month old) exhibits lower absorbance values than the older samples. The 1-, 3-, and 6-month old samples have a more distinct shoulder at 310 - 320 nm (Fig. 2-1c, indicated by arrows) and a less clear peak at 280 nm than all older samples.

In the cell wall of the ground parenchyma cells the lignin content is distinctly lower in the youngest sample. The spectrum of the 3-month old sample has already a high absorbance peak at 310-320 nm, but does not show the typical guaiacyl peak (280 nm) evident in the spectra of older samples (Fig. 2-1d).

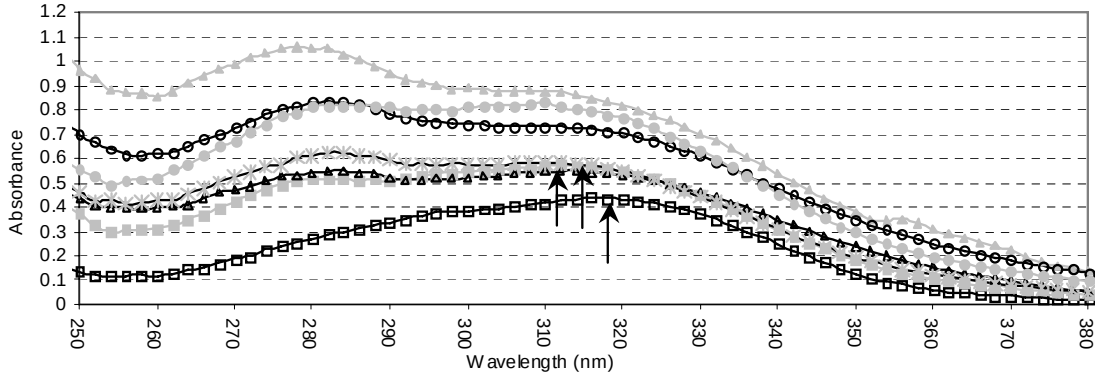
The spectra of CML of ground parenchyma cells of young samples (1- to 9-months old) have only one shoulder at 310-320 nm. The lignin content is higher in the older samples, with two peaks: one at 280-282 nm and one between 310-320 nm.


Absorbance
Wavelength (nm)

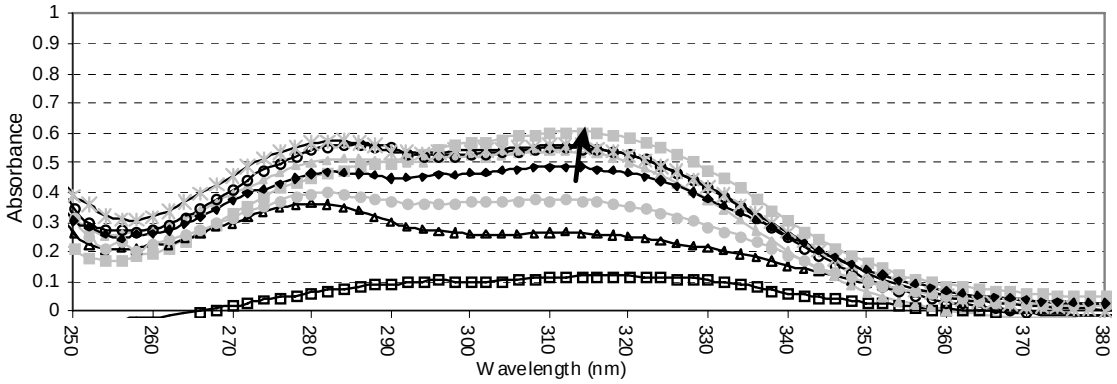
a


Absorbance
Wavelength (nm)

b


Absorbance
Wavelength (nm)

c



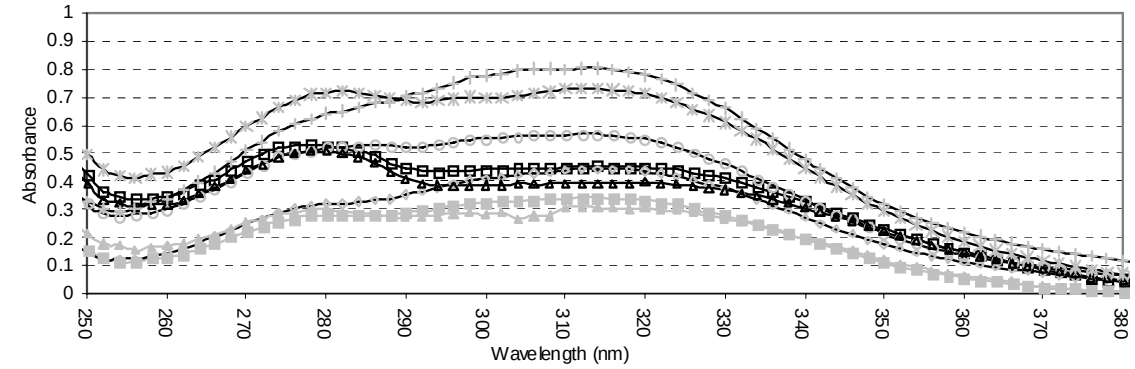
d

Figure 2-1. UV-absorbance spectra. (a) epidermis cell walls. (b) layers of the secondary wall of fibres at the outer part of the culm wall. (c) compound middle lamellae of fibres at the outer part of the culm wall. The arrows indicate the absorption shoulder between 310-320 nm in the younger culms (1-, 3- and 6-months old). (d) layers of the secondary wall of ground parenchyma cells. The arrow indicates the maximum absorbance between 310-320 nm in a 3-month old culm.

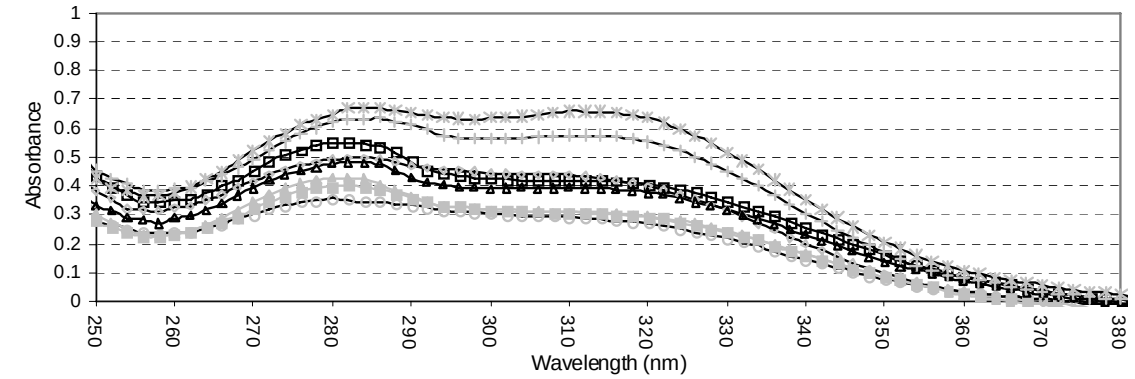
The spectra curves of layers of the secondary wall of early maturing fibres at the xylem and phloem inner side (adjacent to the vascular tissue) of vascular bundles in the middle part of the culm wall are similar (Fig. 2-2), with a maximum at 280-282 nm. Ageing of the bamboo culm does not have an influence on the lignin content of this early maturing fibres (Table 2-1).

Table 2-1. P-values of ANOVA or Kruskall-Wallis (indicated with *) tests between younger (1-, 3- and 6-months old) and older (older than 6 months) samples. Only the cell wall layers of fibres adjacent to the parenchyma (late maturing fibres) at 280 nm are significantly lower (P < 0.05) in the younger samples (bold).

	$P_{280\ nm}$	$P_{312\ nm}$
xyin	0.227	0.757
phlin	0.131	0.923
xyout	0.011	0.727*
phlout	0.032	0.433*



a



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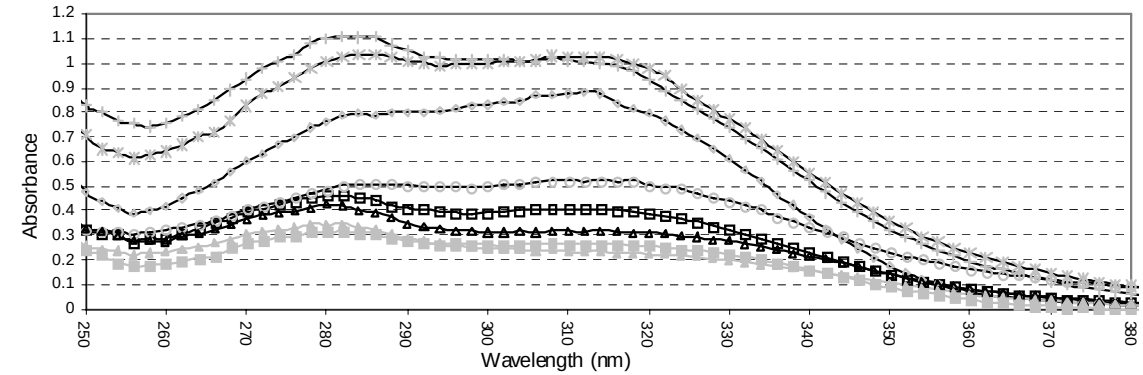


c

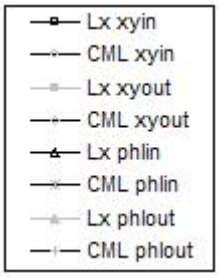


Figure 2-2. UV-absorbance spectra. (a) layers of the secondary wall (L_x) and compound middle lamella (CML) of fibres from a vascular bundle at the middle part of the culm wall of a 3-month old culm. (b) layers of the secondary wall and compound middle lamella of fibres from a vascular bundle at the middle part of the culm wall of a 12-month old culm. (c) layers of the secondary wall and compound middle lamella of fibres from a vascular bundle at the middle part of the culm wall of a 9-year old culm. xyin: fibres at the xylem inner side; xyout: fibres at the xylem outer side; phlin: fibres at the phloem inner side; phlout: fibres at the phloem outer side.

In the fibres at the xylem and phloem outer side (late maturing fibres adjacent to the parenchyma) of the young samples (1-, 3- and 6-months old) the spectra of layers of the secondary wall have an absorbance maximum at 310-320 nm. In the older samples (older than 6 months) there is a maximum peak at 280-282 nm (Fig. 2-2). Significantly less lignin is present in the xylem and phloem outer fibres of young samples in comparison to older samples (Table 2-1).

The fibres adjacent to the vascular tissue in young culms (1-, 3- and 6-months old) have a higher lignin content than those adjacent to the ground parenchyma, whereas in the older culms (older than 6 months) the lignin content of the fibres at both locations is ± equal. Hence, the layers of the secondary wall of fibres at the xylem and phloem inner side lignify faster than at the xylem and phloem outer side.

In the vascular bundles of the inner and outer part of the culm wall, the UV-spectra of layers of the secondary wall and CML of the fibres follow a similar pattern as those from the middle part of the culm wall. The UV absorbance at 280 and 312 nm of the fibre cell walls from the vascular bundles of the outer, middle and inner part of the culm wall are not significantly different (paired t-test, 2-tailed; $P > 0.1$). The UV-spectra from the CML of the fibres have a more distinct shoulder at 310-320 nm and have higher values in comparison to the spectra of the fibre layers of the secondary wall. The CML spectra from young samples (1-, 3- and 6-months old) do not show a guaiacyl peak, whereas older samples do. The spectrum of fibre layers of the secondary wall at the xylem outer side (adjacent to the ground parenchyma) has in the young samples (1-, 3- and 6-months old) an absorbance maximum at 310-320 nm. In the older samples (older than 6 months) the maximum peak is at 280-282 nm.

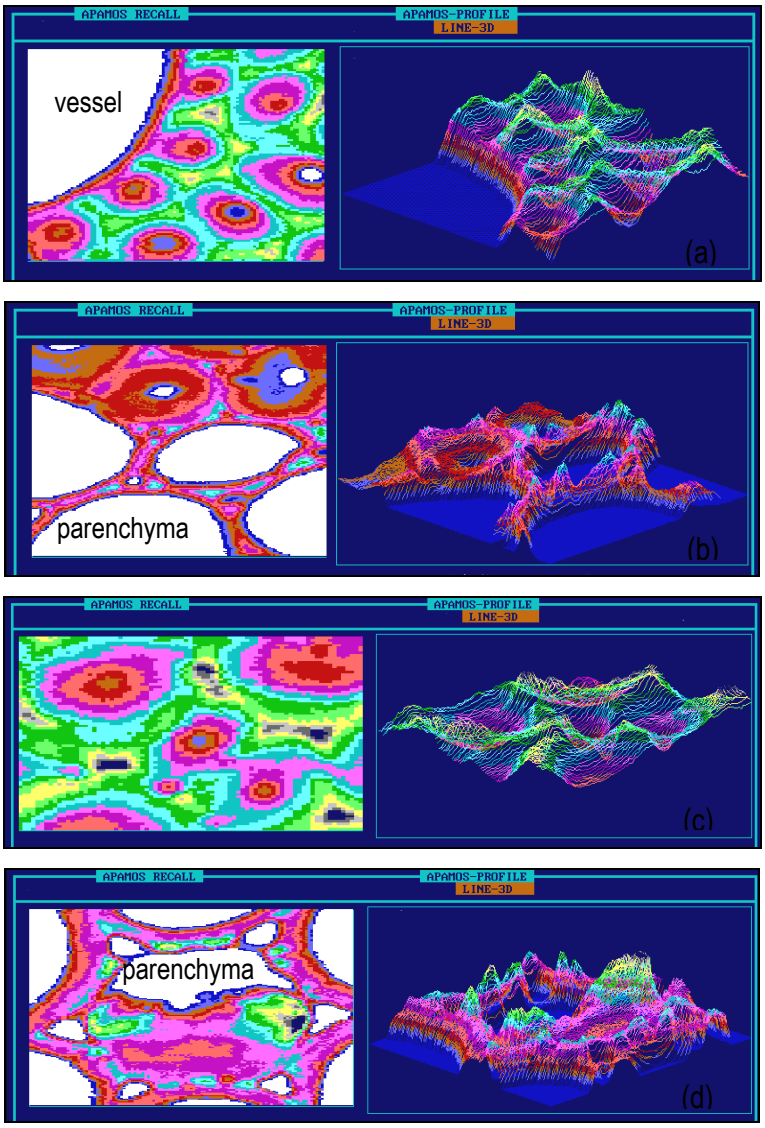
2.3.2. Scanning profiles of different cell types

The colour pixels indicate different intensities of UV absorbance at $\lambda_{280\ nm}$. The high resolution (0.25 μm^2 per pixel) enables a high differentiation of the UV absorbance within individual cell wall layers.

Scanning profiles of the epidermis and hypodermis cells show that the lignin content is low and ± equal in young and older culms. The vessel wall (metaxylem) has a low lignin content, but as demonstrated in figure 2-3a, the surrounding fibres lignify very fast. A difference between image profiles of a 3-month old culm and older culms could not be observed.

The fibres show a lamellar structure with a decreasing lignin content towards the cell lumen. The fibres close to the ground parenchyma are more heavily lignified in a 9-year old culm than in a younger one (Fig. 2-3b and 2-3c). Both inner early maturing and outer late maturing fibres show a lamellar structure.

There are two types of parenchyma cells: short and long cells. The short ones are thin walled and some are filled with phenolic compounds as was confirmed by the UV-spectrum (Fig. 2-3d). These compounds show much higher absorbance values than cell wall associated lignins. Furthermore, their absorbance maxima reveal a bathochromic shift to longer wavelength range of 310 nm (Koch & Kleist 2001). The cell corners are not lignified in contrast to the cell walls in contact with the cell wall of long parenchyma cells.



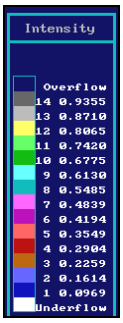


Figure 2-3. UV-micrographs (left) and 3D profiles (right) of cell walls of *P. viridiglaucescens*. (a) metaxylem vessel and the surrounding fibres from a vascular bundle from the outer part of the culm wall of a 3-month old culm. (b) fibres adjacent to parenchyma cells and parenchyma cells from the middle part of the culm wall of a 3-month old culm. (c) fibres at the outer side in a vascular bundle from the middle part of the culm wall of a 9-year old culm. (d) short, thin walled parenchyma cell of a 6-month old culm, the lumen is filled with phenolic compounds.

2.4. Discussion and Conclusion

Bamboo lignin is considered to be composed of guaiacyl, syringyl, and *p*-hydroxyphenylpropan units. As a unique feature, it also contains 5-10 % of *p*-coumaric acid ester (Nakamura & Higuchi 1976, Higuchi 1987), located at the γ-positions of grass lignin, predominantly on syringyl units (Lu & Ralph 1998). The spectra curves of the epidermis, hypodermis, fibre and parenchyma cell walls show a shoulder between 310-320 nm, which can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987). In the spectra, a peak at 280-282 nm is present. He & Terashima (1991) found a similar guaiacyl peak in the spectra from rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.). Fengel & Shao (1985) concluded on the basis of a chemical analysis and the bands in IR-spectra that bamboo is rich in syringyl units. However, they also observed a guaiacyl peak at 280 nm in the UV-spectrum. Lin *et al.* (2002) detected with VIS-microspectrophotometry, after staining with Wiesner and Mäule reagent, that the absorbance curves for bamboo are similar to those of dicotyledons, with a syringyl peak in the spectra.

Ferulic and *p*-coumaric acids are widely distributed in *P. viridiglaucescens* and their content is dependent on the anatomical location and the differentiation phase. The epidermis cells and compound middle lamellae of fibres and parenchyma cells have high absorbance values (maxima) for the wavelengths related with these esters. Younger cell walls have higher ratios of $abs_{310\ nm}$ / $abs_{280\ nm}$ than cell walls in older culms°. The decrease in the $abs_{310\ nm}$ / $abs_{280\ nm}$ can be explained by an increased deposition of lignin structural units with high absorbance at 280 nm, mainly guaiacyl units. A shift from 280 nm toward longer wavelengths and from 310-320 nm toward shorter wavelengths with the progress of lignification (i.e. at a later differentiation stages) in the cell corners of fibres as found in rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.) (He & Terashima 1991) was not observed in *P. viridiglaucescens*.

The lignin content in the epidermis cells is approximately the same in a one-month old sample and in older samples, which indicates that the lignin in the epidermis is deposited early during cell wall development and does not increase with age (Fig. 2-1a). The fibre layers of the secondary wall (L_x) in the outer part of the culm wall in young culms show lower ratios of $abs_{310\ nm}$ / $abs_{280\ nm}$ (more G units are already deposited) than the values of ground parenchyma cells in culms of the same age (Fig. 2-1b and 2-1d), demonstrating that the fibres in the outer parts of the culm wall mature and fully lignify more rapidly than the parenchyma cells. This is in agreement with Itoh (1990) who stated that the lignification starts at the outside of the culm and proceeds inwardly, and that lignification of fibres and epidermal cells precedes that of ground parenchyma cells.

° In Appendix 2 all UV-spectra are given. It is not possible to give values for the ratios because this has to be done for each cell type at a different position and at each culm age seperately.

Lignification

The lignin content of layers of the secondary wall of the fibres adjacent to the vascular tissue (early maturing fibres) is ± equal in all samples, except in the 1-month old sample (results not shown). The UV-spectrum of a 1-month old sample does not have a clear peak in contrast to spectra of samples older than 1 month showing a maximum at 280-282 nm. On the other hand, cell walls of 1-, 3- and 6-month old fibres, adjacent to the ground parenchyma (late maturing fibres), have a maximum at the typical grass lignin peak (310-320 nm). The fibre wall of culms older than 6 months has a maximum peak at 280 nm in the inner early maturing fibres adjacent to the vascular tissue as well as in the outer late maturing fibres adjacent to the ground parenchyma. The presence of early maturing fibres and the progress of lignification outwards from these fibres into more peripheral fibres has already been reported (Murphy & Alvin 1997a; Liese & Weiner 1996, 1997).

Lignin is already present in the cell corners and compound middle lamellae of a 1-month old sample in contrast to the cell walls where almost no lignin is present (results not shown). This indicates that lignification first takes place here and then proceeds into the secondary wall of the fibres.

The secondary fibre walls form a lamellar structure with an increasing lignin content from the lumen side towards the middle lamella as is shown by the scans. Koch & Kleist (2001) observed the same in a three-year old culm of *P. edulis*. The lamellation is generally described as alternating broad and narrow layers with different fibrillar orientation with alternating low and high concentration of lignin (Parameswaran & Liese 1976; Murphy & Alvin 1992). The narrow layers with widths between 0.1 and 0.2 µm are below the geometric resolution of the scanning stage (0.25 μm^2) and could therefore not be displayed as single layers.

Lignin content of the fibre and parenchyma cell walls increases in the first year but not during later years. A difference in lignin content between a 1-year old and a 9-year old culm could not be demonstrated (Fig. 2-d; Fig. 2-2 b, c) whereas an increase in G units in samples up to 6-months old is proved. This is in contrast with the conclusions of several authors (Murphy & Alvin 1997a; Lin *et al.* 2002) but agrees with the findings of Itoh (1990) who stated that lignification is completed within one growing season. However, it is important to consider that the spectra represent only one of several layers of a cell wall. It could be possible that lignification is completed within one cell wall layer while the fibre retains the ability to form new layers and lignify them additionally.

Some short, thin walled parenchyma cells are filled with phenolic compounds. The cell corners are only slightly lignified in contrast to the cell wall adjacent to a cell wall of a long parenchyma cell. He *et al.* (2002) found the same lignification pattern with no lignin deposition in the cell corner of short parenchyma cells and lignin deposition in short parenchyma cell walls in contact with long parenchyma cells in a study of the two types of parenchyma cells in bamboo.

The vessel cell wall has a low lignin content. This is in contrast with most dicotyledons (Musha & Goring 1975; Saka & Goring 1988). Donaldson *et al.* (2001) found a higher lignin concentration in the vessel walls of the monocotyledon *Triticum aestivum* L. (wheat straw). An increased lignin content of the cell wall could be expected, as vessel cell walls must withstand the large compressive forces resulting from the transpiration stream in plants. In bamboos highly lignified walls are present in the fibres adjacent to the vessels. Hence, they reduce the susceptibility of the vascular tissue to collapse.

3. Lignin distribution in the tropical bamboo species *Gigantochloa levis* (Blanco) Merrill[2]

Summary

The lignin distribution within the cell walls of *Gigantochloa levis* (Blanco) Merrill was studied topochemically by means of TEM and cellular UV-microspectrophotometry. The study deals with the distribution of lignin structural units in different anatomical regions and provides a comparison of lignification of the tropical bamboo species *Gigantochloa levis* (Blanco) Merrill with the temperate bamboo species *Phyllostachys viridiglaucescens* (Carr.) Riv. & Riv. Considerable differences were found in cell wall structure between fibres adjacent to the vascular tissue, fibres of free fibre strands and parenchyma cells. The secondary fibre wall in general has a lamellar structure with an increasing lignin content from the centre towards the compound middle lamella. *P*-coumaric and ferulic acids are more widely distributed in *G. levis* and their content depends on the anatomical location. The early maturing fibres adjacent to the vascular tissue and at the outer culm wall reveal a maximum absorbance at 280 nm (guaiacyl peak) whereas the late maturing fibres display a shoulder at 310-320 nm. This is in contrast to *P. viridiglaucescens* where the late maturing fibres also show a maximum peak at 280 nm. The compound middle lamellae show higher absorbance values and are richer in *p* - coumaric and ferulic acid esters in comparison to the layers of the secondary wall. The vessel walls have a lower lignin content. A difference in lignin content between the various ages and between flowering and non-flowering culms could not be observed.

[2] Adapted from:

Bieke Lybeer & Gerald Koch. 2005b. Lignin distribution in the tropical bamboo species *Gigantochloa levis* (Blanco) Merrill. IAWA Journal 26 (4): 443-456.

3.1. Introduction

Culms of many bamboo species are applied in a wide range of economic uses (e.g. constructions, furniture, parquet) and therefore the mechanical properties and durability of bamboo are of great importance. Lignin is a major component of the cell wall of fibres, parenchyma cells, and vessels in woody bamboo tissue and is responsible for many mechanical properties. In their studies on the course of lignification during growth, Murphy & Alvin (1997a) and Lin *et al*. (2002) found an increasing lignin content during maturation in later years. In contrast, Itoh (1990) concluded that lignification increased progressively in both fibres and parenchyma cells and was completed in one growing season. Lybeer & Koch (2005a), studied the fibre and parenchyma secondary walls of *Phyllostachys viridiglaucescens* performing cellular UV point measurements and scanning UV-microspectrophotometry, and demonstrated that the lignin content of fibre and parenchyma cell walls increases in the first year but not during later years. They could clearly demonstrate the lamellar structure of the fibres with an increasing lignin content from the lumen towards the middle lamella. This layering is generally described as alternating broad and narrow layers with different fibrillar orientation and with alternating low and high concentration of lignin (Parameswaran & Liese 1976; Murphy & Alvin 1992). Lybeer & Koch (2005a) emphasize the importance to consider that UV-spectra (point measurements of 1 µm²) represent only one of several layers of a cell wall. It is possible that lignification is completed within one cell wall layer while the fibre retains the ability to form new layers and lignify them additionally.

Studies on lignification in bamboos have been carried out on species of the genus *Phyllostachys* characteristically lacking free fibre strands (Itoh 1990; Yoshizawa *et al*. 1991; Murphy & Alvin 1997a; Lin *et al*. 2002; Lybeer & Koch 2005a). This study examined aspects of lignification in *Gigantochloa levis* (Blanco) Merrill, representing tropical bamboos characterized by possessing free fibre strands associated with the vascular bundle but entirely surrounded by parenchyma. The vascular bundles are characteristically of either type III or IV. In type III, the bundle, with its four small fibre caps (one adjacent to the protoxylem, one to the phloem and one to each of the two large metaxylem vessels) is associated with a usually large, centripetally placed free fibre strand at the protoxylem side (Grosser & Liese 1971; Liese & Grosser 2000). Type IV has a central vascular strand with four smaller sclerenchyma sheaths and in addition two isolated fibre strands, located at the phloem and protoxylem side (Grosser & Liese 1971; Liese & Grosser 2000). *G. levis* has notably more type III than type IV vascular bundles.

Using cellular UV-microspectrophotometry, Koch & Kleist (2001) and Lybeer & Koch (2005a) observed typical UV-spectra for bamboo fibres and parenchyma cells with a guaiacyl peak at 280 nm and a shoulder between 310-315 nm, which can be linked to the presence of *p*-coumaric acid esters. Lybeer & Koch (2005a) revealed that *p*-coumaric and ferulic acids are widely distributed and that their content depends on the anatomical location and the differentiation phase.

Transmission electron microscopy using potassium permanganate staining is as well applied for localization of lignin in woody cell walls (e.g. Donaldson 1992; Singh & Daniel 2001; Schmitt & Melcher 2004).

The present paper describes a study of the lignin distribution of *G. levis*. It deals with the distribution of lignin structural units in different anatomical regions and makes a comparison of lignin distribution of the tropical bamboo species *G. levis* with the temperate bamboo species *P. viridiglaucescens*.

3.2. Materials and Methods

3.2.1. Bamboo samples

Bamboo culms of the tropical species *Gigantochloa levis* (Blanco) Merrill were sampled at the 4-year old plantation in Real Quezon, the Philippines. All plant samples had been marked with the year of emergence giving precise ageing (Table 2-2). Both flowering and non-flowering culms have been harvested. Blocks of about 1-2 cm along the grain were cut from the middle part of the 6th internode (numbered from the ground level) and preserved in a mixture of 50 % alcohol, 10 % glycerine and 40 % water.

3.2.2. Preparation

Transmission electron microscopy

Sections of 50 µm of some samples (Table 2-2) were cut using a Microm-HM440E sliding microtome. From the middle culm wall, a small part containing a vascular bundle and parenchyma cells was dissected using a sharp scalpel. These specimens were dehydrated in a graded series of acetone and subsequently washed out in a graded series of alcohol and impregnated with LR white™ (Polysciences, Eppelheim, Germany) through a series of alcohol/LR white™ mixture, followed by immersion in pure resin. 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 with potassium permanganate according to Donaldson (1992) with a 1 % solution of potassium permanganate dissolved in double distilled water containing 0.1 % sodium citrate. Duration of on-grid-staining was three minutes, followed by washing twice in double distilled water. The sections were observed with a Jeol-1010 transmission electron microscope operating at 60 kV.

Table 2-2. Sampled culms of *G. levis*.

Age	*Flowering*	*UMSP*	*TEM*
8-months old	no	x	x
8-months old	yes	x	
21-months old	no	x	x
22-months old	yes	x	
40-months old	no	x	
40-months old	yes	x	x

UV-microspectrophotometry

Small blocks (1 x 1 x 5 mm^3) were cut from the sampled material. The specimens were dehydrated in a graded series of acetone and impregnated with Spurr's resin (Spurr 1969) through a series of propylenoxide/spurr resin mixture, followed by immersion in pure resin.

Transverse sections of 1 µm in thickness were cut with a Reichert Ultracut ultramicrotome using a diamond knife. The sections were transferred to quartz microscope slides, immersed in a drop of non-UV absorbing glycerine and covered with a quartz cover slip. The immersion solution consisted of a glycerine/water mixture n_D = 1.46. The sections were observed using the immersion ultrafluar objectives 32:1 and 100:1.

Examination of the sections was carried out using a ZEISS UMSP 80 microspectrophotometer equipped with a scanning stage enabling the determination of image profiles at defined wavelengths. The specimens were investigated by point measurements with a spot size of 1 μm^2 between 240 and 400 nm wavelength using the programme LAMWIN® (Zeiss). Image scan profiles at a constant wavelength of 280 nm were generated using the scan programme APAMOS® (Zeiss). This programme digitizes rectangular fields of the tissue with a geometrical resolution of 0.25 μm^2 and a photometrical resolution of 4096 grey scale levels, which are converted in 14 basic colours to visualize the absorbance intensities.

Epidermis, hypodermis, cell wall layers in the middle of the fibre and parenchyma secondary wall (L_x)° and compound middle lamellae (CML) were measured at the outer, middle and inner part of the culm wall. In a vascular bundle, inner early maturing fibres adjacent to the vascular tissue and late maturing fibres of free fibre strands at the xylem and phloem side were measured. For each sampled culm, one measurement for each position in the culm wall and for each position within a vascular bundle was performed.

° In this work the term '*secondary wall*' is used when all cell wall layers (except the compound middle lamella) are indicated. When a specific layer is meant, the term L_x is used (See Introduction p. 23-24).

3.3. Results

3.3.1. Transmission electron microscopy

A typical type IV vascular bundle of *G. levis* with four smaller fibre sheaths adjacent to the vascular tissue and two free fibre strands, one located at the phloem and one at the protoxylem side is shown in figure 2-4.

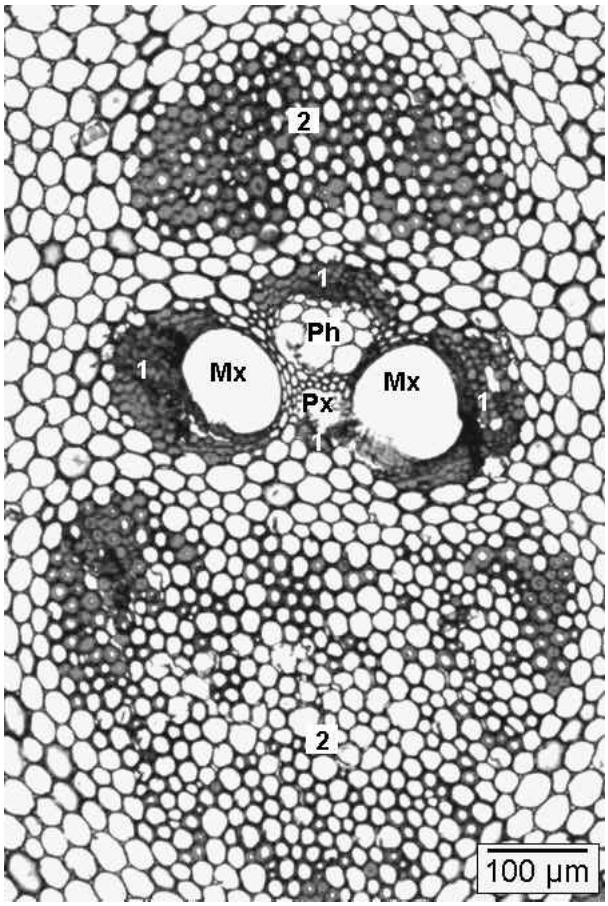


Figure 2-4. Typical type IV vascular bundle of *G. levis* with four smaller fibre sheaths adjacent to the vascular tissue (1) and two free fibre strands (2), located at the phloem and protoxylem side. Mx: metaxylem, Px: protoxylem, Ph: phloem.

The folds in the sections (Fig. 2-5) are 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 demonstrated by the absence of folds in the parenchyma cells. Fibres of vascular fibre caps adjacent to the vascular tissue are smaller in diameter and thicker in cell wall than those of the free fibre strands (Table 2-3). This is reflected in the 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.

Table 2-3. Comparison of cell wall thickness and cell diameter of fibres adjacent to the vascular tissue and of fibres of free fibre strands of *G. levis*.

Age (in months)	*Cell wall thickness vascular fibre cap (µm)*	*Cell diameter vascular fibre cap (µm)*	*Cell wall thickness free fibre strand (µm)*	*Cell diameter free fibre strand (µm)*
8	4.40	12.21	1.86	17.44
21	4.45	11.01	2.96	15.11
40	3.60	8.34	1.82	17.55

The differences in cell wall structure between fibres adjacent to the vascular tissue, fibres of free fibre strands and parenchyma cells are clear (Fig. 2-5). Fibres adjacent to the vascular tissue have a lamellar secondary wall with alternating small and broad layers. The small layers are darker coloured, which indicates higher lignin content. The cell lumen is small and the number of layers as well as the thickness of the layers is variable between different fibres. The fibres of free fibre strands have thinner cell walls and larger cell lumens than the fibres adjacent to the vascular tissue. The wall consists of alternating small and broad layers. Mostly, the broad layers are smaller compared to the broad layers of fibres adjacent to the vascular tissue. The number of layers is highly variable. Often, close to the middle lamella, several smaller layers are present. The number of layers of fibres of free fibre strands is lower than in the fibres adjacent to the vascular tissue. In comparison, the parenchyma cell wall is thin with several small cell wall layers. The compound middle lamellae and cell corners are darker coloured, which indicates the higher lignin content. Within a cell wall, small layers have more lignin than broad layers.

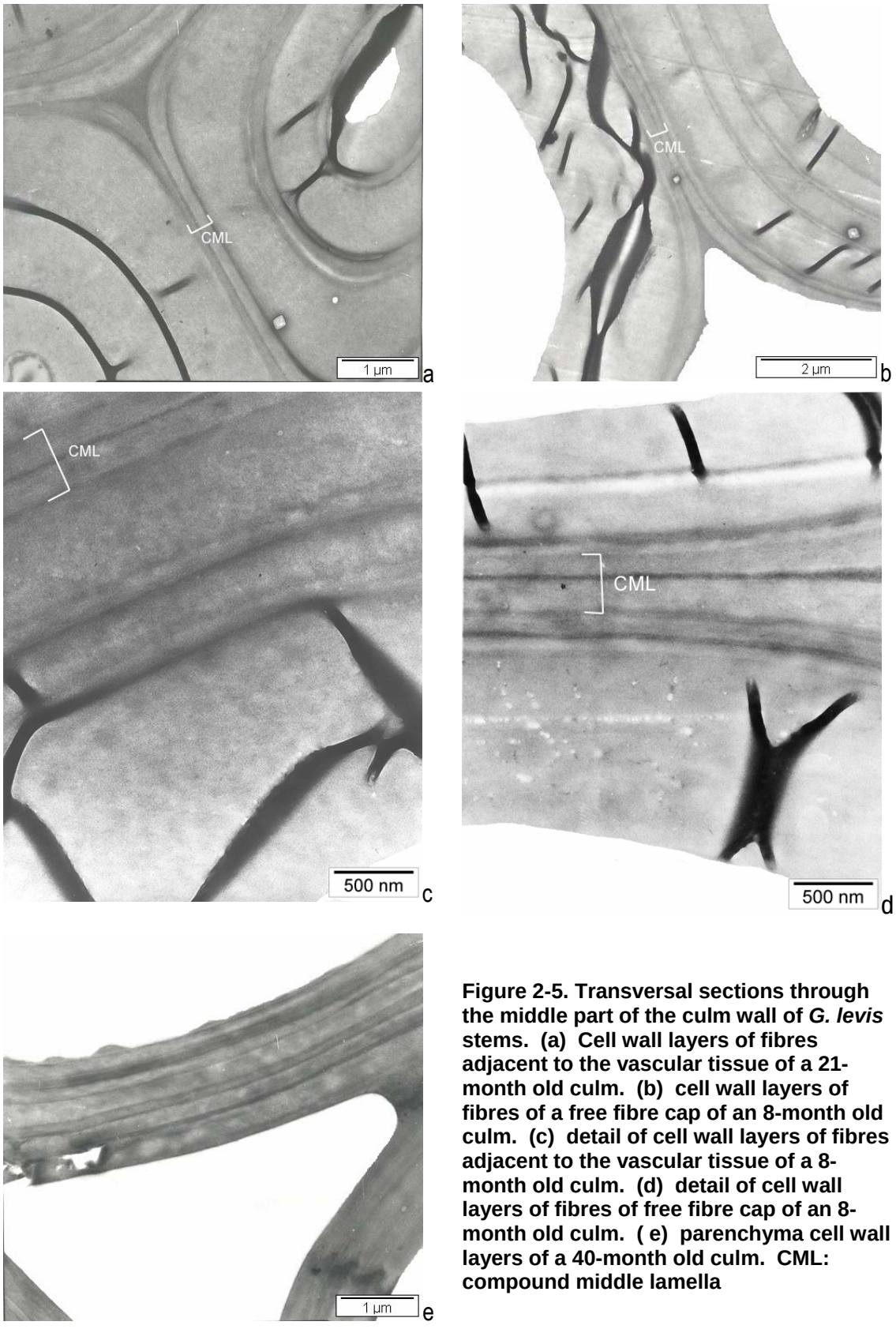


Figure 2-5. Transversal sections through the middle part of the culm wall of *G. levis* stems. (a) Cell wall layers of fibres adjacent to the vascular tissue of a 21-month old culm. (b) cell wall layers of fibres of a free fibre cap of an 8-month old culm. (c) detail of cell wall layers of fibres adjacent to the vascular tissue of a 8-month old culm. (d) detail of cell wall layers of fibres of free fibre cap of an 8-month old culm. (e) parenchyma cell wall layers of a 40-month old culm. CML: compound middle lamella

3.3.2. UV-absorbance spectra of individual cell wall layers

The cell wall of the epidermis could only be measured in an 8-month old sample. The spectra reveal an unclear peak at 280-282 nm and a broad shoulder between 310-320 nm. The spectra of a hypodermis cell have a clear peak at 280-282 nm and a less clear shoulder between 310-320 nm in comparison to the spectra of an epidermis cell.

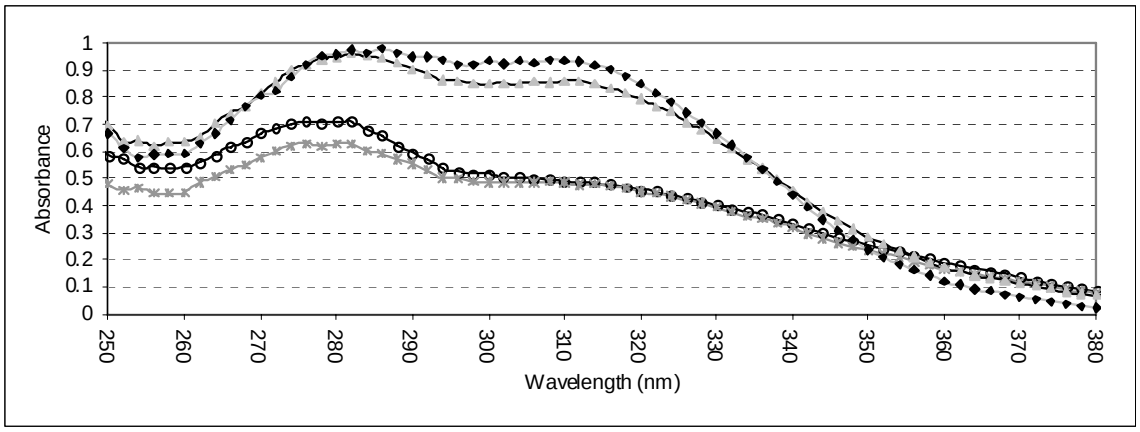
The layers of the secondary wall (L_x) of fibres at the outer part of the culm wall adjacent to the vascular tissue and of free fibre strands have similar UV-spectra with a clear peak at 280-282 nm and a slight shoulder at 310-320 nm (paired t-test, 2-tailed; $P_{280\ nm}$=0.297; $P_{312\ nm}$=0.814) (Fig. 2-6a). There is no difference in absorbance behaviour and absorbance values between the different ages and between flowering and non-flowering culms. The compound middle lamellae (CML) of the fibres adjacent to the vascular tissue have mostly a similar type of spectra as the layers of the secondary wall (L_x), but sometimes the shoulder at 310-320 nm is more pronounced. The CML of fibres of free fibre strands have spectra with almost no or no peak at 280-282 nm and a clear shoulder at 310-320 nm. Both have higher absorbance values than the absorbance values of the layers of the secondary wall (L_x).

The parenchyma cells at the outer part of the culm wall show spectra with an unclear peak at 280-282 nm and a broad shoulder at 310-320 nm. The spectra of the CML are similar but have higher absorbance values. There is no difference between the different ages.

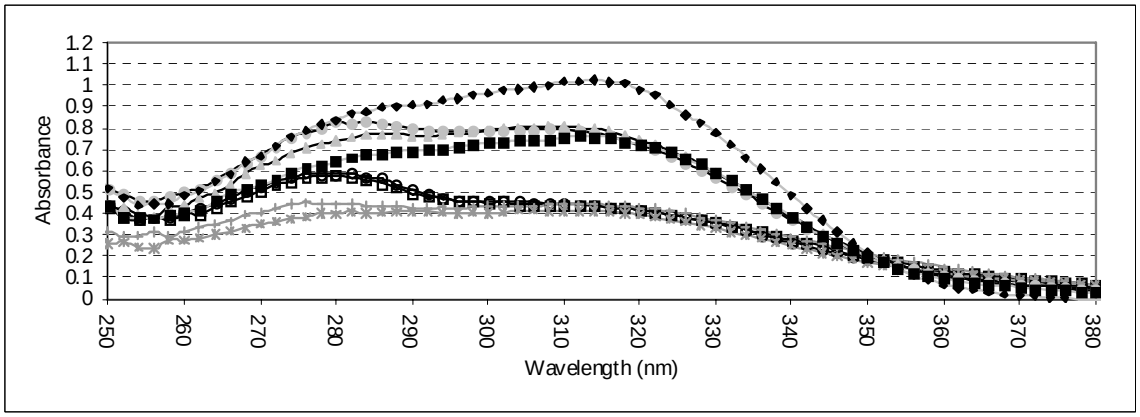
Layers of the secondary wall of fibres at the middle part of the culm wall adjacent to the vascular tissue are characterized by spectra with a clear peak at 280-282 nm and a shoulder between 310-320 nm. Spectra of layers of fibres of the free fibre strands exhibit a less clear peak at 280-282 nm and a broader shoulder at 310-320 nm (Fig. 2-6b). The ratio of $abs_{310\ nm}$ / $abs_{280\ nm}$ [°] is higher in fibres of the free fibre strands than in the fibres adjacent to the vascular tissue. The spectra are equal in samples of different ages and in flowering and non-flowering plants. The CML of the fibres of the free fibre strands have a higher ratio $abs_{310\ nm}$ / $abs_{280\ nm}$ than the CML of the fibres adjacent to the vascular tissue. So, the spectra of the CML of fibres adjacent to the vascular tissue show a clearer peak at 280-282 nm in comparison to the fibres of the free fibre strands (Fig. 2-6b). The absorbance values of the spectra of the CML are higher than of spectra of the layers of the secondary wall (L_x).

The UV-spectra of parenchyma cell wall layers at the middle part of the culm wall have an unclear peak at 280-282 nm and a clear shoulder at 310-320 nm. There is no difference between the spectra of different ages. The spectra of the CML of the parenchyma cells at the middle part of the culm wall show almost no peak at 280-282 nm and a clear shoulder at 310-320 nm. The absorbance values are higher in the CML than in the layers of the secondary wall.

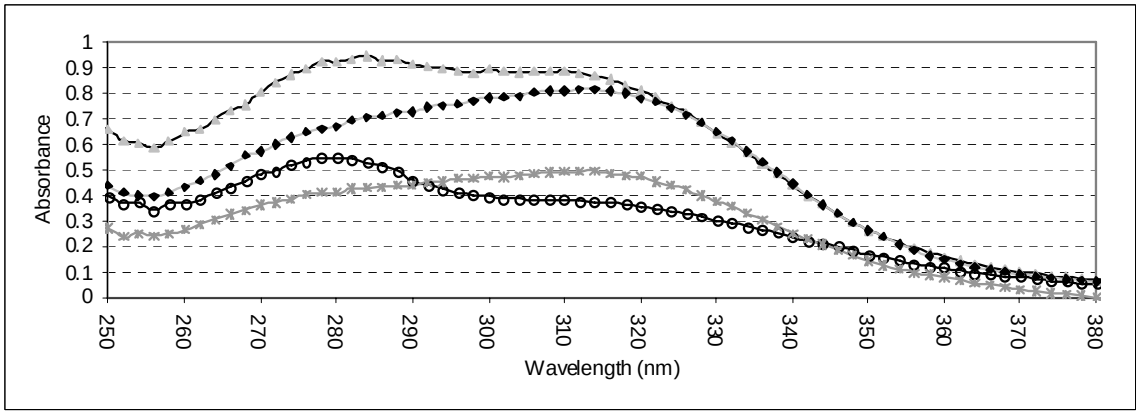
[°] In Appendix 2 all UV-spectra are given. It is not possible to give values for the ratios because this has to be done for each cell type at a different position and at each culm age seperately.


Absorbance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250 260 270 280 290 300 310 320 330 340 350 360 370 380
Wavelength (nm)

a


Absorbance
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250 260 270 280 290 300 310 320 330 340 350 360 370 380
Wavelength (nm)

b


Absorbance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250 260 270 280 290 300 310 320 330 340 350 360 370 380
Wavelength (nm)

c

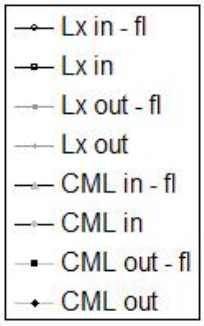


Figure 2-6. (a) UV-absorbance spectra of layers of the secondary wall (L_x) and compound middle lamellae (CML) of a 22-month old non-flowering culm at the outer part of the culm wall of *G. levis*. (b) UV-absorbance spectra of layers of the secondary wall (L_x) and compound middle lamellae (CML) of a 40-month old flowering (fl) and non-flowering culm at the middle part of the culm wall. (c) UV-absorbance spectra of layers of the secondary wall (L_x) and compound middle lamellae (CML) of an 8-month old flowering (fl) culm at the inner part of the culm wall. in: fibres at the inner side (adjacent to the vascular tissue); out: fibres of free fibre strands.

In the inner part of the culm wall the spectra of layers of fibres (Fig. 2-6c) adjacent to the vascular tissue have a clear peak at 280-282 nm and only a slight shoulder between 310-320 nm. There is no difference in lignin content between the different ages and between flowering and non-flowering plants. The spectra of the fibres in the free fibre strand have almost no peak at 280-282 nm and a clear broad shoulder at 310-320 nm. The spectra of the CML of the fibres at the inner part of the culm (Fig. 2-6c) wall close to the vascular tissue show a more clear shoulder at 310-320 nm and higher absorbance values in comparison to the L_x. The CML of the fibres in the free fibre strand have similar spectra of the L_x but the absorbance values are higher.

The spectra of the parenchyma cells at the inner part of the culm wall display no or an unclear peak at 280-282 nm and a clear shoulder at 310-320 nm with no differences between the various ages. Between the parenchyma cells near the lacuna and the parenchyma cells further at the inner part of the culm wall there is no difference in lignin content. The CML have similar spectra but higher absorbance values.

The spectra of the layers of the secondary wall at the outer, middle and inner part of the culm wall show a difference in absorbance behaviour but not in absorbance values (Fig. 2-7). The layers of fibres adjacent to the vascular tissue all have a clear peak at 280-282 nm and a shoulder at 310-320 nm. Nevertheless, the spectra of the fibre layers at the inner part of the culm wall display a more clear shoulder at 310-320 nm in comparison to the fibre layers at the middle and outer part of the culm wall. The spectra of the layers of the secondary wall of the free fibre strands at the outer culm wall show a clear peak at 280-282 nm. The spectra of the fibre layers at the middle part of the culm wall have a less clear peak at 280-282 nm and the spectra of the fibre layers at the inner part of the culm wall almost show no peak at 280-282 nm. The spectra display a broad shoulder at 310-320 nm at the middle and inner part of the culm wall, but a less clear shoulder at 310-320 nm at the outer part of the culm wall. Within one fibre, inner layers (i.e. layers close to the cell lumen) have lower absorbance values than layers near the compound middle lamellae.

The spectra of the parenchyma cells do not show a distinctive difference in absorbance behaviour. The spectra of the parenchyma cells at the outer part of the culm wall have a clearer peak at 280-282 nm than the spectra of the parenchyma cells at the middle and inner part of the culm wall, which have similar spectra.

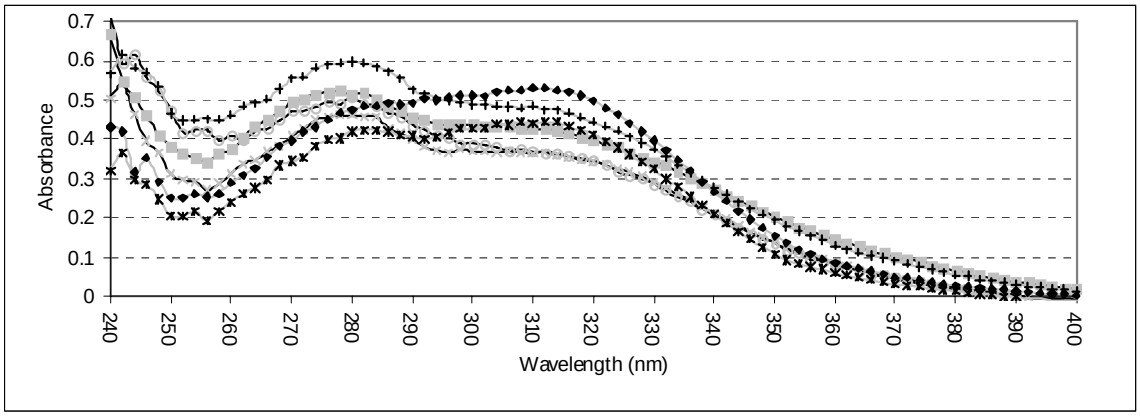


Figure 2-7. UV-absorbance spectra of layers of the secondary wall (L_x) of a 21-month old culm at the outer, middle and inner part of the culm wall of fibres adjacent to the vascular tissue (in) and of free fibre strands (out) of *G. levis*.

3.3.3. Scanning profiles of different cell types

The topochemical distribution of lignin within the individual cell wall layers was also studied by the evaluation of scanning profiles with a resolution of 0.25 µm². The cell walls of the vessels (metaxylem) are characterized by a lower lignin content in comparison to the surrounding fibres. The fibres show a lamellar structure with a decreasing lignin content towards the cell lumen. There is no difference between an 8-month old culm and a 22-month old culm in lignification at the outer part of the culm wall. The fibres of the free fibre strands have higher lignin content at the outer part of the culm wall but about the same lignin content in the middle and inner part of the culm wall. There is no difference in lignin content between the different ages.

3.4. Discussion and Conclusion

Potassium permanganate represents a powerful tool in TEM for the localization of lignin on the subcellular level. However, Schmitt & Melcher (2004) recommend carrying out parallel analyses by means of UV-microscopy for verification of the results, although the latter technique only provides information on the cellular level. The typical lamellate structure of a bamboo cell wall with alternating small and broad layers (Parameswaran & Liese 1976; Parameswaran & Liese 1980) can be clearly visualized by means of TEM photographs but not by means of UV scanning profiles. The narrow layers with widths between 0.1 and 0.2 µm are below the geometric resolution of the scanning stage (0.25 µm²) and could therefore not be displayed as single layers.

Nonetheless, the UV-micrographs provide additional information on the semiquantitative lignin content within the cell wall and demonstrate the increasing lignin content from the lumen towards the middle lamella. Using both techniques, a high variability in lignin content between the different wall layers can be shown. The difference in lignin content between the alternating broad and narrow layers with respectively low and high concentration of lignin was already described by Parameswaran & Liese (1976) and Murphy & Alvin (1992).

Bamboo lignin is considered to be composed of guaiacyl, syringyl, and *p*-hydroxyphenyl units. As a unique feature, it also contains 5-10 % of *p*-coumaric acid ester (Higuchi 1987), located at the γ-positions of grass lignin, predominantly on syringyl units (Lu & Ralph 1999). All spectra curves of the epidermis, fibre and parenchyma cell wall layers show a shoulder between 310-320 nm, which can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987). Most spectra curves also have an absorbance peak at 280-282 nm, which indicates the presence of the strong absorbing guaiacyl lignin (Fergus & Goring 1970b; Musha & Goring 1975). He & Terashima (1991) found a similar guaiacyl peak in the spectra from rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.). Fengel & Shao (1985) concluded on the basis of a chemical analysis and the bands in IR-spectra that *Phyllostachys makinoi* is rich in syringyl units. However, they also observed a guaiacyl peak at 280 nm in the UV-spectrum. Lin *et al.* (2002) detected with VIS-microspectrophotometry, after staining with Wiesner and Mäule reagent, that the absorbance curves for *Phyllostachys pubescens* are similar to those of dicotyledons, with a syringyl peak in the spectra. Lybeer & Koch (2005a) observed a guaiacyl peak at 280-282 nm and a shoulder at 310-320 nm due to the presence of esters of *p*-coumaric and ferulic acid in lignin units of *Phyllostachys viridiglaucescens*. The UV-spectra of *G. levis* show for most cell types a less clear G peak but a more apparent shoulder between 310-320 nm comparing to the same cell types of *P. viridiglaucescens* (higher ratio $abs_{310\ nm}$ / $abs_{280\ nm}$) (Table 2-4). This difference is especially clear in the fibres of free fibre strands and in the parenchyma cells at the inner part of the culm wall. The spectra of the epidermis and hypodermis cell walls of *G. levis* form an exception as they have a clearer peak at 280-282 nm in comparison to the spectra of epidermis and hypodermis cell walls of *P. viridiglaucescens*. The absorbance values, which indicate the lignin content, are higher in *G. levis* or similar in both species.

Table 2-4. Comparison of absorbance behaviour within individual cell wall layers of *G. levis* and *P. viridiglaucescens*. + means higher values than in *P. viridiglaucescens*, ++ means much higher values than in *P. viridiglaucescens* °

Anatomical region	*Gigantochloa levis* versus *Phyllostachys viridiglaucescens*
	Absorbance behaviour
	ratio abs_{310nm}/abs_{280nm}
Layers of the secondary fibre wall of early maturing fibres, inner part of the culm wall	+
Layers of the secondary fibre wall of late maturing fibres, inner part of the culm wall	++
Layers of the secondary fibre wall of early maturing fibres, middle part of the culm wall	+
Layers of the secondary fibre wall of late maturing fibres, middle culm wall	++
Layers of the secondary fibre wall of early maturing fibres, outer part of the culm wall	+
Layers of the secondary fibre wall of late maturing fibres outer part of the culm wall	+
Parenchyma cells, inner part of the culm wall	++
Parenchyma cells, middle part of the culm wall	+
Parenchyma cells, outer part of the culm wall	+

Ferulic and *p*-coumaric acids are more widely distributed in *G. levis* than in *P. viridiglaucescens* and their content is dependent on the anatomical location. Hydroxycinnamic acid moieties cause the cross-linkage of cell wall polysaccharides and participate with lignin to generate polysaccharide-lignin complexes, which lead to an increase in wall rigidity (Ishii 1997; Morrison *et al*. 1998). Hydroxycinnamic acids thus have an important effect on the wall mechanical properties. Ferulic acid esters are suggested as lignin initiation sites and direct cell wall cross-linking during plant growth and development. The role of *p*-coumaric acid occurs later and serves to bind together the growing lignin polymer (Morrison *et al*. 1998).

° In Appendix 2 all UV-spectra are given. It is not possible to give values for the ratios because this has to be done for each cell type at a different position and at each culm age seperately. This table gives an overall evaluation.

Morrison *et al*. (1998) studying the cell wall composition of maize internodes of varying maturity found that rind tissue (cuticle, epidermis, xylem elements, and phloem) of maize generally have greater ferulic acid and *p*-coumaric acid ester concentrations than pith tissue (parenchyma cells and randomly distributed vascular strands). As ferulic acid esters act as lignin initiation sites and direct cell wall cross-linking during plant growth and development this is as they had expected because rind vascular tissues lignify to a greater extent to support conductive and supportive tissue of the internode. Differences between pith and rind concentrations of ferulic acid esters diminished as internode tissues further differentiated and added cell wall components.

Our results reveal only distinctly higher content of hydroxycinnamic esters in the epidermis and hypodermis cells and not in the fibres at the outer part of the culm wall. This may be due to the fact that Morrison *et al*. (1998) did not study separate cell types but only made a division in rind and pith. Higher content of hydroxycinnamic esters is also found in the CML, the parenchyma cell walls and the layers of the secondary wall of fibres of free fibre strands. He & Terashima (1991) found that the absorption maximum reach 314 nm when the ratio *p*-coumaric acid ester to ferulic acid esters is equal or higher than 1.14. They recorded a shift from longer toward shorter wavelengths with maturation in the region of 320-310 nm indicating the presence of more ferulic acid esters in the younger differentiation stage (the secondary wall is not highly lignified and multilayered). The maximum in this study is 312 nm, which indicates that more *p*-coumaric acid esters are present than ferulic acid esters (ratio is higher than 1.14). As very young samples were not available, a shift during maturation could not be studied. However, early maturing fibres (fibres adjacent to the vascular tissue and at the outer part of the culm wall) show lower absorbance values at 312 nm. These fibres show early in the maturation process cell wall thickening and lignification (Murphy & Alvin 1997a; Liese & Weiner 1996, 1997; Lybeer & Koch 2005a). In *P. viridiglaucescens* the difference between UV-spectra of early and late maturing fibres disappears in culms of 6- to 12-months old (Lybeer & Koch 2005a) whereas the difference in absorbance behaviour between early and late maturing fibres is still present in culms of *G. levis* of 40-months old. A possible explanation could be that culms of *P. viridiglaucescens* have to harden more quickly than culms of *G. levis*, growing in a more stable climate. However, more temperate as well as tropical species should be studied.

The vessel cell wall of *G. levis* has low lignin content, which is comparable to the vessel cell wall of *P. viridiglaucescens* (Lybeer & Koch 2005a). The function of the early maturing fibres adjacent to the vessels is probably to reduce the susceptibility of the vascular tissue to collapse. The lignin concentration in the cell corners and compound middle lamella is higher than in cell wall layers of the secondary wall which is comparable to *P. viridiglaucescens* where it is clear from young samples that lignification first takes place here and then proceeds into the secondary wall of the fibres (Lybeer & Koch 2005a).

Lignification

A difference in lignin content between the different ages and between flowering and non-flowering culms could not be observed. This is in contrast with the conclusions of several other authors (Murphy & Alvin 1997a; Lin *et al.* 2002) that lignification increases with ageing but agrees with the findings of Itoh (1990) who stated that lignification is completed within one growing season. However, it is important to consider that the spectra represent only one of several layers of a cell wall. It could be possible that lignification is completed within one cell wall layer while the fibre retains the ability to form new layers and lignify them additionally. Between the parenchyma cells close to the lacuna and parenchyma cells still located in the inner culm wall but further away from the lacuna there is no difference in lignin content. That could be expected as the parenchyma cells bordering the lacuna have a different cell shape and must give support at the inner side of the culm wall.

4. Concluding discussion

In the discussion on the lignin distribution in *G. levis* (3.4. Discussion and Conclusion, p. 66) a detailed comparison between the lignin content and distribution in *P. viridiglaucescens* and *G. levis* is given (cf. Table 2-4, p. 68). The UV-spectra of most layers of the secondary wall of *G. levis* have a more apparent shoulder between 310-320 nm, which is caused by the presence of more hydroxycinnamic acids than in layers of the secondary wall of *P. viridiglaucescens*. Furthermore, the absorbance values are equal or higher in *G. levis*, indicating an equal or higher lignin content.

An exception to this general observation is the epidermis and hypodermis cell wall. In contrast to the cell wall layers of fibre and parenchyma cells where a less obvious guaiacyl peak in *G. levis* is present, the epidermis and hypodermis display a clearer peak at 280 nm in *G. levis* than in *P. viridiglaucescens* (Fig. 2-8).

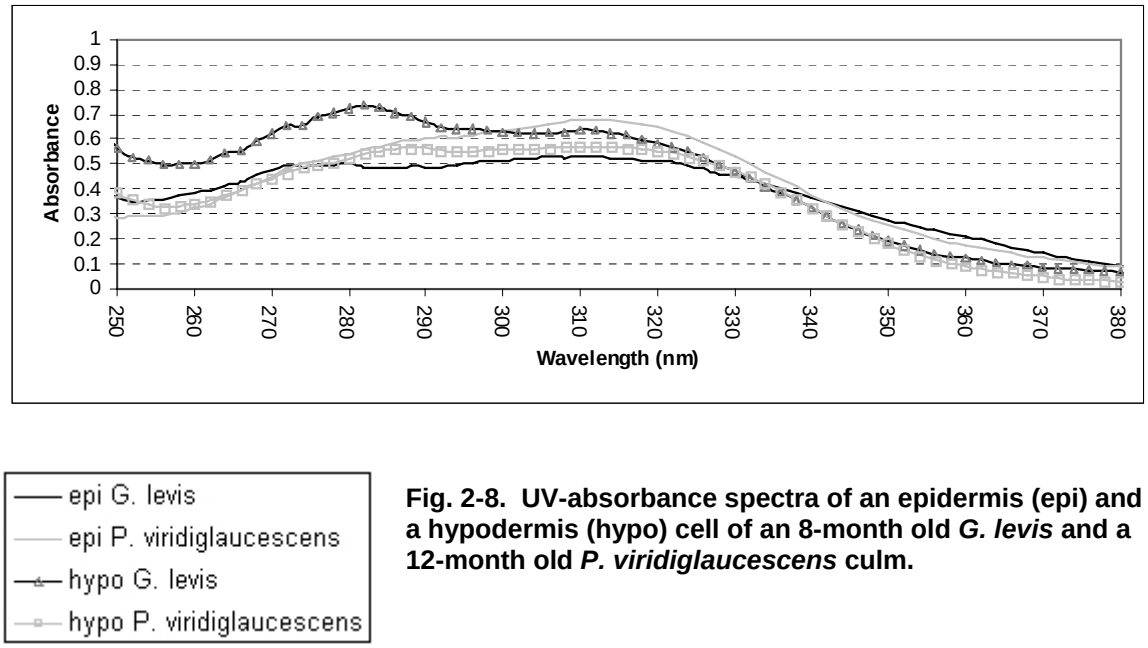


Fig. 2-8. UV-absorbance spectra of an epidermis (epi) and a hypodermis (hypo) cell of an 8-month old *G. levis* and a 12-month old *P. viridiglaucescens* culm.

Higher content of hydroxycinnamic acid esters (i.e. higher ratio $abs_{310\ nm}$ / $abs_{280\ nm}$) in *G. levis* are found in the compound middle lamella, the parenchyma cell wall layers and the cell wall layers of secondary walls of late maturing fibres (fibres of free fibre strands) of all samples. Similarly, a higher ratio $abs_{310\ nm}$ / $abs_{280\ nm}$ is found in young (1- to 6-months old) compound middle lamella, young (3-months old) parenchyma cells and young (3-months old) late maturing fibres of *P. viridiglaucescens* samples.

Ferulic acid esters are suggested as lignin initiation sites and direct cell wall cross-linking during plant growth and development. The role of *p*-coumaric acid occurs later and serves to bind together the growing lignin polymer (Morrison *et al*. 1998). Azuma *et al.* (1996) showed that the esterified *p*-coumaric acid content in bamboo is closely related to the lignin content, whereas ferulic acid was rich in upper immature portions of the culm shoot. He & Terashima (1991) found that the absorption maximum reach 314 nm when the ratio *p*-coumaric acid ester to ferulic acid esters is equal or higher than 1.14. They recorded a shift from longer toward shorter wavelengths with maturation in the region of 320-310 nm indicating the presence of more ferulic acid esters in the younger differentiation stage (the secondary wall is not highly lignified and multilayered). The maximum in this study of *G. levis* is 312 nm, which indicates that more *p*-coumaric acid esters are present than ferulic acid esters. Very young samples should be studied to see if there is also higher content of ferulic acid in young *G. levis* culms. Similar shift from 310-320 nm toward shorter wavelengths related with these higher amount of ferulic acid was not observed in *P. viridiglaucescens*. Perhaps younger more immature samples still surrounded by culm sheaths should be studied to observe this shift in the UV-spectra.

Koch & Kleist (2001; Fig.1 p. 564) compared UV-absorbance spectra of *Fagus sylvatica*, *Picea abies* and *Phyllostachys edulis*. The absorbance maximum of *F. sylvatica* is at 278 nm (Abs_{278nm} 0.32) because the S_2 wall of hardwood fibres is composed of syringyl - guaiacyl lignin in varying ratios characterised by a more variable shifted maximum between 270-278 nm. The UV-spectrum of the S_2 layer of the spruce tracheid shows a generally higher absorbance (Abs_{280nm} = 0.49) compared to the hardwood fibre. The maximum absorbance at 280 nm indicates the presence of the strongly absorbing guaiacyl lignin. The UV-spectrum of the fibre layers of bamboo gives a 'guaiacyl' peak with similar absorbance as in spruce (Abs_{280nm} = 0.47). A broad shoulder is also present between 300-315 nm due to hydroxycinnamic esters. The age and location of the bamboo fibres is not given but according to this study it concerns a mature fibre of an older culm.

The method used here and in the study of Koch & Kleist (2001) gives only a semiquantitative idea about the lignin content. Lin & Dence (1992) mention that it is important to keep in mind that none of the methods for the determination of lignin are totally satisfactory because lignin should be isolated in a pure condition, what is impossible. The most widely applied method for directly measuring the lignin content is the determination of acid - insoluble (Klason) lignin. However, such direct methods do not give an idea on the topochemical variation in the different anatomical regions. Abd. Latif *et al*. (1996) reported values between 20.6 % and 22.5 % Klason lignin in culms between half-year and four-year old *Bambusa heterostachya* culms. Jamaludin *et al*. (1995) gives average values of culms of 2.5-years old of 23.4 % for *Bambusa vulgaris*, of 25.2 % for *Gigantochloa levis* and of 28.4 % for *Gigantochloa scortechinii*. Murphy & Alvin (1997a) mention values of 13.3 % in a 1-year old *P. viridiglaucescens* culm and of 26.0 % in a 3-year old *P. viridiglaucescens* culm. However, they dated the culms on culm appearance. So, it is possible that the 1-year old culm is in fact younger than 1 year, which can certainly influence the overall lignin content. They give reference values of 27.1 % lignin for pine and 24.9 % lignin for birch. In general, it can be concluded that mature bamboos culms have lignin content comparable with wood species.

Gritsch *et al.* (2004b) demonstrated that the multilayered cell wall of phloem fibres (early maturing fibres) in *Dendrocalamus asper* is mainly formed during the first year of growth. However, mature 3-year old culms show an increased number of cells with nine or more layers in comparison with a 1-year old culm. As bamboo fibre and parenchyma cells retain a living protoplast, they can lignify these newly formed layers. The question, however, is to what extent this has an impact on the overall lignin content. If only these newly formed layers lignify in older bamboo culms, and the lignin content of already present layers is constant, then it is likely that the impact of the overall lignin content is minimal. Especially because a layering pattern with decreasing lignin content towards the cell lumen is demonstrated.

Figure 2-9 shows UV-micrographs of early maturing fibres (close to the vascular tissue) (a, b) and of late maturing fibres (fibres of free fibre strands) (c, d) of an 8-month old and a 40-month old *G. levis* culm. The early maturing fibres show no visual difference in lignin content. A cell lumen is mostly not present so additional deposition of layers is impossible. The late maturing fibres still have a large cell lumen so additional deposition is possible. However, the scans show no difference in number of cell wall layers or in lignin content.

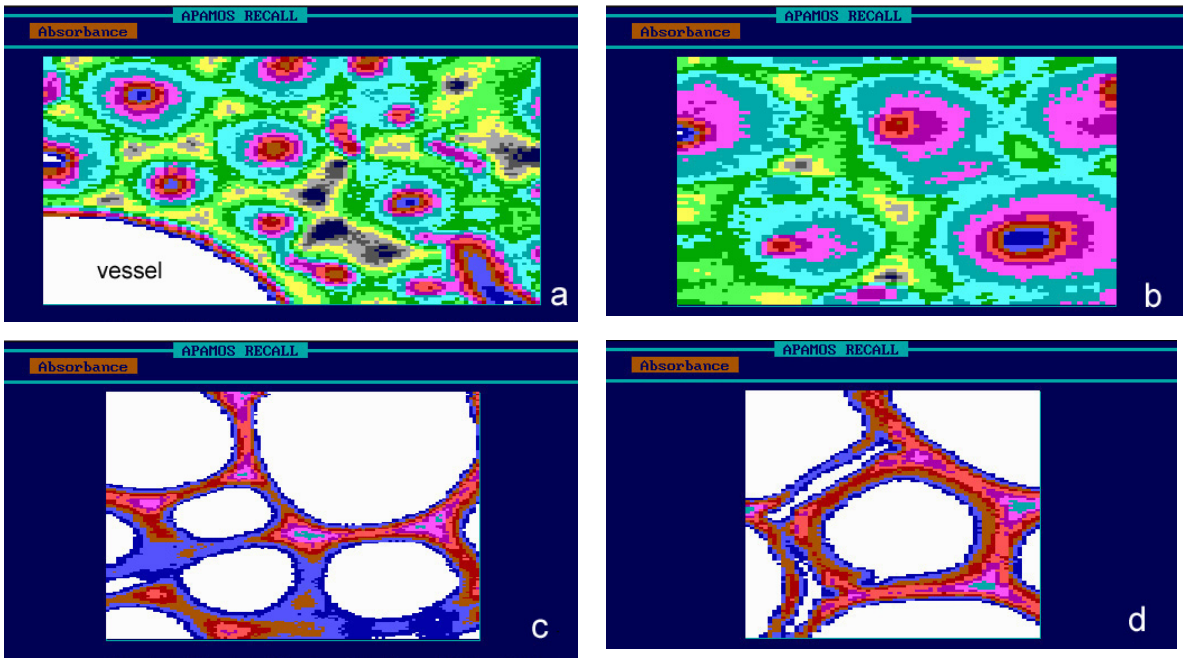


Figure 2-9. UV-micrographs of cell walls of *G. levis*. (a) Fibres close to the vascular tissue of an 8-month old culm, vascular bundle of the middle part of the culm wall. (b) Fibres close to the vascular tissue of a 40-month old culm, vascular bundle of the middle part of the culm wall. (c) Fibres of free fibre strand of an 8-month old culm, vascular bundle of the inner part of the culm wall. (d) Fibres of free fibre strand of a 40-month old culm, vascular bundle of the inner part of the culm wall.

The early maturing fibres correspond to the fibres studied by Gritsch *et al.* (2004b). These authors also observed that most fibre cell walls of a 1-year old culm are close to their maximum thickness. Figure 2-9c and d demonstrate that although the late maturing fibres still have a large cell lumen, probably no additional cell wall layers are deposited. These cells are not studied by Gritsch & Murphy (2005). However, it is possible that fibres at specific places within the fibre strand deposit more cell wall layers than others.

The discussion in Chapter III (p. 96) gives a comparison between TEM photographs of *G. levis* and *P. viridiglaucescens*. The discussion on lignification in combination with cell wall thickening is continued there.

CHAPTER III

CELL WALL THICKNESS OF TEMPERATE AND TROPICAL BAMBOO CULMS OF DIFFERENT AGES

CHAPTER III

CELL WALL THICKNESS OF TEMPERATE AND TROPICAL BAMBOO CULMS OF DIFFERENT AGES

1. Variability in fibre and parenchyma cell walls of temperate and tropical bamboo culms of different ages[3]

Summary

Fibre and parenchyma cell wall thickness of bamboo culms both from temperate (*Phyllostachys* spp.) and tropical (*Gigantochloa levis* and *Dendrocalamus asper*) species and of different ages have been investigated in detail for the middle part of the culm wall of the 6th and 12th internode above the ground level. 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 (adjacent to the parenchyma) 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 one 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.

[3] Adapted from:

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Variability in cell walls

1.1. Introduction

Bamboos are one of the most important non-timber forest products and one of the more important agricultural perennial plants in the world. During the last decade, increased knowledge and research of 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-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 & Murphy 1988; Liese & Weiner 1996,1997; Murphy & 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 & Murphy 1988; Liese 1998). Murphy & Alvin (1997a) demonstrated that culm density, and fibre cell 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 & 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 toward the inner side. Furthermore, it is influenced by the position of the fibres within the vascular bundle. Several authors (Alvin & Murphy 1988; Liese & Weiner 1996, 1997; Murphy & Alvin 1997a) described fibre maturation in bamboo species with type I vascular bundles. They describe the fibres close to the vascular tissue as early maturing fibres and the fibres adjacent to the parenchyma as late maturing fibres. Murphy & Alvin (1997b) studied fibre maturation in the bamboo *Gigantochloa scortechinii* with type III and IV vascular bundles and they paid special attention to aspects of fibre maturation in free fibre strands. They describe the fibres of the vascular fibre caps as thickening to almost their maximum during the first year of culm 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 toward the inner side is apparently clear, the cell wall thickening of fibres and parenchyma cells in the middle part 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.

1.2. Materials and Methods

1.2.1. Plant samples

Samples of culms with different ages were taken of *Phyllostachys viridiglaucescens* (Carrière) A. et 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 Ghent University Botanical Garden (*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 of emergence giving precise age at the date of sampling (Table 3-1).

Table 3-1. Collected samples. Samples indicated with * were used for TEM studies.

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

1.2.2. Light microscopy

Transverse sections of 18-25 µm 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 in a graded alcohol series and clearing in Parasolve (Prosan 88001-0, Ghent, Belgium), 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 x 400.

1.2.3. Transmission electron microscopy

First, sections of 50 µm of some samples of *P. viridiglaucescens* and *G. levis* (Table 3-1) were cut using a Microm-HM440E sliding microtome. From the middle part 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™ (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 three minutes, followed by washing twice in double distilled water. The sections were examined with a Jeol-1010 transmission electron microscope operating at 60 kV.

1.2.4. Cell wall measurements

For the cell wall measurements the image analysis program analySIS 3.0 (Soft Imaging System) was used. For each characteristic, 25 measurements were recorded in one vascular bundle of the middle part of the culm wall. 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 part of the 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 *Dendrocalamus asper* and *Gigantochloa 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 part of the of the culm wall.

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

1.2.5. 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.

For the tropical species *G. levis* and *D. asper*, age classes were made to perform the analyses. For *G. levis*, the ages 19-, 21-, and 22- months old, 29-, 31-, and 32-months old and 39-, and 40-months old were treated as age classes. For *D. asper*, the ages 29-, 31-, 32-, and 35-months old and 41-, 42-, 43-, and 45-months old were treated as age classes.

1.3. Results

1.3.1. Cell wall thickness at different ages

The *Phyllostachys* species have vascular bundles consisting of central vascular tissue with four fibre caps as supporting tissue (Fig. 3-1a). This type I vascular bundle has been described for the genus *Phyllostachys* by Liese & Grosser (2000). Some minor differentiation between the *Phyllostachys* 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. *Gigantochloa levis* and *Dendrocalamus asper* have type III and type IV vascular bundles (Fig. 3-1b). Type III 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 & Grosser 2000). *G. levis* has notably more type III bundles. In *D. asper*, both bundle types are equally present. Liese & Grosser (2000) described the genera *Dendrocalamus* and *Gigantochloa* to have type III or IV vascular bundles.

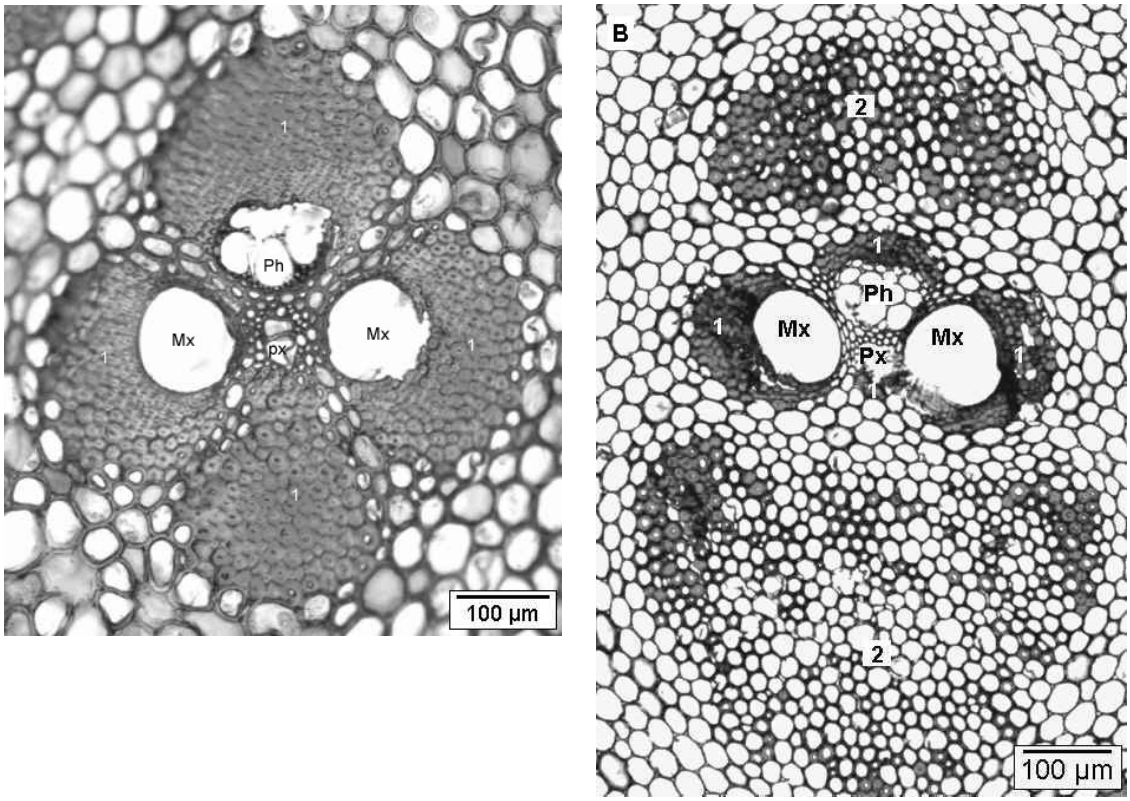


Figure 3-1. Transversal sections through internode 6 in the middle part of the culm wall. (A) Type I vascular bundle of *P. viridiglaucescens* with four fibre caps (1) surrounding the vascular bundle. (B) Type IV vascular bundle of *G. levis* with four smaller fibre caps (1) adjacent to the vascular tissue and two free fibre strands (2), located at the phloem and protoxylem side. Mx: metaxylem, Px: protoxylem, Ph: phloem.

This study focuses on the cell wall thickening of fibres and parenchyma cells in the middle part of the culm wall. The tropical species *G. levis* and *D. asper* have free fibre strands in contrast to the temperate *Phyllostachys* spp. who are characterized by larger fibre caps surrounding the vascular tissue. The phloem and xylem fibre caps of *Phyllostachys* spp. can be divided in inner early maturing fibres located adjacent to the phloem and xylem and outer late maturing fibres at the periphery of the phloem and xylem cap. In the tropical species, the walls of fibres adjacent to the phloem and xylem completed their thickening at the same time as fibre walls at the periphery of the phloem and xylem cap (early maturing). However, the fibres of the free fibre strands are late maturing.

Figure 3-2 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 *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. 3-2a). 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-months old sample (Fig. 3-2b, c, d).

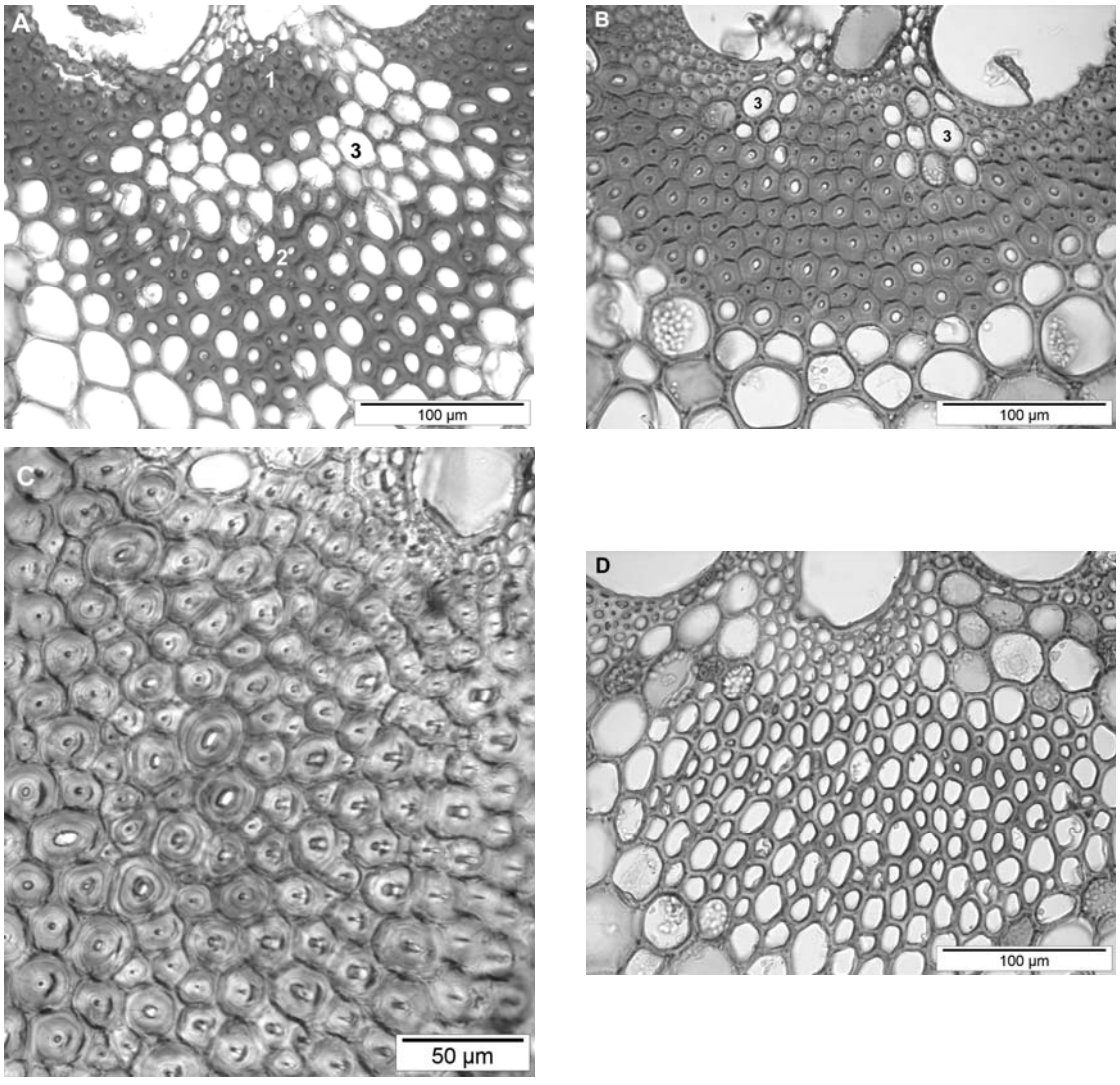


Figure 3-2. Transversal sections through internode 6 in the middle part of the culm wall. Fibre caps at the protoxylem side from the inner toward the outer fibres of a 3-month old (a) a 6-month old (b) a 20-month old (c) and a 44-month old culm (d) of *P. viridiglaucescens*. 1: inner early maturing fibres, 2: outer late maturing fibres, 3: parenchyma cells that are interspersed between the fibres.

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. 3-3). A greater variability and a thinner cell wall are observed in free fibre strands of *G. levis* and *D. asper*.

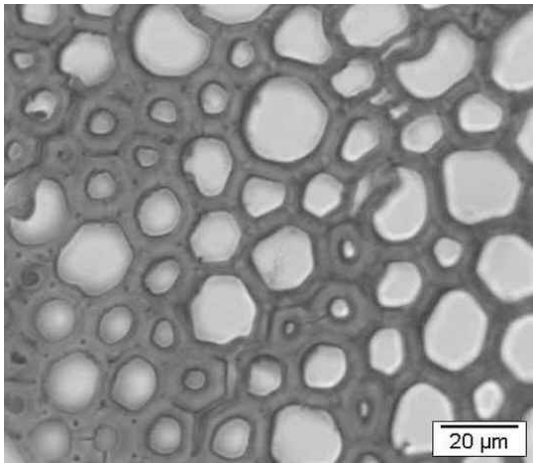


Figure 3-3. Transversal sections through internode 6 in the middle part of the culm wall. Fibres of a free fibre cap of an 8-month old *G. levis* culm showing the large variability in cell diameter and cell lumen size within the same fibre cap.

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*. *Dendrocalamus asper* and *Gigantochloa 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 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* (Table 3-2). 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 ($P < 0.01$).

Table 3-2. 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. 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 *Dendrocalamus asper* only culms of 8-month old and older were sampled. * indicate significant difference at P = 0.01

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

Although the fibre wall thickening is not significant during the first year of development, an upward trend from a 1-month old culm toward 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. 3-4). This upward trend is not found 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. Figure 3-4 shows scatter-plots visualizing nested design ANOVA's from late maturing fibres of *P. nigra* and *D. asper*. They illustrate that 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.

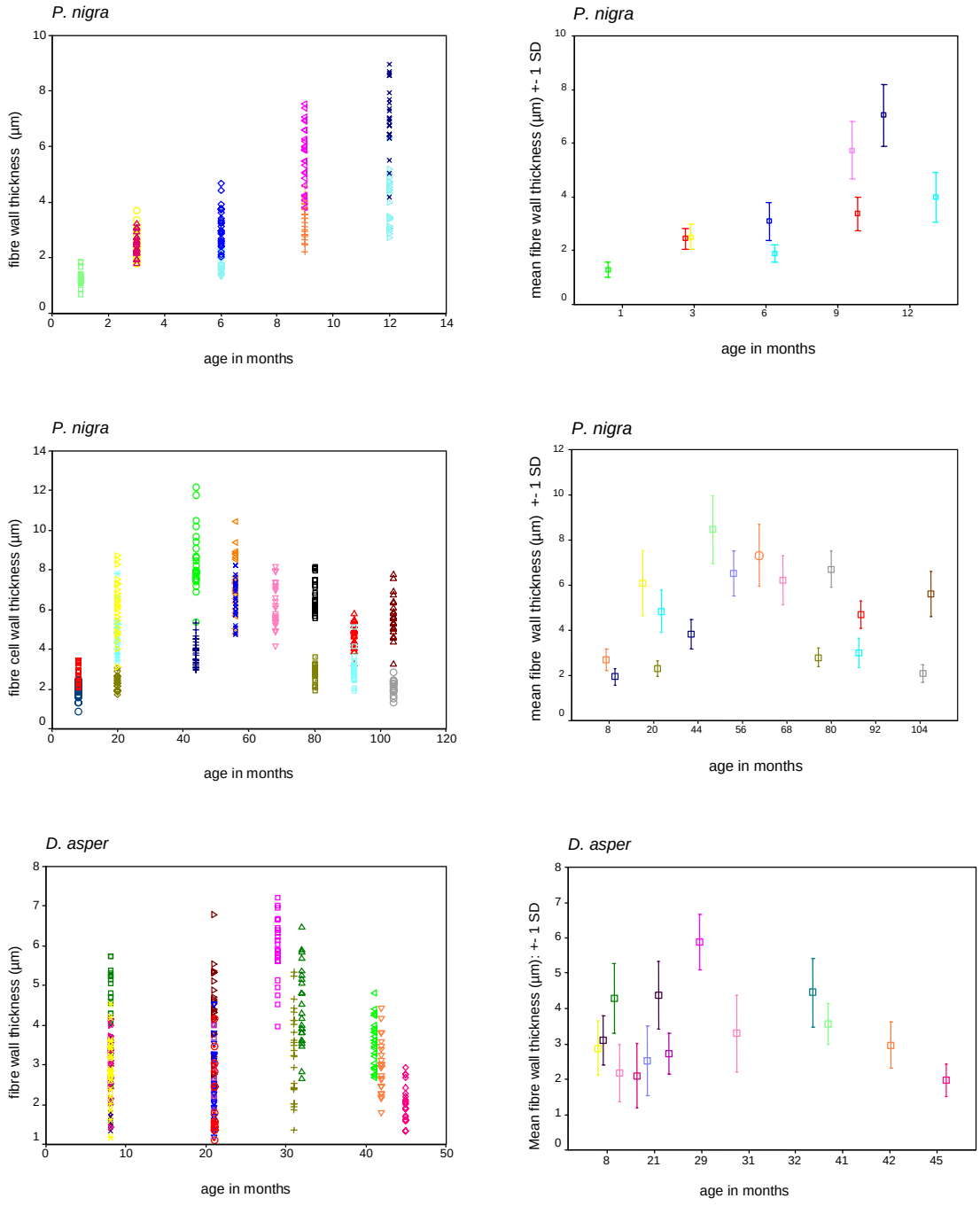


Figure 3-4. Scatter-plots visualizing the nested design ANOVA's (left) and mean values with standard deviation (right) during the first year of development (*P. nigra*) and during later years (*P. nigra* and *D. asper*) from late maturing fibres at the xylem side. Each color corresponds to measurements of one culm. From each culm, 25 late maturing fibres at the xylem side were measured.

Table 3-3 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 old or older for the *Phyllostachys* species. 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. 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 significant different ($P < 0.05$). However, in that case the variability within one culm is not taken into account. Nonetheless, younger culms can show high values for mean cell wall thickness and some older culms also may have low cell wall thickness, indicating the high variability between culms.

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 of 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 part 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 %.

Table 3-3. 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. 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

Species	*Origin*	*Age in months*	*xyin (µm)*	*xyout (µm)*	*phlin (µm)*	*phlout (µm)*	*par (µm)*
P. viridiglaucescens	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
		12	5.85	5.97	4.68	5.50	4.19
	France	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
		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

Species	*Origin*	*Age in months*	*xyin (µm)*	*xyout (µm)*	*phlin (µm)*	*phlout (µm)*	*par (µm)*
P. nigra	Belgium	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
		12	5.29	5.52	4.28	5.26	3.50
	France	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
		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
P. viridis	France	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
		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
D. asper	Philippines	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
G. levis	Philippines	8	4.20	3.45	4.26	4.15	1.79
		22	4.27	3.13	4.29	3.92	1.96
		29-32	4.56	3.14	4.20	3.35	1.79
		39-40	4.36	3.77	4.31	4.60	2.18

1.3.2. Cell wall layering

The fibre and parenchyma wall is thickening due to the deposition of additional cell wall layers (Parameswaran & Liese 1976; Murphy & Alvin 1992, 1997a; Gritsch *et al.* 2004b).

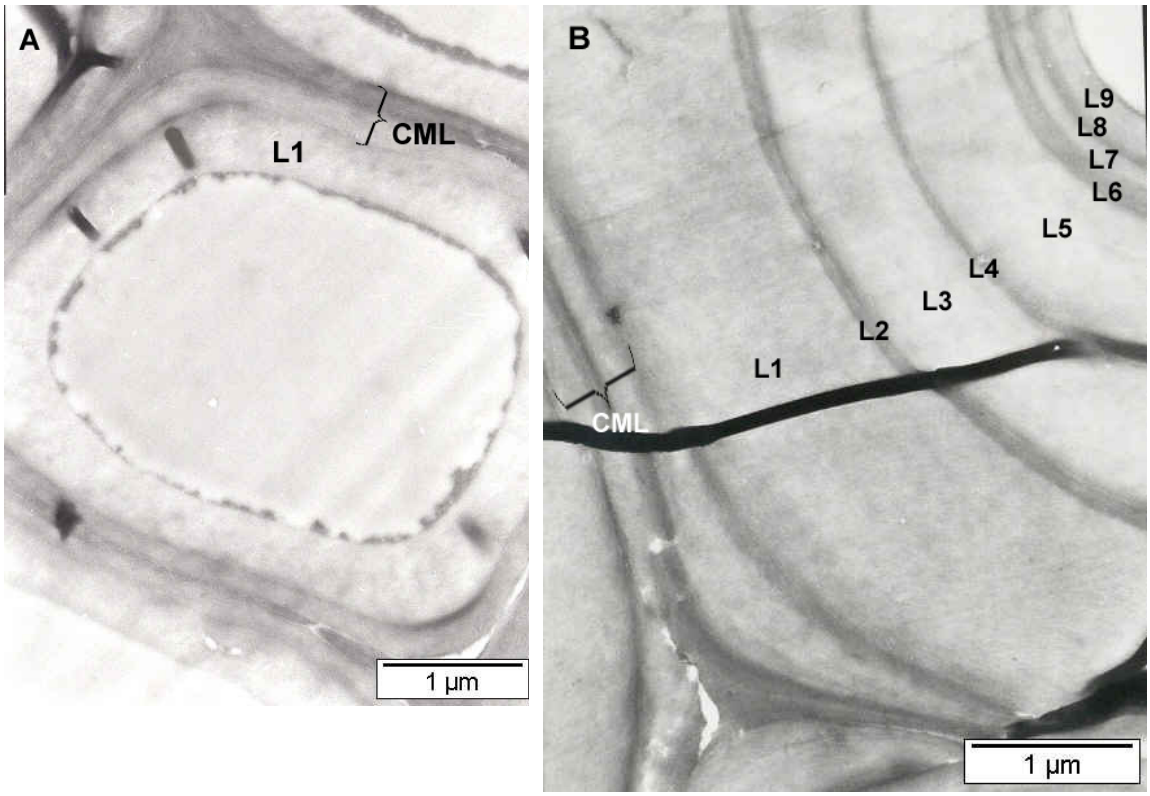


Figure 3-5. Transversal sections through fibres from the inner side of a phloem fibre cap of a 1-month old *P. viridiglaucescens* culm (A) with only few layers in comparison to a fibre from the inner side of a xylem fibre cap of a 3-year old *P. viridiglaucescens* culm (B).

Figure 3-5 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 secondary wall with alternating small and broad layers°.

° In this work the term '*secondary wall*'' is used when all cell wall layers (except the compound middle lamella) are indicated. When a specific layers is meant, the term L_x is used (See Introduction p. 23-24).

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. 3-6). The variability in layer thickness is most obvious in the broad layers; the narrow layers are more uniform in thickness.

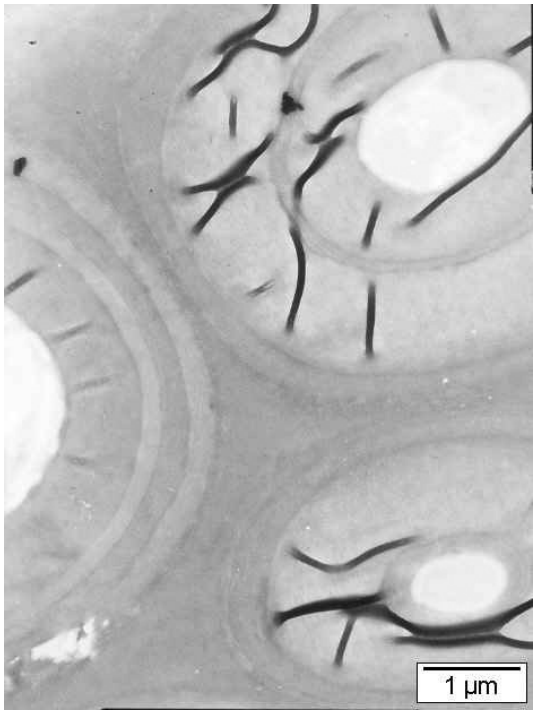


Figure 3-6. Transversal sections through fibres of a phloem fibre cap of a 6-month old *P. viridiglaucescens* sample

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. 3-7). 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 figure 3-8. The parenchyma cell wall is composed of different smaller layers with alternating lignin content. The number and thickness of layers is variable. The compound middle lamellae and cell corners are darkstained with potassium permanganate indicating higher lignin content.

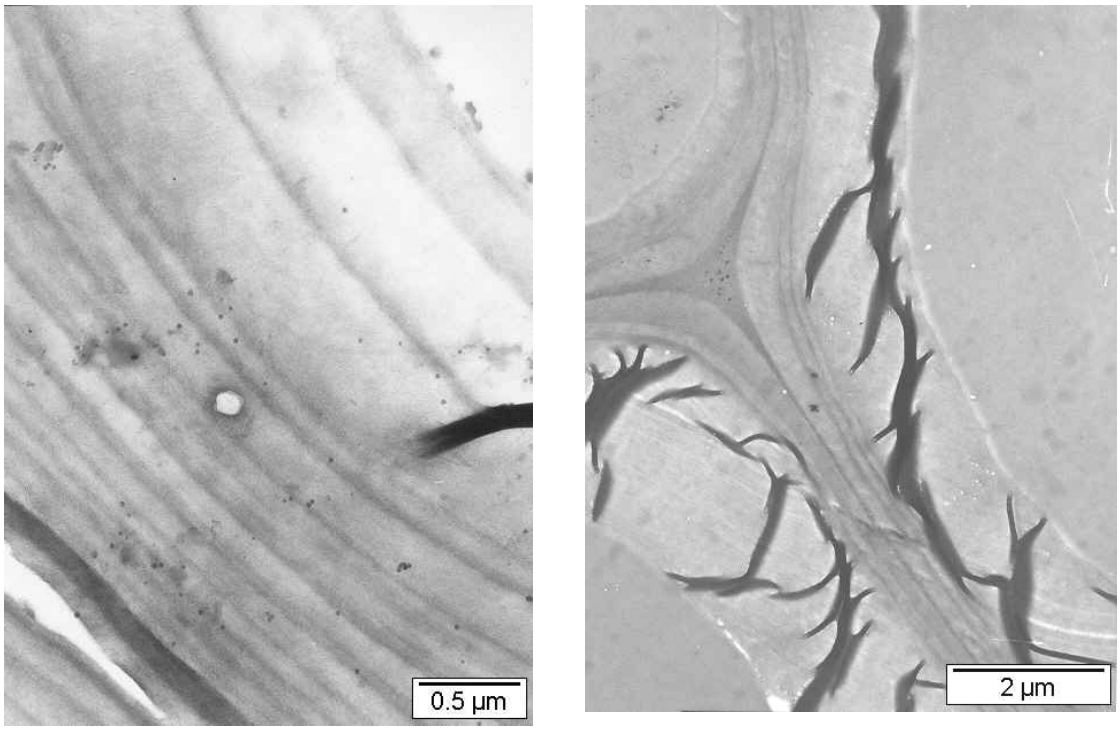


Figure 3-7. Comparison between late maturing fibres of a *P. viridiglaucescens* culm (3-years old) (left) with late maturing fibres of a *G. levis* culm (21-months old) (right). Late maturing fibres of *P. viridiglaucescens* culms have a thicker cell wall with more layers.

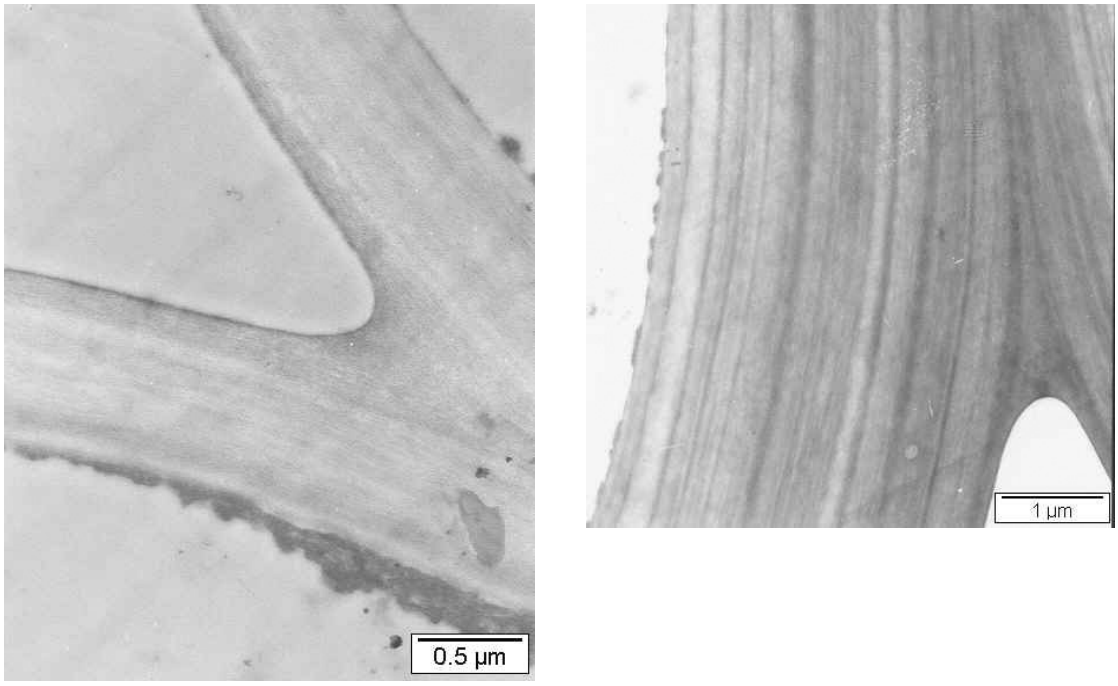


Figure 3-8. Parenchyma cell walls of a 1-month old (left) and 3-year old (right) sample of *P. viridiglaucescens*.

1.4. 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 as demonstrated in scatter-plots. These results contradict the general accepted idea that bamboo fibres have a prolonged maturation with an increasing cell wall thickness over several years (Alvin & Murphy 1988; Liese & Weiner 1996, 1997; Murphy & Alvin 1997a, b). Liese & Weiner (1996) published the mean fibre wall thickness of vascular bundles at the 4th internode from *Phyllostachys viridiglaucescens* (Table 1 in Liese & Weiner 1996) from which they ascertain the increase in fibre wall thickness. Table 3-4 compares the mean values from measurements in this study from the 6th internode of *P. viridiglaucescens* with their measurements.

Table 3-4. Comparison between measurements of fibre wall thickness of *P. viridiglaucescens* made by Liese & Weiner 1996 at the 4th internode and measurements made at the 6th internode from material from Prafrance used in this study.

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

The table does not support the conclusion of Liese & Weiner (1996) of an increasing fibre wall thickness with ageing. The fact that the variability, which we have demonstrated to be very important, was taken into account for the new data set could explain partly 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. 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 three 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. Nonetheless, some younger culms also display high values indicating the high variability between culms. 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 & Alvin (1997b) who observed that many fibres of free fibre strands of *G. scortechinii* after three years still remained thin walled. Lybeer & Koch (2005b) 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 difference in lignification might be related to the stable tropical climate for the bamboo species *G. levis*. For the tropical species sampled at Real

Qezon, it has to be remarked that the plantation was only 4-years old and as a consequence the oldest samples are culms taken from non-mature bamboo plants. The youngest culms are harvested from clumps approaching maturity. This fact can have consequences on the maturation (cell wall thickening and lignification) of these bamboos. Furthermore, as no material younger than 8-months old and older than 40 months could be sampled, no conclusions can be formulated on the cell wall thickening during the first year of development and of culms older than 3 years. Nevertheless, these periods are shown to be important for the *Phyllostachys* spp.

Parameswaran & 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. Murphy & 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. Gritsch *et al.* (2004b) concluded that the multilayered structure of the fibre cell walls was formed mainly 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 secondary 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.

In all studied species the proportion of parenchymatous tissue in the middle part of the culm wall at internode 6 is about 56 %. Grosser & 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 part of the culm wall at internode 6 correspond to those mentioned in Grosser & 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 parenchyma 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 significant 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 diameter. A possible explanation for the difference in culm wall thickness between the bamboos sampled in Belgium and those in France can be the more favourable climate for the growth of bamboo culms in the south of France, which is a typical Mediterranean climate with higher mean temperatures. Another possibility can be the maintenance of the fully developed bamboo plants. In France, the plants are thinned every year resulting in more growing space for each individual culm.

1.5. 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 one 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 (*Phyllostachys* spp.). These results suggest that some kind of late cell wall maturing can take place and can explain that for practical uses culms of three years and older are being used. However, some younger culms also display high values. So, not only culms of three year and older could be used, but culms older than one year would be appropriate for some industrial uses in respect of the parameters studied here. However, from the tropical culms, very young (younger than 8-months old) and old material (older than 40 months) could not be sampled. Furthermore, the sampled culms came from non-mature bamboo plants which certainly can have an impact on fibre and parenchyma maturation in bamboo culms.

2. Concluding discussion - Lignification (chapter II) and cell wall thickening in bamboo internodes

It can be concluded that both lignification and cell wall thickening of fibre and parenchyma cell walls mainly take place during the first year of growth. Probably, further thickening and lignification can take place in cell walls of older bamboo culms but it seems impossible to detect a significant increase for a population of culms. This can be attributed to the high variability present within one culm and even within one fibre cap. It is possible that cells, which still have a cell lumen further deposit and subsequently lignify cell wall layers, it is however unlikely that this will have a major impact on the overall proportion of cell walls or lignin content.

However, from the tropical culms, young and older material could not be sampled. Furthermore, the sampled culms came from non-mature bamboo plants which certainly can have an impact on fibre and parenchyma maturation in bamboo culms.

Gritsch *et al.* (2004b) measured all fibres of a phloem fibre cap in the inner culm wall region of *Dendrocalamus asper* culms of three different ages (< 6-months old, 1-year old and 3-years old) resulting in a significant increase in average cell wall thickness. It has to be noted that when comparing mean cell wall thicknesses the high variability of the wall thicknesses within one culm and between different culms is not taken into account. As is mentioned in the article, the average thickness of the fibre cell wall in the mature culm was 4.5 µm but the maximum wall thickness measured was up to 12 µm or more in some individual cells.

Bamboo cell wall ultrastructure was studied by Gritsch & Murphy (2005) revealing a primary wall consisting of 2 layers. The fibre secondary wall began to be laid down while cells were still showing some elongation, suggesting that it may cause the slow-down and eventual cessation of cell elongation. As only young material was used, no folds are present in the sections. In this study also 9-year old material was examined. As is clear in figure 3-6b (3-years old) folds are present in the sections due to the anatomical differences between parenchyma cells and fibres. Parenchyma cells have a larger diameter, a larger cell lumen and a thinner cell wall compared to fibres. As a consequence, the impregnation of the parenchyma cell wall is easier than of the fibre cell wall, which is demonstrated by the absence of folds in the parenchyma cells. Test samples impregnated with Spurr's resin (Spurr 1969) gave sections with even more folds, probably because it is more viscous than LR white™ resin.

The first trials with dehydratation in an alcohol series and subsequently impregnation with Spurr resin resulted in floating samples which were not impregnated. England *et al.* (1997) suggest that the bubbles, responsable for floating of the specimens in the resin, are vaporized solvent. Because of the different permeability of the cell walls, resin molecules are either totally excluded from the cells or enter only very slowly. Solvent molecules, however, move freely through the walls. Also Crow (2000) found that resin infiltration and polymerisation was perfect in young tissue, but as soon as a secondary wall was present, the cells became impervious to resin resulting in incomplete polymerisation. In this study the problem of incomplete impregnation was

solved by dehydration of the samples with acetone and subsequent impregnation with the less viscous LR white™ resin. Crow (2000) referred to Tewari (1992) who reported that bamboo contains a fine quality wax, which is partly retained in the fibre cell and creates a barrier to bleaching agents in the pulp and paper process. It is possible that acetone impregnation removes the waxes from the cells and improves impregnation with LR white™ resin.

The TEM photographs clearly show the high heterogeneity present as well in number as in thickness of cell wall layers. This variability is present in both cell wall layers of temperate bamboos with type I vascular bundles and tropical bamboos with type 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 type III an 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 type III an IV vascular bundles have a thinner cell wall with fewer cell wall layers. Only Murphy & Alvin (1997b) studied free fibre strands in *Gigantochloa scortechinii*. They observed a higher heterogeneity in terms of their diameter and even after three 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.

Gritsch *et al.* (2004b) observed a degree of 'order' in the distribution of multilayered fibres within phloem caps of *Dendrocalamus asper* with multilayered cell walls common in fibres adjacent to the phloem elements and around the edge of the fibre cap. A similar study on fibres of free fibre strands would be useful.

CHAPTER IV

LIGNIFICATION AND CELL WALL THICKENING IN BAMBOO NODES

CHAPTER IV

LIGNIFICATION AND CELL WALL THICKENING IN BAMBOO NODES

1. Lignification and cell wall thickening in nodes of *Phyllostachys viridiglaucescens* and *P. nigra*[4]

Summary

Bamboos are among the most important plants in the world. In contrast to the well-documented anatomical structure and mechanical properties of the culm internode, fewer details are available of the culm node. The aim of this study was a topochemical investigation on lignification and cell wall thickening in the outer part of the node and in the diaphragm of developing and maturing *P. nigra* culms and of mature *P. viridiglaucescens* culms of different age classes. The lignification was studied topochemically by means of cellular UV-microspectrophotometry. A combination of light microscopy and image analysis techniques were used to measure cell wall thickness. In shoots, the lignin content of hypodermis and fibre cell walls at the outer part of the node are higher than the lignin content of fibre and parenchyma cells of the diaphragm. In older nodes, the lignin content values of the outer part of the node and of the diaphragm are more similar but the fibre and parenchyma cell wall layers of the diaphragm have higher content of *p*-coumaric and ferulic acids than in the outer part of the node. Fibre and parenchyma cell wall thickness does not significantly increase during ageing. In the diaphragm, the cell walls are thinner and the cell diameter is larger than in the outer part of the node.

It was hypothesized that the combination of thinner cell walls in combination with higher cell diameters and more hydroxycinnamic acids in the diaphragm than in the outer part of the node may play an important role in the biomechanical function of the node to act as a spring-like joint to support the culm by bending forces.

[4] Adapted from:

Bieke Lybeer, Gerald Koch, Joris Van Acker & Paul Goetghebeur. 2006. Lignification and cell wall thickening in nodes of *Phyllostachys viridiglaucescens* (Carrière) A. et C. Rivière and *Phyllostachys nigra* (Loddiges ex Lindley) Munro. Annals of Botany 97: 529-539.

Bamboo nodes

1.1. Introduction

Because of their economic importance and multiple uses in daily life bamboo culms and more specifically bamboo internodes have been investigated thoroughly. In contrast to the rather straightforward anatomy of the internodes, the composition and structural details of the more complex nodes have been poorly analysed so far (Zee 1974; Liese & Ding 1994; Ding & Liese 1995, 1997; André 1998). Nevertheless, the nodes have special significance for the culm function. Owing to the lack of radial conduction cells, they enable the necessary communication for cross-transport of both water and nutrients. Furthermore, the nodal structure is important for liquid movement during drying and preservation as well as for physical and mechanical properties of the culm. The functional role of nodal diaphragms is to act as braces to resist wall invagination or buckling (Niklas 1997, 1998). Because nodal diaphragms brace internodal walls against lateral contraction, they increase the effective stiffness of stems. They also function as spring-like joints that store energy to elastically restore stems to their original shapes when bending forces are removed (Niklas 1997, 1998). In internodes, cell wall thickening and lignification was considered to take place over several years (Alvin & Murphy 1988; Majima *et al.*, 1991; Liese & Weiner 1996, 1997; Murphy & Alvin 1997a, b). However, Lybeer *et al.* (Chapter III, p. 77-95) found an increasing cell wall thickness during the first year of development, but not during later years. Nevertheless, they recorded the highest mean values for fibre wall thickness in culms of 44-months old or older, which suggest that some kind of late cell wall maturing can take place. This ability of prolonged cell wall thickening of fibres and parenchyma cells may provide an excellent mechanism for further strengthening the culm as it ages (Murphy & Alvin 1997a). Nonetheless the importance of cell wall thickening and lignification for the mechanical strength of the culm has been investigated, it has never been studied in detail in the nodes of bamboo.

1.2. Materials and Methods

1.2.1. Bamboo samples

Samples of the nodal area and of the culm sheath from shoots of *Phyllostachys nigra* (Loddiges ex Lindley) Munro were taken in the Ghent University Botanical Garden, Belgium (Table 4-1). Older culms of *Phyllostachys nigra* were sampled both in the Ghent University Botanical Garden, Belgium (1-, 3-, 6-, 9-, 12- months old) and at the Bambuseraie in Prafrance, France (8-, 20-, 32-, 44-, 56-, 68-, 80- and 104-months old) (Table 4-1).

Bamboo culms of the species *Phyllostachys viridiglaucescens* (Carrière) A. et C. Rivière from 1-, 3-, 6-, 9-, 12- months old were harvested in the National Botanical Garden of Belgium (Meise). Older culms (8-, 20-, 32-, 44-, 56-, 68-, 80- and 104-months old) were sampled at the Bambuseraie in Prafrance (France). Blocks of the nodal area between the 6th and 7th internode (numbered from the ground level) were cut and preserved in a mixture of 50 % alcohol, 10 % glycerine and 40 % water.

Table 4-1. Samples used for this study. The shoots of *P. nigra* were used to study the structure of the nodes and early lignification. Samples marked by * were used for UV-microspectrophotometry.

Species	Origin	Age (in months)	Remarks
P. nigra	Ghent	shoot I	12 cm height, completely surrounded by culm sheaths
		shoot II	360 cm height, nodes 1-6*: culm sheaths not present; nodes 7-15: surrounded by culm sheaths, internode elongated; nodes 15-top*: completely surrounded by culm sheaths
		1, 3, 6, 9, 12	
	Prafrance	8, 20, 32, 44, 56, 68, 80, 92, 104	
P. viridiglaucescens	Meise	1, 3, 6*, 9, 12*	
	Prafrance	8, 20, 32, 44, 56, 68, 80, 92, 104*	

1.2.2. Microscopy and cell wall measurements

Young, soft nodes and culm sheaths of *P. nigra* shoots were dehydrated in a graded series of alcohol and embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany). Transversal and longitudinal sections of 5 µm thickness were cut using a rotary microtome (Minot-Mikrotom1212, Leitz, Wetzlar, Germany) and stained with toluidine blue O. Toluidine blue O stains lignified cell walls blue-green and unlignified walls reddish purple (O'Brien *et al*. 1964). The sections were permanently mounted in DePeX (Merck, Lutterworth, UK).

From the older samples, transversal and longitudinal sections of 18 – 20 µm thick were cut using a sliding microtome (Microm HM440E) and double stained with safranin and astrablue. After dehydration and clearing in Parasolve (Prosan 88001-0, Ghent, Belgium), the sections were permanently mounted in Entellan (Merck, Darmstadt, Germany).

All observations were made on an AHBS-21 Vanox Olympus universal microscope using bright field illumination. For the cell wall measurements the image analysis program analySIS 3.0 (Soft Imaging System) was used. Fibre wall thickness was measured in the middle of the outer part of the node and both fibre and parenchyma wall thicknesses were measured in the diaphragm. For each characteristic, 50 measurements were recorded on transversal-sectioned cells.

1.2.3. UV-microspectrophotometry

Small blocks (1 x 1 x 5 mm^3) were cut from selected material of *P. nigra* and *P. viridiglaucescens* (Table 4-1; *P. nigra* shoot II: node between 2nd and 3rd and between 18th and 19th internode; *P. viridiglaucescens*: node between 6th and 7th internode). These specimens were dehydrated in a graded series of acetone and impregnated with Spurr's resin (Spurr 1969) through a series of propylenoxide/spurr resin mixture, followed by immersion in pure resin.

Transverse sections through the node of 1 µm in thickness were cut with a Reichert Ultracut ultramicrotome using a diamond knife. The sections were transferred to quartz microscope slides, immersed in a drop of non-UV absorbing glycerine and covered with a quartz cover slip. The sections were observed using the immersion ultrafluar objective 32:1. The used immersion oil consisted of a glycerine/water mixture n_D = 1.46.

The sections were examined under a ZEISS UMSP 80 microspectrophotometer equipped with a scanning stage enabling the determination of image profiles at defined wavelengths. The specimens were investigated by point measurements with a spot size of 1 μm^2 between 240 and 400 nm wavelength using the programme LAMWIN® (Zeiss). Epidermis and hypodermis, layers in the middle of the fibre and parenchyma secondary wall $(L_x)^{\circ}$, compound middle lamellae (CML) and vessel cell walls were measured at the outer part of the node and at the diaphragm. For each sampled culm, one measurement for each position in the culm wall and for each position within a vascular bundle was performed.

Image scan profiles at a constant wavelength of 280 nm were generated using the scan programme APAMOS® (Zeiss). This programme digitizes rectangular fields of the tissue with a geometrical resolution of 0.25 μm^2 and a photometrical resolution of 4096 grey scale levels, which are converted in 14 basic colours to visualise the absorbance intensities.

$^{\circ}$ In this work the term '*secondary wall"* is used when all cell wall layers (except the compound middle lamella) are indicated. When a specific layer is meant, the term L_x is used (See Introduction p. 23-24).

1.3. Results

In the following description, the morphological terms as defined by McClure (1966) are used. The sheath scar is called the nodal ridge and the bulge formed by the intercalary meristem the supranodal ridge (See fig. 1.4 p. 9).

1.3.1. Structure of the node

During culm growth, an internodal central cavity called the lacuna is formed. In young, still elongating shoots, this central part consists of parenchyma cells that will die and disappear upon completed elongation. Figure 4-1a shows a longitudinal section through the diaphragm of a node (shoot II – between internode 6 and 7) of an almost completely elongated part of the culm. The parenchyma cells adjacent to the parenchyma cells, which will die, become sclerified. These cells will form the border between the diaphragm and the lacuna. In the node, the normal arrangement of the vascular bundles with four fibre caps vanishes and a sclerenchyma sheath can be observed. Some bundles only have one vessel element. In the same section, both longitudinally and transversely sectioned vascular bundles are present indicating that they run criss-cross in the nodal structure. Branching vascular 'anastomoses' are developed as can be seen in figure 4-1a, b and c. The branching of phloem and xylem does not always start simultaneously. The fibres and parenchyma cells in the diaphragm have an irregular form.

Figure 4-1b and c illustrate that cell wall lignification proceeds from the bottom towards the top of the internode. Figure 4-1b shows a longitudinal section through a node between the 2nd and the 3rd internode. In comparison to a longitudinal section through a node between the 18th and the 19th internode (Fig. 4-1c), the cell walls are blue colored (staining with toluidine blue) which indicates higher lignin content.

At the nodal ridge the vascular bundles mostly have two fibre caps, one at the xylem and one at the phloem side. The ramification has already begun at this portion. At the supranodal ridge, the vascular bundles have a different form. The fibre caps are larger at the phloem and protoxylem side and smaller at the metaxylem side. More hypodermis cells are present compared to the number of cells in the internodes.

The culm sheath has a hypodermis with thick walled, lignified cells. The inner cells opposite the vascular bundle are also sclerified. So, the culm sheath forms a hard structure that protects the underlying still developing culm.

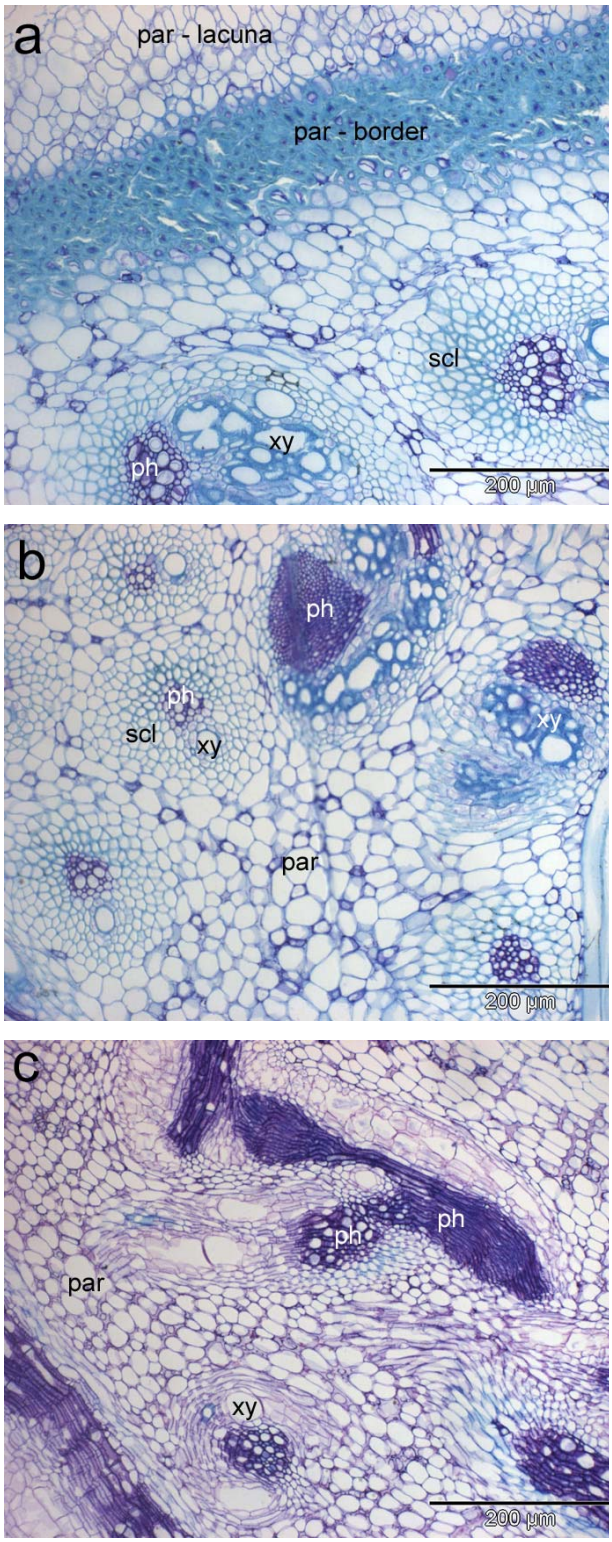
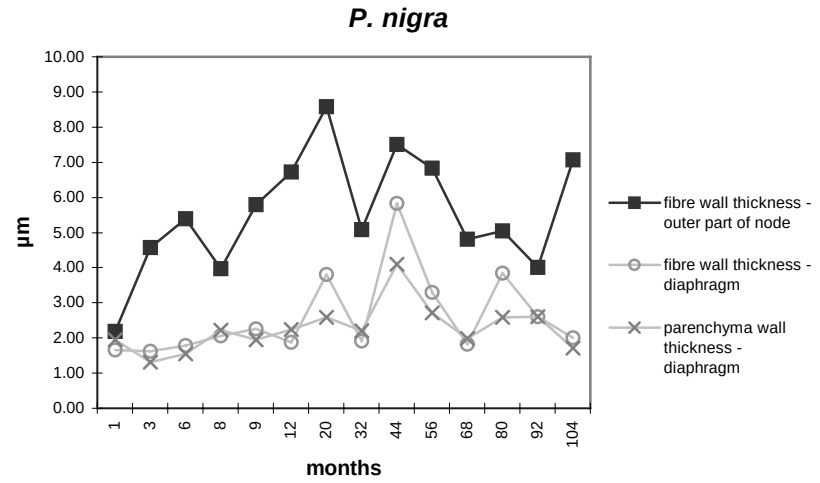
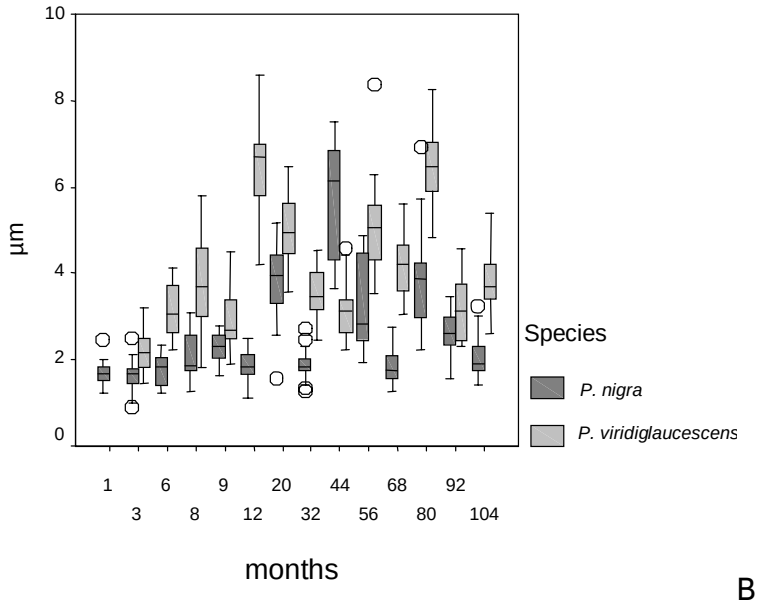


Figure 4-1. Longitudinal sections through nodal diaphragms of *P. nigra* shoot II. (a) Longitudinal section through the diaphragm of node between internode 6-7. The upper parenchyma cells (par-lacuna) will die and disappear, forming the lacuna. The sclerified parenchyma cells will form the border between the lacuna and the tissue of the diaphragm (par-border). (b) Longitudinal section through the diaphragm of node between internode 2-3 (culm sheath lost; internodes completely elongated). The cell walls are blue which indicates the presence of lignin. Ramification of xylem and phloem cells can be seen. Some vascular bundles have only one vessel bundle. (c) Longitudinal section through the diaphragm of node between internode 18-19 (culm sheath present; internodes not completely elongated). The cells are purple which indicates the absence of lignin. Both longitudinal and transverse sectioned vascular bundles are present indicating that they run criss-cross in the nodal structure. par: parenchyma; ph: phloem; scl: sclerenchyma sheath; xy: xylem

1.3.2. Fibre and parenchyma wall thickness in the nodal structure



A



B

Figure 4-2. Parenchyma and fibre wall thickness. (a) Comparison between parenchyma and fibre wall thickness of the outer part of the node and the nodal diaphragm. The cell walls are thinner in the nodal diaphragm. (b) Box-plots of fibre wall thickness in the diaphragm per age. Cell wall thickness does not significantly increase during ageing.

Figure 4-2a shows mean cell wall thickness of fibre and parenchyma cells in the nodal structure of *P. nigra* culms. Fibre and parenchyma cell wall thickness is lower in the diaphragm than in the outer part of the node. Nonetheless, the fibre diameter is higher in the nodal structure, which implies a potential for thicker cell walls in the diaphragm. The same observations are made in culms of *P. viridiglaucescens*. Box-plots (Fig. 4-2b) reveal an overlap between the measurements

indicating that age does not have a significant influence on cell wall thickness of both fibre and parenchyma cells in the nodal structure. It can be deduced that the values of *P. viridiglaucescens* are mostly higher than those of *P. nigra*. But again, due to the overlap of the boxes, this difference is not significant. It has to be remarked that the samples older than 12-months and the 8-month old samples are harvested in southern France, while the others are collected in Belgium. The difference in climate and soil could also have an impact.

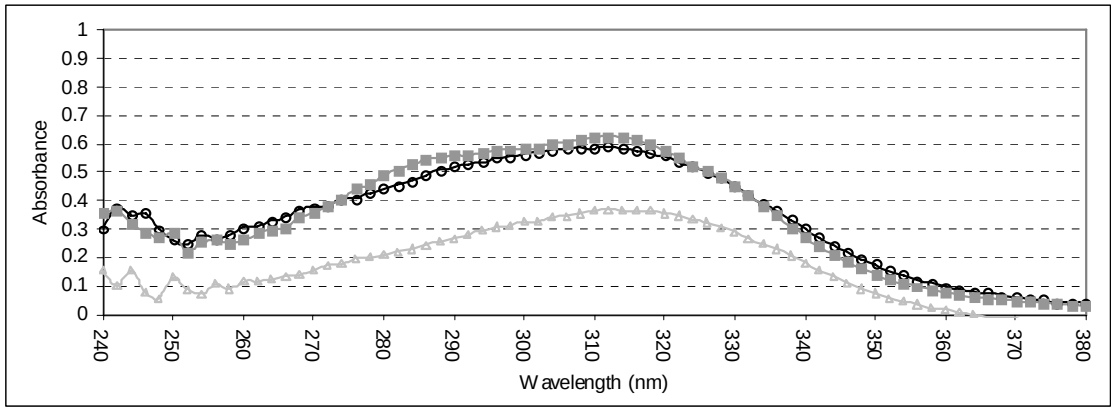
1.3.3. UV-absorbance spectra of individual cell wall layers of the tissue

The cellular UV-spectra of the epidermis cell walls show a clear broad shoulder with absorbance maxima between 310-320 nm (Fig. 4-3a). This shoulder is typical for grass lignin and can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987). There is a significant difference between nodes surrounded by culm sheaths and nodes without culm sheaths. Epidermis cells of nodes protected by culm sheaths are less lignified, whereas epidermis cells of nodes without culm sheaths show similar absorbance behaviour in very young (shoots) and older (12-month old) nodes.

Hypodermis cell walls have different lignin content in samples of different ages (Fig. 4-3b). In nodes surrounded by culm sheaths the UV-spectra have relative low absorbance values. The spectra from nodes not surrounded by culm sheaths show a maximum absorbance at 310-320 nm, due to the esters of *p*-coumaric and ferulic acid. There is no shoulder at 280-282 nm, indicating the absence of the strong absorbing guaiacyl (G) lignin (Fergus & Goring 1970; Musha & Goring 1975). Older samples (6-, 12-, and 104-months old) have higher lignin content characterised by spectra with a clearer peak at 280-282 nm and a shoulder between 310-320 nm. The absorbance values of a 6-month old node are lower than those of 12-, and 104-month old nodes. The spectra of the young samples (shoots) have lower absorbance values compared to the absorbance values of the epidermis cells, whereas the older samples (12- and 104-months old) have higher values.

The UV-spectra of layers of the secondary wall of fibres at the outer part of the node all follow a similar pattern (Fig. 4-3c). There is no significant difference between the spectra of shoots (with and without culm sheaths) and of 6-, 12-, and 104-month old samples. The spectra curves have a clear peak at 280-282 nm and a broad shoulder at 310-320 nm. Comparing to the epidermis and hypodermis cell walls, the ratio $abs_{310\ nm}$ / $abs_{280\ nm}$ is lower.

The absorbance values at 280 nm and 312 nm are significantly higher in the compound middle lamellae than in the layers of the secondary wall (paired t-test, 1-tailed; $P_{280\ nm} = 0.042$; $P_{312\ nm} = 0.023$). The spectra of the compound middle lamellae have a more clear shoulder at 310-320 nm, indicating the presence of more *p*-coumaric and ferulic acids in comparison to the layers of the secondary wall.



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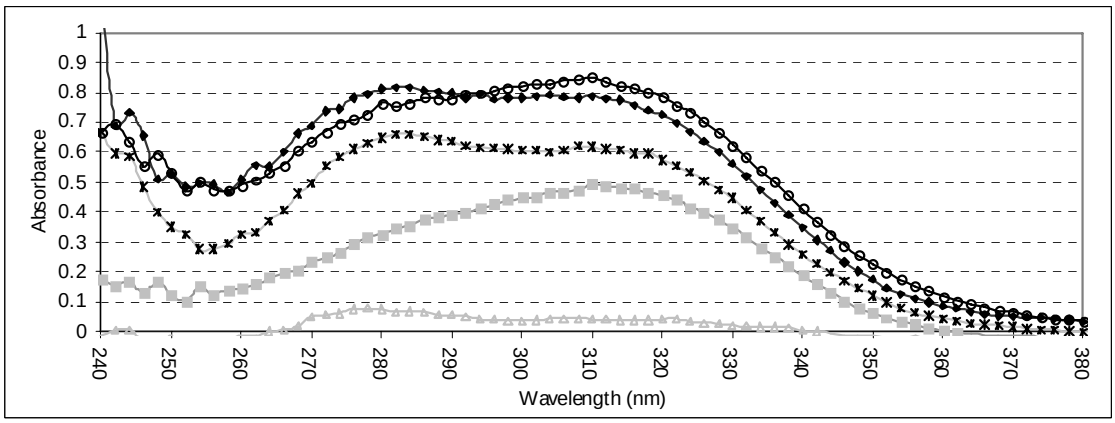

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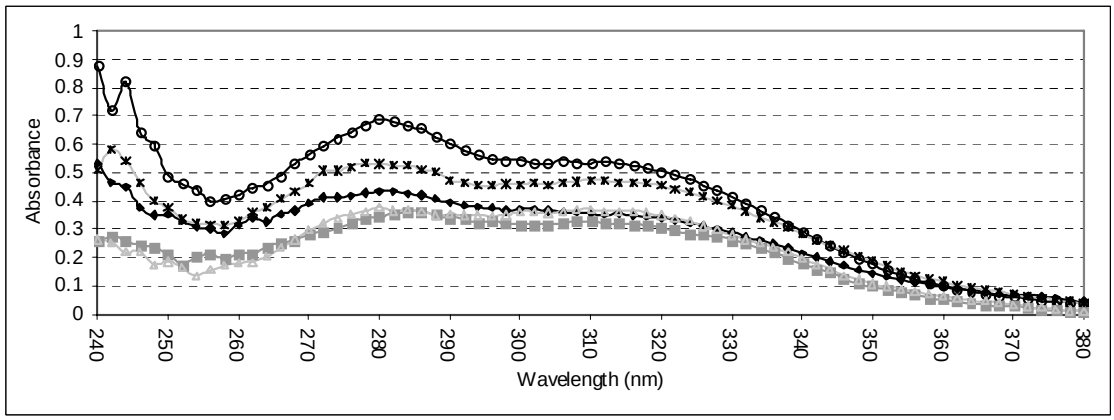

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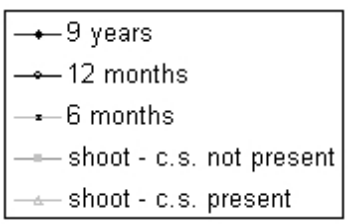
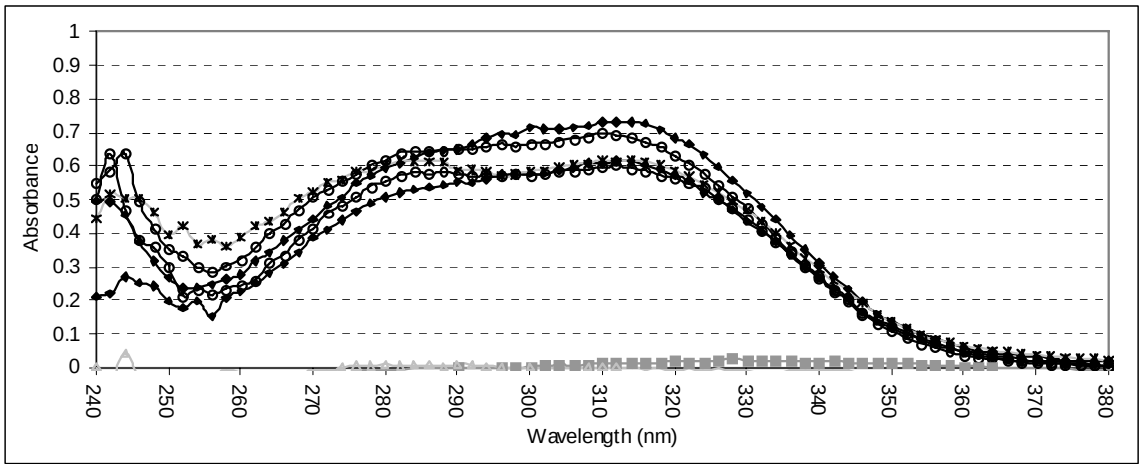


Figure 4-3. UV-absorbance spectra (a) UV-absorbance spectra of epidermal cell walls. (b) UV-absorbance spectra of hypodermal cells. (c) UV-absorbance spectra of cell wall layers of fibres at the outer culm wall.

Cell wall layers of parenchyma cells located in the diaphragm of very young nodes (shoots) are almost not lignified showing very low absorbance values without pronounced maxima (Fig. 4-4a). The UV-spectra of older nodes (6-, 12-, and 104-months old) exhibit similar absorbance values with a maximum shoulder between 310-320 nm. A second peak is present between 284-288 nm. Compared to the layers of the secondary wall of fibres at the outer part of the node, this means a shift of absorption maximum from 280-282 nm toward longer wavelengths. The absorbance values are higher in the compound middle lamellae (paired t-test, 1-tailed; $P_{280\ nm} = 0.037$; $P_{312\ nm} = 0.055$) but the spectra are similar to those of the cell walls. There is no significant (paired t-test, 1-tailed; $P > 0.05$) difference between the ratio $abs_{310\ nm}/abs_{280\ nm}$ in the layers of the secondary wall and the compound middle lamellae.

The spectra curves of the fibres in the diaphragm of the young samples (shoots) do not have high absorbance values, although the UV absorbance of the shoots is higher compared to the UV absorbance of the parenchyma cells of the shoots (Fig. 4-4b). The older nodes (6-, 12-, and 104-months old) show similar absorbance behaviour, with a peak at 280-282 nm and a shoulder between 310-320 nm. No shift of the absorbance maximum from 280-282 nm to longer wavelengths could be observed. The compound middle lamellae have approximately the same absorbance values for the young samples but higher absorbance values for the older samples (paired t-test, 1-tailed; $P_{280\ nm}=0.033$; $P_{312\ nm}=0.02$). The ratio $abs_{310\ nm} / abs_{280\ nm}$ is the same in the secondary wall layers and the compound middle lamellae of the fibres in the diaphragm (paired t-test, 1-tailed; $P > 0.05$).



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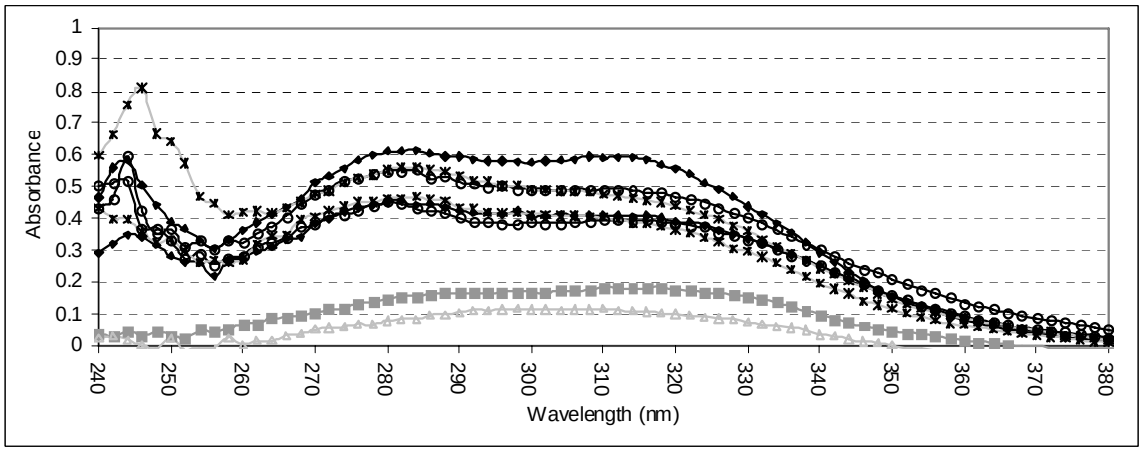

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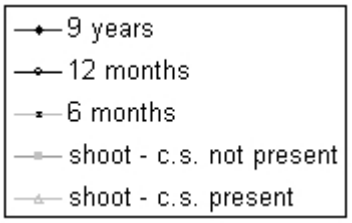
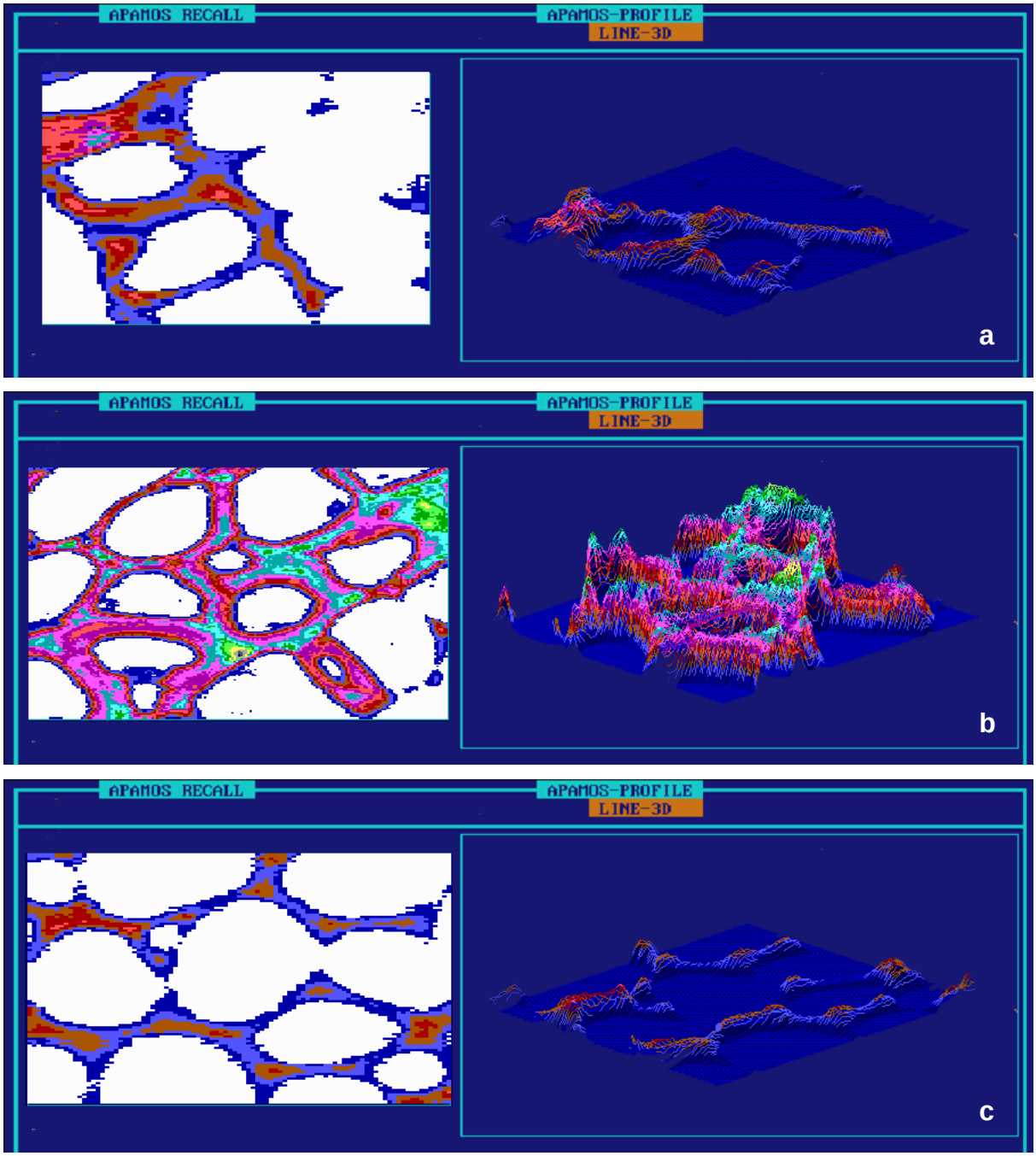
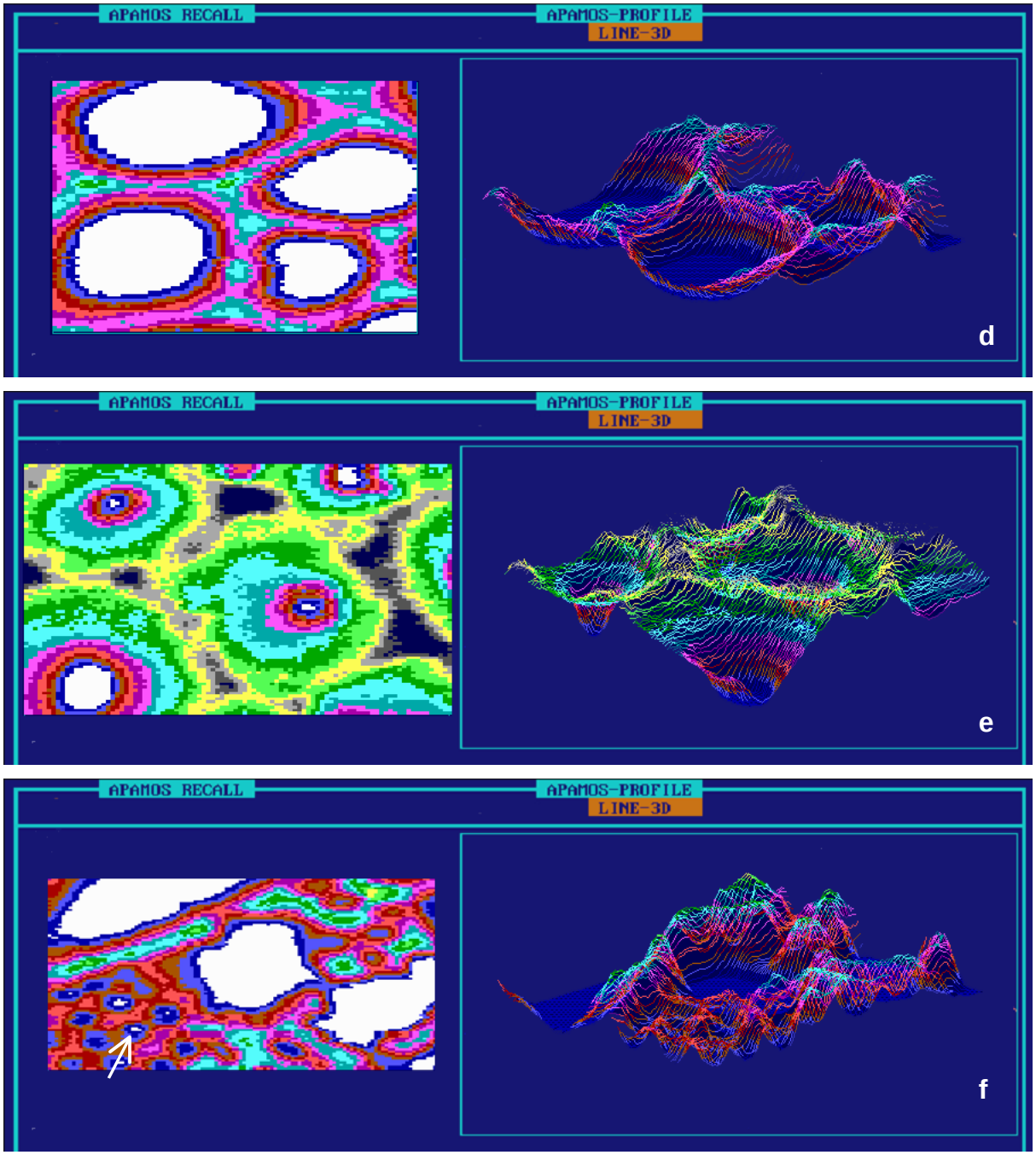


Figure 4-4. UV-absorbance spectra (a) UV-absorbance spectra of parenchyma cells in the diaphragm. (b) UV-absorbance spectra of cell wall layers of fibres in the diaphragm.

1.3.4. Scanning profiles of different cell types of the tissue





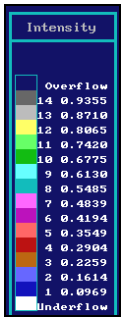


Figure 4-5. UV-micrographs (left) and 3D profiles (right) of cell walls of *P. nigra* (a, b, c, d) and *P. viridiglaucescens* (e, f). The color pixels indicate the different UV-absorbance values at a wavelength of 280 nm. (a) epidermis cells of a *P. nigra* shoot with culm sheath. (b) epidermis cells of a *P. nigra* shoot without culm sheath. (c) outer fibres of a *P. nigra* shoot with culm sheath. (d) outer fibres of a *P. nigra* shoot without culm sheath. (e) outer fibres of a 104-months old *P. viridiglaucescens* culm. (f) xylem cells of a *P. viridiglaucescens* culm of 12-months old (arrow point to pits).

The colour pixels of the scanning profiles indicate different intensities of UV absorbance at $\lambda_{280\ nm}$. The high resolution (0.25 µm^2 per pixel) enables a high differentiation of the UV absorbance within individual cell walls. Scanning profiles of epidermis and hypodermis cells of a node surrounded by a culm sheath and a node where the culm sheath is already lost, show a clear difference in lignin content within individual cell wall layers (Fig. 4-5a, b).

Fibres at the outer part of the node of older samples (6-, 12-, and 104-months old) show a lamellar structure with a decreasing lignin content towards the cell lumen. This lamellar structure can already be observed in a node without protecting culm sheath at the outer part of the node, but not yet in fibres of nodes still surrounded by culm sheaths (Fig 4-5c, d, e). The compound middle lamella and especially the cell corners are more lignified. Fibres in the diaphragm of a node without protecting culm sheath have just started to form wall layers and to lignify them. Older samples have already fibres with cell wall layers with decreasing lignin content towards the cell lumen. A scanning profile of a longitudinal section of a fibre revealed that the middle and the end part of a fibre are equally lignified. Figure 4-5f illustrates xylem cells. Near the pits there is only relative low lignin content.

1.4. Discussion

1.4.1. Structure of the node

In the nodes the structure is less organised than in the internodes. Since no radial conducting system exists in the internodes of bamboo culms because they are hollow, the transverse distribution of water and nutrients occurs in the nodal region. Therefore, the typical composition of a vascular bundle in the internode is changed in the nodal region. In the node branching of the xylem and phloem can be observed. Ding & Liese (1997) studied the anatomy of bamboo nodes. They describe that at the branching point, the metaxylem consists of abundant small vessels, which may have more apertures. Between the vessels there are many small cells with pits resembling those of the vessels, but without perforations. The protoxylem vessels exhibit intensive branching and these structures facilitate the liquid movement in different directions. In the phloem, abundant lateral sieve areas enhance connection of sieve elements. A spindle-like glomerulus structure consisting of filiform elements connects phloem of the main vascular bundles to that of the branched ones (phloem ganglion). André (1998), who carried out microcasting of vascular bundles in the nodes of bamboo, gives a more complete picture of the vessel ramification. He stated that the vessels that ramify form cell clusters, which consist of many densely pitted vessel elements. This author demonstrated that the 'xylem transfer cells' (intensively pitted small cells lying between the vessels) as described by Zee (1974) and the 'cells derived from these xylem transfer cells' as described by Ding & Liese (1997) are in fact densely pitted vessel elements of the ramification forming cell clusters. Also the transfer cells as described for wheat (Patrick 1972; Busby & O'Brien 1979) and other plant species (Gunning *et al.* 1970) are probably the vessel elements as described by André (1998). Ding *et al.* (2000) studied the development and ultrastructure of the phloem ganglion in bamboo node. They distinguished two cell types, one with pointed ends but many pits in the lateral wall and another one at both

ends of the spindle that possess an intermediate form between the filiform cell and the normal sieve tube. They state that the cells of the phloem ganglion have the character of transfer cells. Gunning *et al.* (1970) give as definition of transfer cells: plant cells with wall ingrowths thereby increasing the area over which the plasma membrane is in contact with the extra-cytoplasmic environment and specialized in relation to short distance transport of solutes.

1.4.2. Cell wall thickening during ageing

In the literature, no studies on cell wall thickening in the nodal structure of bamboos were found. This study reports that cell wall thickening does not significantly take place during ageing. In the diaphragm, cells with thinner walls are present than in the outer part of the node where the cell wall thicknesses are similar to those of the internode. The latter can be explained by the fact that these fibres probably surround vascular tissue passing directly from one internode to another and as a result have similar cell wall thicknesses as in the adjacent internodes.

Not only thinner cell walls but also wider cell diameters were found in the diaphragm. Both elements indicate that the density is lower in the diaphragm. This can be related to the fact that nodes store energy to elastically restore stems after bending forces are removed; thick cell walls and high density would make the diaphragm too stiff to have this feature.

1.4.3. Lignin distribution and lignification during ageing

Lybeer & Koch (2005a) characterized UV-spectra of *P. viridiglaucescens* internodes as having an absorbance shoulder between 310-320 nm and a peak at 280-282 nm. The presence of a shoulder between 310-320 nm can be linked to the presence of *p*-coumaroylation as demonstrated by Higuchi (1987). An absorbance peak at 280-282 nm indicates the presence of the strong absorbing guaiacyl lignin (Fergus & Goring 1970; Musha & Goring 1975).

Epidermis, hypodermis and fibre cell wall layers at the outer part of the node have similar spectra and absorbance values of cell wall layers of fibres at the outer culm side of the internode (Lybeer & Koch 2005a). Only the UV-spectra of parenchyma and fibre cell wall layers of the diaphragm are different. In *P. viridiglaucescens* internodes, ferulic and *p*-coumaric acids are widely distributed and their content is dependent on the anatomical location and the differentiation phase. The epidermis cells and compound middle lamellae of fibres and parenchyma cells of bamboo internodes have high absorbance values (maxima) for the wavelengths related with these esters. Younger cell walls have higher ratios of $abs_{310\ nm}$ / $abs_{280\ nm}$ than cell walls in older culms (Lybeer & Koch 2005a). In the nodes, hydroxycinnamic acids are even more widespread as they do not only occur in epidermis cells and compound middle lamella, but also in parenchyma and fibre cell wall layers of the diaphragm of older culms. Hydroxycinnamic acid moieties cause the cross-linkage of cell wall polysaccharides and participate with lignin to generate polysaccharide-lignin complexes, which lead to an increase in wall rigidity (Ishii 1997; Morrison *et al.* 1998).

Hydroxycinnamic acids thus have an important effect on the wall mechanical properties. Ferulic acid esters are suggested as lignin initiation sites and direct cell wall cross-linking during plant growth and development. The role of *p*-coumaric acid occurs later and serves to bind together the growing predominantly syringyl lignin polymer (Morrison *et al.* 1998). Morrison *et al.* (1998) studying the cell wall composition of maize internodes of varying maturity concluded that rind tissue (cuticle, epidermis, xylem elements, and phloem) of maize generally has greater ferulic acid and *p*-coumaric acid ester concentrations than pith tissue (parenchyma cells and randomly distributed vascular strands). As ferulic acid esters are suggested as lignin initiation sites and direct cell-wall cross linking during plant growth and development this was expected because rind vascular tissues lignify to a greater extent to support conductive and supportive tissue of the internode. Differences between pith and rind concentrations of ferulic acid esters diminished as internode tissues further differentiated and added cell wall components. Nodes have an important mechanical function for the bamboo culm as they act as braces to resist wall invagination or buckling and as they increase the effective stiffness of stems (Niklas 1997, 1998). This could be a possible explanation for the higher presence of hydroxycinnamic acid moieties in the nodal diaphragm as hydroxycinnamic acids increase the wall rigidity due to cross-linking with cell wall polysaccharides. This increase in wall rigidity by generating polysaccharide-lignin complexes perhaps increases the stiffness more than the deposition of guaiacyl units with lower linking. Furthermore, as thick cell walls and high density would make the diaphragm too stiff to elastically restore stems after bending forces are removed, the cell walls themselves need to give enough support to resist to mechanical stresses.

He & Terashima (1991) found a shift from 280 nm toward longer wavelengths and from 310-320 nm toward shorter wavelengths with the progress of lignification (i.e. at a later differentiation stages) in the cell corners of fibres in rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.). They worked out that this shift is due to the increase in the total content of hydroxycinnamic acid esters. A similar shift from 280-282 nm toward longer wavelengths could be observed in the parenchyma cells of the diaphragm but not in other cells.

There is a clear difference in lignin content between cells of shoots and of older culms. This difference is especially clear in the fibre and parenchyma cells of the diaphragm, which illustrates that the lignification process starts at the outside and proceeds towards the centre of the diaphragm. In the epidermis and hypodermis cells, shoots with culm sheath have lower lignin content than shoots without culm sheath. This is probably because the hard structure of the culm sheath protects the weak, less lignified underlying structures. Significant differences in lignin content between 6-month old, 12-month old and 9-year old culm could not be observed. Lybeer & Koch (2005) demonstrated the same situation in internodes of *P. viridiglaucescens*. Their results are in contrast with the conclusions of several authors (Murphy & Alvin 1997a; Lin *et al.* 2002) but agree with the findings of Itoh (1990) who stated that lignification is completed within one growing season. However, the authors mention that it is important to keep in mind that the spectra represent only one of several layers of a cell wall. It could be possible that lignification is completed within one cell wall layer while the fibre retains the ability to form new layers and lignify them additionally. The scanning profiles showed a lamellar structure of fibre cells with a decreasing lignin content towards the cell lumen.

The xylem transfer cell wall has low lignin content. This is in contrast with most dicotyledons (Musha & Goring 1975; Saka & Goring 1988) and the monocotyledon *Triticum aestivum* L. (Donaldson *et al.* 2001) but agrees with the findings of a low lignin content in bamboo vessel cell walls in the internode (Lybeer & Koch 2005a). Near the pits there is only low lignin content.

1.5. Conclusion

Nodes are not only important for the lateral transport of water and nutrients within the culm and between the culm, the branches and the leaves but they also have a major mechanical function. During development, the hard culm sheath protects the weak, non-lignified structures. In a fully elongated stem, the nodal structure can act as a spring-like joint to support the culm by bending forces as is demonstrated by Niklas (1998). This function is reflected in its anatomy. The combination between on the one hand larger diameters and thinner cell walls and on the other hand presence of more hydroxycinnamic acid moieties in the diaphragm makes it a flexible but strong structure.

2. Concluding discussion - Comparison between cell wall thickness and lignification in bamboo nodes and internodes (chapter II & III)

The lignification and cell wall thickening of bamboo nodes is studied in *Phyllostachys* species. Because of the different structure of the node in comparison with the internode, the nodal structure can not be divided into an outer, middle and inner part of the culm wall. The outer part of the node can be compared to the outer part of the culm wall of the internode. The middle and inner part of the culm wall of the internode are not present in the nodal structure but instead a diaphragm is present. This diaphragm originates from the central cells and provide the transversal connection of the conductive tissue.

The lignification in the nodes proceeds as in the internodes: first at the outside of the culm (epidermis, hypodermis and fibre wall layers at the outer part of the culm wall). In the diaphragm, as in the internodes the lignification of the fibre wall layers precedes that of the parenchyma wall layers.

The topochemical structure of the lignin is different in the diaphragm: more hydroxycinnamic acids (higher ratio abs_{312nm} / abs_{280nm}) are present, leading to an increase in wall rigidity (Fig. 4-6). The topochemical structure of fibre wall layers at the outer part of the node is comparable to that of the fibre cells at the outer part of the culm wall of internodes (Fig. 4-7).

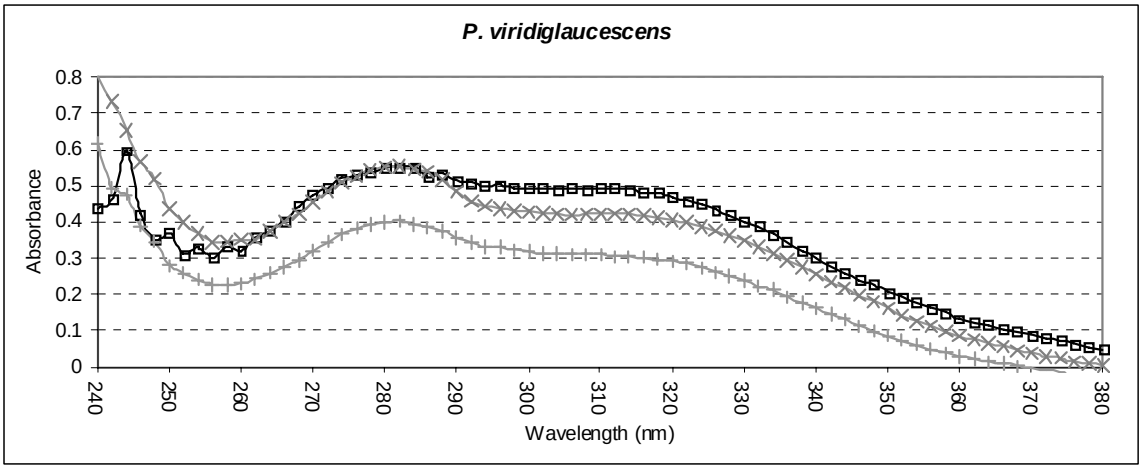


Figure 4-6. UV-absorbance spectra of a 12-month old *P. viridiglaucescens* culm comparing UV-spectra of a fibre wall layer in the diaphragm of the node (L_x dia) with UV-spectra of fibre wall layers of early (L_x xyin) and late (L_x xyout) maturing fibres at the middle of the culm wall of the 6th internode.

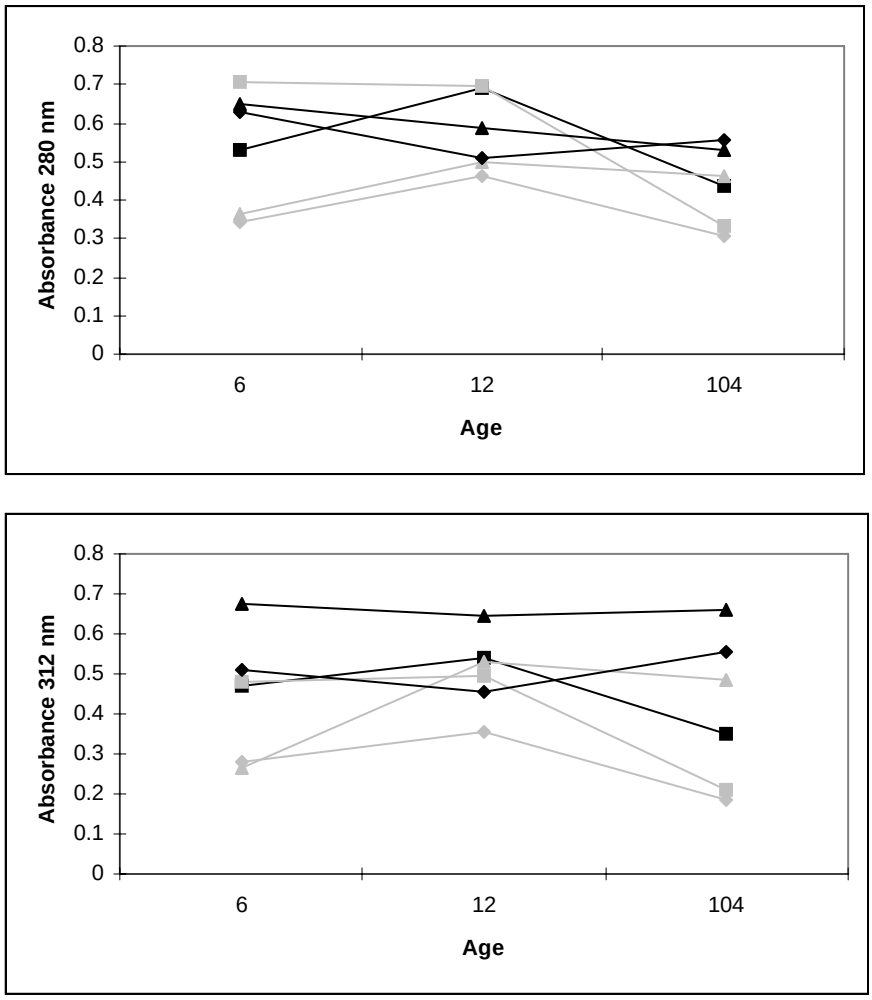


Figure 4-7. Absorbance values of *P. viridiglaucescens* culm of different ages at 280 and 312 nm of wall layers of parenchyma and fibre cells at the node and internode of the same culm. fibre out: fibre layer absorbance values of the outer part of the culm wall. par: parenchyma wall layer absorbance values of the diaphragm or of the ground parenchyma in the middle part of the culm wall. fibre: fibre wall layer absorbance values of the diaphragm or mean fibre wall layer absorbance of late and early maturing fibres of the middle part of the culm wall.

Bambang (1996) reported that the Klason lignin content of the nodal portion is higher than that of the internodal portion. Figure 4-7 compares absorbance values at 280 and 312 nm of parenchyma and fibre walls in the node (between internode 6 and 7) and internode (internode 6) of *P. viridiglaucescens* culms. Only the absorbance values at 280 nm of fibre wall layers at the outer part of the culm wall are comparable for both. In all other cases the absorbance values are higher in cell wall layers of nodal cells indicating higher lignin content.

In the diaphragm, thinner cell walls and cells with higher diameter are found compared to the cell walls and cell diameters of the corresponding cells in the internodal structure (Fig. 4-8). The combination of higher diameters and thinner cell walls indicate that the density will be lower in the nodal diaphragm. This makes them probably less stiff than internodal structures. It is hypothized that the combination of lower density and higher hydroxycinnamic acids induces the nodal diaphragm to become a flexible but strong structure, which can resist to higher bending stresses.

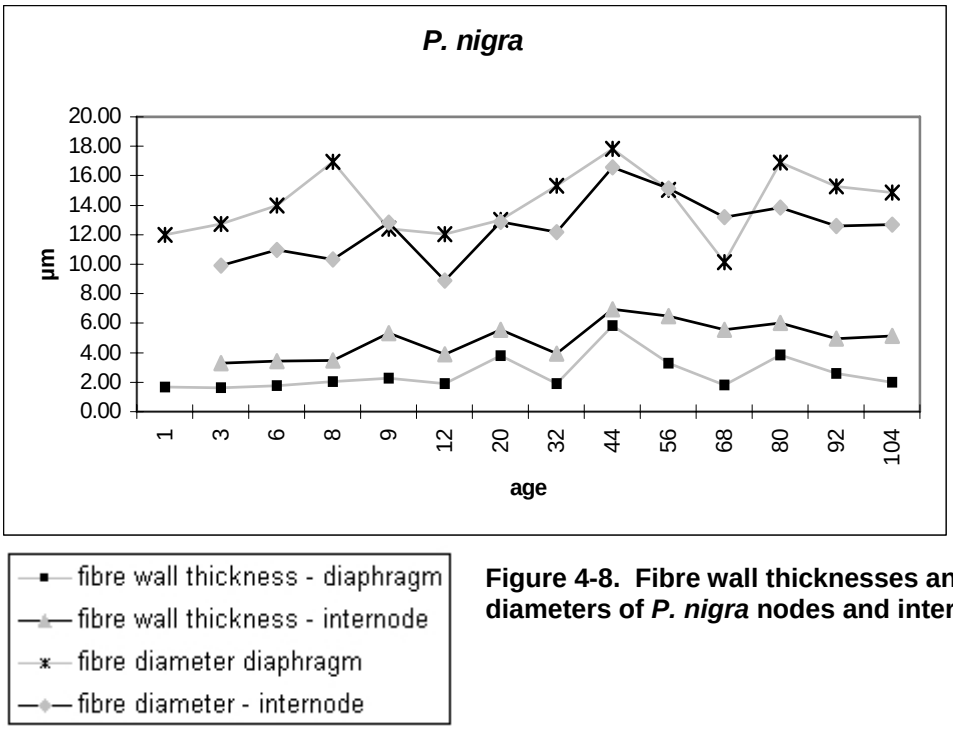


Figure 4-8. Fibre wall thicknesses and diameters of *P. nigra* nodes and internodes.

The fibre and parenchyma cell wall thickness in the node does not significantly increase with age. The lignin content is only significantly lower in the nodal cell walls of shoots. This is in agreement with the conclusion that both lignification and cell wall thickening of fibre and parenchyma cell walls in the internode mainly take place during the first year of growth. It has to be noticed that further thickening and lignification probably can take place in cell walls of older bamboo culms by further deposition and subsequently lignification of cell wall layers.

As no literature on cell wall thickening or lignification in bamboo nodes was found, it is impossible to discuss our results with conclusions from other authors.

CHAPTER V

SILICA CONTENT AND DISTRIBUTION IN SOME WOODY BAMBOO CULMS

CHAPTER V

SILICA CONTENT AND DISTRIBUTION IN SOME WOODY BAMBOO CULMS[5]

Summary

The overall silica content and distribution of bamboo culms from different ages both from temperate (*Phyllostachys viridiglaucescens* and *Phyllostachys nigra*) and tropical (*Gigantochloa levis* and *Dendrocalamus asper*) species was analysed. Regardless of diameter class, height and age, silica accounts for 0.04 to 0.11 % dry weight for *Phyllostachys* spp. and 0.08 to 0.11 % dry weight for *G. levis* and *D. asper* which is much lower than values mentioned in previous studies. Silica is concentrated on the outer epidermal layer. *Gigantochloa levis* and *Dendrocalamus asper* also contain, in contrast to the studied *Phyllostachys* spp., Si in the hypodermis cells. The EDX-maps showed an unequal distribution of Si in the epidermis of *Phyllostachys* spp. and higher Si concentrations in the outer epidermal cell wall.

[5] Adapted from:

Bieke Lybeer, Joris Van Acker, Paul Goetghebeur. Silica content and distribution in some woody bamboo culms. Submitted for publication in Journal of Bamboo and Rattan.

1. Introduction

Numerous studies have shown that grasses accumulate Si in their tissues, and the highest content of this element is deposited in leaves in a majority of the species studied (e.g. Metcalfe 1960; Motomura *et al.* 2004). Several authors have studied the silicon deposition in bamboo leaves and roots (Bennett & Sangster 1981; Lux *et al.* 2003; Motomura *et al.* 2000; Motomura *et al.* 2002; Motomura *et al.* 2004). In leaves, the foremost place of silicon deposition is the epidermis, with the highest concentration in silica cells. In bamboo roots, the deposition of Si is found only in endodermis cell walls. The content of Si on dry weight basis measured by gravimetric quantification was 7.6 % in leaves and 2.4 % in roots, respectively (Lux *et al.* 2003). Londoño & Kobayashi (1991) compared the silica bodies of the leaves between *Bambusa* and *Guadua* and concluded that size and distribution of the silica bodies rather than form can be used to distinguish both genera. Silica is considered to increase natural durability and strength (Sanyal *et al.* 1988) and to offer mechanical support (Lux *et al.* 2003). Previous studies showed that silica influences the cuticular transpiration and CO_2 uptake of plants (Kupfer & Kahnt 1992; Sakae *et al.* 1998). It also protects the plant from attacks of other organisms (Samuels *et al.* 1993) as well as from unfavourable climatic conditions, e.g. frost (Larcher *et al.* 1991). Indeed, silica though not considered an essential element is very important to the plant. However, one important element to the efficient processing of timber as well as non-timber materials is the abundance of silica within its structure. Amorphous silica either as a cell wall encrustment or impregnation or as translucent bodies can cause severe dulling of tools, which impedes the processing of the materials. In timber, silica is present as silica bodies commonly associated with the parenchyma cells, fibres and tyloses of vessels (Furuno & Côté 1983). In bamboo, silica is considered to be major component of the culm epidermis and it is mainly found in the form of small silica cells (cells containing silicon dioxide crystals) associated with cork cells (Liese 1998). Schmitt *et al.* (2002) localized silica polymers as extracellular deposits within the wall of epidermal cells of bamboo culms (*Sasa palmata* and *Sinoarundinaria* spp.). Young internodes showed distinct silica deposition in epidermal wall regions underneath the cuticle. In the outer wall regions silica granular deposits were found in increasing amounts during maturation but were only present in inner wall regions in late developmental stages. Gritsch *et al.* (2004a) showed in a study on *Guadua angustifolia* that there is a large amount of silica cells concentrated on the outer epidermal layer but they also observed silica bodies embedded amongst fibres in the periphery of the vascular bundles and in the intercellular spaces between parenchyma cells in the ground tissue. According to their SEM-EDX observations the concentration of silica bodies in the middle part of the culm wall seemed to increase with maturity. However, the silica content was not quantified to prove these observations.

The aim of the present work was to examine the silica content and distribution within culms of *Phyllostachys nigra*, *Phyllostachys viridiglaucescens*, *Dendrocalamus asper* and *Gigantochloa levis*. Quantification of the silica content was based on the molybdenum blue method and the distribution was observed with a scanning electron microscope (SEM) coupled with X-ray microanalysis. Precise distributional knowledge of silica deposition and silica content in the culm could give a good estimate of its possible impact on the processing of the bamboo culm. This may in turn improve the processing techniques.

2. Materials and Methods

2.1. Plant material

Samples of culms from different ages of *Phyllostachys viridiglaucescens* (Carrière) A. et C. Rivière and *Phyllostachys nigra* (Loddiges ex Lindley) Munro were taken in the Bambuseraie in Prafrance, France. Culms in the first year of development and 2-year old culms were harvested in the Belgian National Botanical Garden, Meise (*P. viridiglaucescens*) and in the Ghent University Botanical Garden (*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 of emergence giving precise ageing (Table 5-1).

Table 5-1. Samples used for quantitative analysis. The samples marked by * were also studied by SEM-EDX.

Species	Origin	Age (in months)	Remarks
P. nigra	Ghent	3*, 9*, 12*	int 6
		1, 3, 9	int 6– only epidermis
		24*	int 2 up to int 22 and node 2/3 up to node 18/19
	Prafrance	8, 32*, 56*, 104*	int 6
		8, 32, 56, 104	int 6– only epidermis
P. viridiglaucescens	Meise	1, 3*, 9*, 12*	int 6
		1, 3, 9, 12	int 6– only epidermis
		24	int 2 up to int 22 and node 2/3 up to node 22/23
	Prafrance	8, 32, 56	int 6 – only epidermis
		8, 32*, 56*, 104*	int 6
G. levis	Real Quezon	8*, 21*, 40*	int 6
D. asper	Real Quezon	8*, 21*, 43*	int 6

2.2. Quantitative Si determination : molybdenum blue method

The silicon content was determined using the molybdenum blue method (Sulthoni 1989; Motomura 2000, 2002). Small bamboo blocks were cut from the middle part of the sampled internodes (Table 5-1). These blocks were grinded to bamboo powder. For some samples, powder of the epidermis was made (Table 5-1). Bamboo powder (0.5 g) was fused with 5 ml 10 % NaOH and pyrolysed at 700 °C during 4h. After cooling, the residue was solved in distilled H_2O and transferred in a volumetric flask of 250 ml. Then 5 ml HCl 12N was added and this mixture was diluted with H_2O until the grade mark. A 20 ml of this bamboo powder solution was transferred in a volumetric flask of 50 ml and 2 ml ammoniummolybdate reagent was added (5g $(NH_4)_6Mo_7O_{24}.4H_2O$ (ammoniumheptamolybdatetetrahydrate) solved in 50 ml water with 5 ml H_2SO_4 95-97 % and diluted until 100 ml with distilled water). After shaking, 2 ml tartaric acid solution, 5 ml H_2SO_4 10N and 2 ml reducing reagent (solve 30 g $Na_2S_2O_5$ and 3 g Na_2SO_3 in 200 ml distilled water; solve 0.5 g metol (p-methylaminofenolsulfate) in 25 ml distilled water; fuse both solutions and dilute until 250 ml with distilled water) were added. After diluting until 50 ml with distilled water, the absorbance at 828 nm was measured. The Si content was calculated on a dry weight basis by comparing the absorbance with silicon standard solutions. The SiO_2 content was calculated by multiplying the Si content with 2.139.

2.3. Microscopy and X-ray analysis

Air-dried bamboo samples were smoothened using razor blades. The samples were fixed on stubs using adhesive tape, and gold coated in a coater (Balzers SCD 030) before being examined under a SEM (FEI Quanta 200 F) equipped with an EDX (Energy dispersive X-ray microanalysis) system (Genesis 4000) at 20 kV.

3. Results

3.1. Si content in bamboo culms

Table 5-2 shows the mean values of percentage Si and SiO_2 on a dry weight basis for the different species and the different ages. Internode 6 of *P. nigra* and *P. viridiglaucescens* samples contain 0.06 to 0.11 % and 0.04 to 0.10 % Si on a dry weight basis, respectively. The tropical bamboo species *D. asper* and *G. levis* contain around 0.08 % Si and 0.08 to 0.11 % Si, respectively. The species are not significantly different (one-way ANOVA, P = 0.677) and within one species there is no significant difference between the different ages (one-way ANOVA, P >> 0.05). Furthermore, no significant difference between culms grown in Belgium and culms grown in France could be demonstrated (one-way ANOVA, P >> 0.05). Less than 1 % (r^2 = 0.017) of the variation in Si content can be explained by the variation in culm wall thickness. So, no significant correlation (P = 0.436) between culm wall thickness and percentage Si was observed in all species and ages.

Table 5-3 illustrates that the Si content is high in the epidermis in comparison to the Si content of the whole culm wall showing that the Si is concentrated here. When comparing the Si content of the nodes with that of the internodes (Fig. 5-1) it can be concluded that the content is slightly higher in the nodes. However, this is not true for all measured nodes and internodes. Therefore, the Si content is not significantly higher in the internodes (paired t-test, P = 0.054).

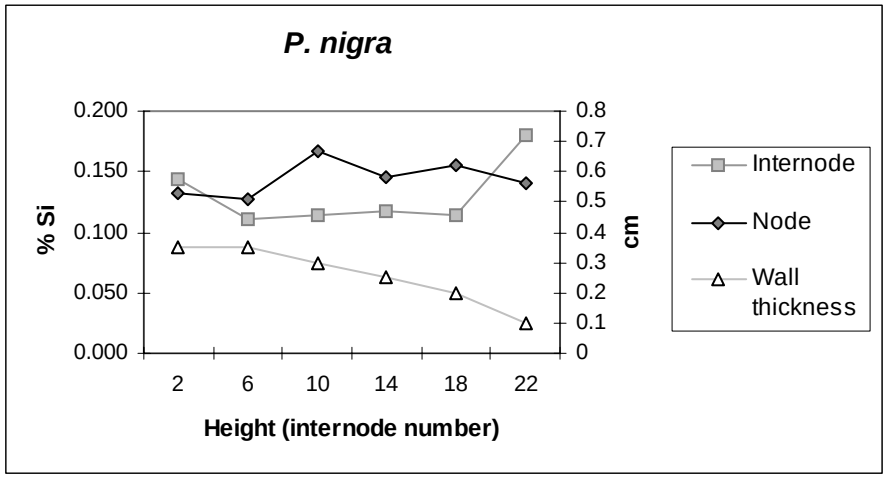
Table 5-2. Mean % Si and SiO_2 on a dry weight basis of internode 6 for the different species and the different ages

Species	Origin	Age (in months)	% Si	% SiO_2
P. nigra	Ghent	3	0.066	0.142
		9	0.078	0.167
		12	0.071	0.152
		24	0.110	0.235
	Prafrance	8	0.081	0.173
		32	0.086	0.185
		56	0.058	0.124
		104	0.104	0.223
P. viridiglaucescens	Meise	1	0.066	0.142
		3	0.042	0.089
		9	0.078	0.167
		12	0.061	0.131
		24	0.081	0.174
	Prafrance	8	0.081	0.173
		32	0.073	0.155
		56	0.098	0.209
		104	0.076	0.162
G. levis	Philippines	8	0.081	0.173
		21	0.078	0.166
		40	0.080	0.171
D. asper	Philippines	8	0.082	0.174
		21	0.082	0.174
		42	0.118	0.252

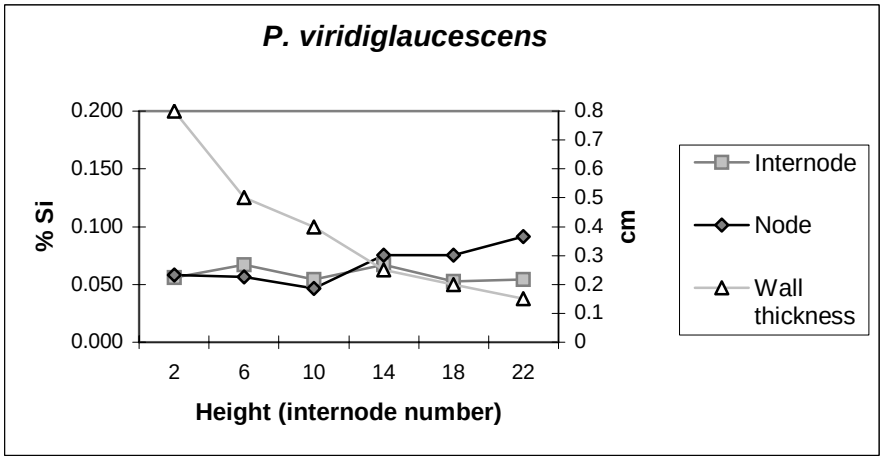
Table 5-3. % Si on a dry weight basis of internode 6 of the same *P. nigra* culms.

Origin	Age (in months)	only epidermis	whole culm wall
Ghent	3	0.085	0.066
	9	0.116	0.078
Prafrance	8	0.155	0.081
	32	0.233	0.086
	56	0.181	0.058
	104	0.163	0.104

Figure 5-1 illustrates that higher internodes have a smaller culm wall thickness. However, only in the culm of *P. nigra* the Si content is higher in the highest internodes.



A



B

Figure 5-1. Comparison between internode and node of a 24-month old culm of *P. nigra* and of *P. viridiglaucescens*.

3.2. Si distribution in bamboo culms

The culm walls of *P. nigra* observed using SEM-EDX displayed a concentration of Si in the epidermis (Fig. 5-2). Si mapping indicated unequal accumulation of Si in the epidermis; some parts of the epidermis have a high concentration of Si, some parts contain less silica and some cells do not include any Si at all. X-ray microanalysis of some cells of the epidermis showed a Si signal in the cell walls, with the highest values found in the outer epidermal cell wall of one cell (Fig. 5-2c, d).

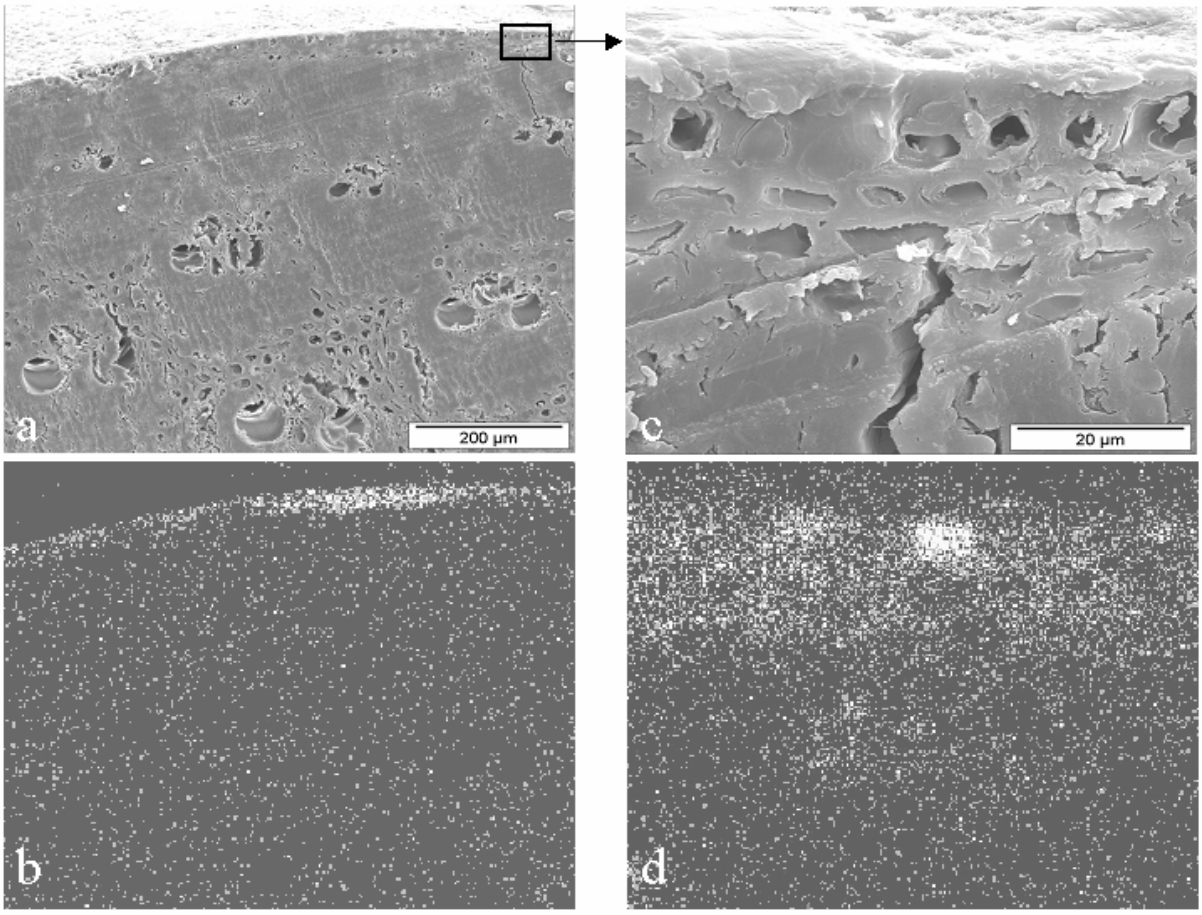


Figure 5-2. SEM - micrographs (above) and EDX - maps of the same area (below) of *P. nigra* (24-months old, internode 20); bright abundant white dots indicate the location of silicon. (a-b) Si is deposited in the epidermis and is not equally distributed. Some cells contain more Si than others and some cells do not contain any Si at all. (c-d) detail of the epidermis of (a-b) showing that Si is mainly concentrated in the outer epidermis cell wall.

Figure 5-3 shows SEM-micrographs and EDX-mappings of Si distribution in *P. viridiglaucescens* culms indicating that most cells do not contain a considerable amount of Si although some cells have higher Si concentration. Si is exclusively accumulated in the epidermal layer and no Si signal was detected in other tissues. No difference in Si concentrations was observed between culms of different ages (Fig. 5-3).

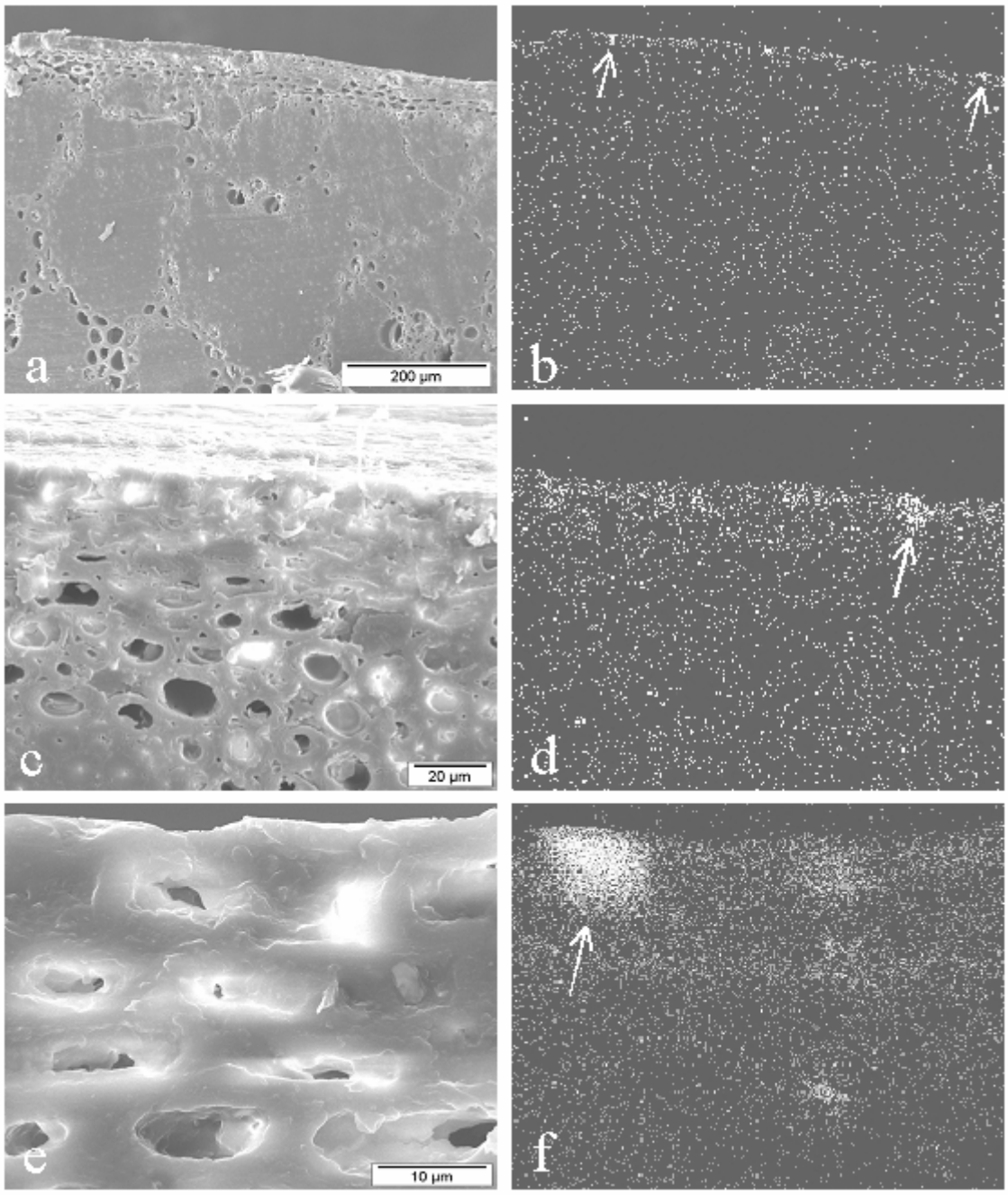


Figure 5-3. SEM - micrographs (left) and EDX - maps of the same area (right) of *P. viridiglaucescens*; bright abundant white dots indicate the location of silicon. (a-b) 9-months old, (c-d) 56-months old, (e-f) 3-months old. The arrows indicate silica cells in the epidermis. Si is not equally distributed. Some cells contain more Si than others and some cells do not contain any Si at all.

The tropical species *G. levis* and *D. asper* have Si deposited in both epidermis and hypodermis cells (Fig. 5-4 and 5-5). In contrast to *P. nigra* and *P. viridiglaucescens*, the Si mappings indicate a more equal distribution of Si with higher accumulation in epidermis cells than in hypodermis cells. The highest Si signal was detected in the outer epidermal cell wall as is illustrated in the EDX-mapping of Si in *D. asper* (Figure 5-5d).

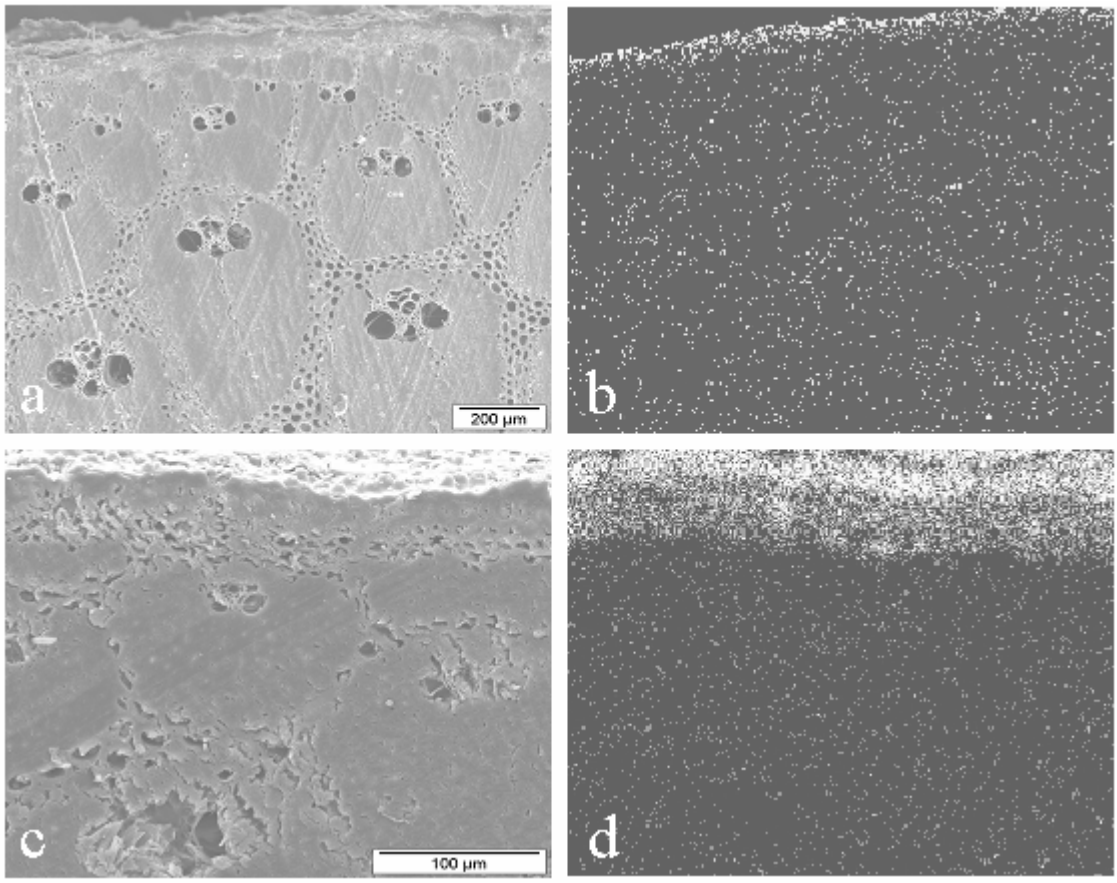


Figure 5-4. SEM - micrographs (left) and EDX - maps of the same area (right) of *G. levis*; bright abundant white dots indicate the location of silicon. (a-b) 21-months old, (c-d) 40-months old. Si is not only present in the epidermis cells, but also in the hypodermis cells and is equally distributed.

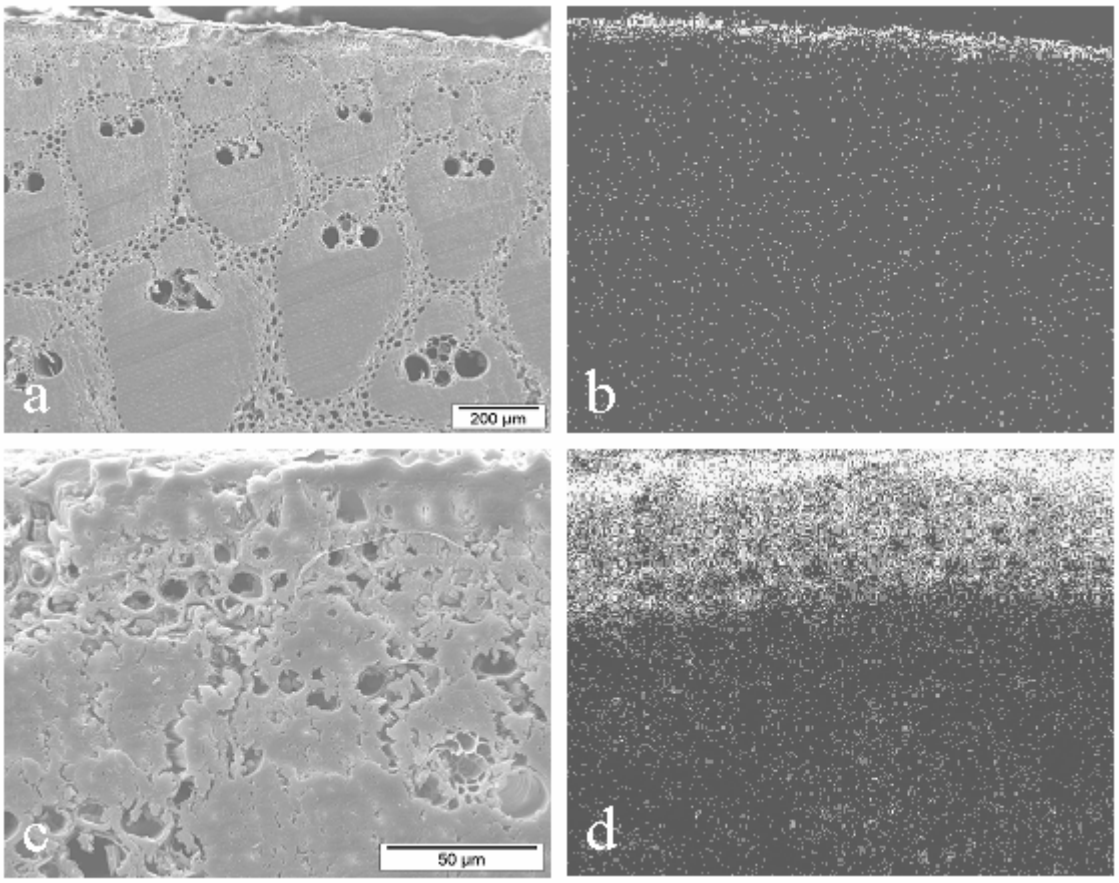


Figure 5-5. SEM - micrographs (left) and EDX - maps of the same area (right) of *D. asper* (8-months old); bright abundant white dots indicate the location of silicon. (a-b) Si is located in the epidermis and hypodermis cells and is equally distributed. (c-d) A detail of the epidermis and hypodermis cells showing that Si is mainly concentrated in the outer epidermis cell wall.

4. Discussion

Silica though not considered an essential element is very important to the plant. However, in the point of view of utilization, silica content over 0.3 % can reduce the production rate due to the frequent replacement of cutting tools (Haygreen & Bowyer 1987). However, Thibaut *et al.* (2004) give much lower values of 0.014 % silica by which there is no influence of Si on tool wear. In bamboo culms, silica is considered to be a major constituent of the epidermis with values between 1.5 % (*Bambusa vulgaris*) and 6.4 % (*Schizostachyum lumampao*) (Liese 1998). Ueda (1960) reported that in *Phyllostachys edulis* most silica was concentrated in the exodermis (4.20 – 5.10 %), with average for the whole culm at 0.29 - 0.33 %. Istas & Raekelboom (1962) mention values between 0.07 - 4.7 % for *Bambusa vulgaris*, between 0.59 – 1.83 % for *Oxytenanthera abyssinica*, around 0.16 % for *Arundinaria alpina* and between 0.19 – 0.49 % for *Gigantochloa*

aspera. Van Acker *et al.* (2000) reported values of 0.066 % in a lower part and 0.082 % in an upper part of *P. praecox* culms and values of 0.120 % in a lower part and 0.355 % in an upper part of *P. nigra* culms. The results of the present study indicate lower values between 0.04 and 0.11 % Si for *P. nigra* and *P. viridiglaucescens* and 0.08 to 0.11 % Si for the tropical species *G. levis* and *D. asper*. The molybdenum blue method, as applied in this study, can be used to detect small amounts of Si present (Bennett & Reed 1971). The minimal detectable concentration is about 1µg/g, which is much lower than the values obtained in this study (mg/g) and the study of Van Acker *et al.* (2000). So, it seems that the low values are not an artefact of the used technique. It is, however, impossible to compare the method used here with the methods used in the cited literature, as they do not mention which technique is applied to determine the silica content. More work is required on the Si content in bamboo species to understand the great differences between the values. It would be helpful to cross-check the values by using different methods (e.g. ICP-MS). Furthermore, environmental condition (e.g. soil) should be taken into account when comparing the Si content.

The EDX-maps showed that indeed there is a large amount on silica cells concentrated in the outer epidermal layer. The studied tropical species *G. levis* and *D. asper* also contain, in contrast to the studied *Phyllostachys* spp., Si is the hypodermis cells. So, it would be expected that the tropical species have higher Si content. Nevertheless, the measured values do not indicate a significantly higher Si percentage for the tropical species. It is not clear to what extent this can be an artefact of the used technique. According to the EDX-maps the amount Si measured is highly depending on the part used for the analysis as some parts of the epidermis contain hardly any Si at all. This might partly explain the differences measured between double samples of the same culm and between the values measured by Van Acker *et al.* (2000) for *P. nigra* and the values measured in this study for *P. nigra.*

Tamolang *et al.* (1980) reported increasing silica content toward the upper culm parts in tropical species. In this study a similar trend could not be observed, although it was expected as the upper internodes are smaller and have in volume percentage more epidermis than the lower culm parts. The unequal distribution of Si in the epidermis could partly explain this outcome. A significant difference between the Si content in the nodes and that of the internodes was not found but usually, the content was somewhat higher in the nodes. Only Liese (1998) mentioned that the culm tissue itself contains hardly any silica and the nodes only small amounts. However, it is not clear if the author means the whole node or only the nodal tissue without the epidermis. The latter would explain the somewhat higher Si content of the nodes as observed here.

Schmitt *et al.* (2002) localized silica polymers as extracellular deposits within the wall of epidermal cells of bamboo culms (*Sasa palmata* and *Sinoarundinaria* spp.). Young internodes showed distinct silica deposition in epidermal wall regions underneath the cuticle. In the outer wall regions silica granular deposits were found in increasing amounts during maturation but were only present in inner wall regions in late development stages. The EDX-maps show that higher concentrations can be found in the outer epidermal cell wall. The studied culms all correspond to the late developmental stages as studied by Schmitt *et al.* (2002).

Gritsch *et al.* (2004a) showed in a study on *Guadua angustifolia* that there is a large amount on silica cells concentrated on the outer epidermal layer but they also observed silica bodies embedded amongst fibres in the periphery of the vascular bundles and in the intercellular spaces between parenchyma cells in the ground tissue. According to their SEM-EDX observations the concentration of silica bodies in the middle of the culm wall seemed to increase with maturity. However, the silica content was not quantified to prove these observations. As no silica bodies are present in the middle culm wall of the species studied here, their concentration cannot increase. In *Gigantochloa scortechinii*, the silica content increased from 2.1 % in the 1-year old culm to 2.6 % (2-year old) and 3 % in 3-year old culms (Yussof *et al.* 1994). Such an increasing trend with age could not be determined in this study.

5. Conclusion

Haygreen & Bowyer (1987) state that silica content higher than 0.3 % can reduce the production rate due to the continuous replacement of cutting tools. However, Thibaut *et al.* (2004) give much lower values of 0.014 % silica by which there is no influence of Si on tool wears. In contrast to Liese (1998) who mentions that in bamboo culms the silica content amounts up to 5% and more depending on species and affects the cutting and pulping properties, this study concludes that the Si content is less than 0.3 % (0.04 to 0.11 % Si for *Phyllostachys* spp. and 0.08 to 0.11 % Si for the tropical species *G. levis* and *D. asper*). In terms of utilization, the Si should have no influence on the production rate of the studied species. Furthermore, as it is only located in the epidermis and hypodermis, removing the outer layers should solve the problem.

CHAPTER VI

GENERAL CONCLUSIONS

CHAPTER VI

GENERAL CONCLUSIONS

In this final part conclusions on the studied age-related anatomical aspects of bamboos are formulated and discussed in respect to the use of bamboo in the wood processing industry. The chapter is finalized with perspectives for future research.

1. Comparison between temperate and tropical bamboo internode anatomy

Although the overall anatomy of woody bamboos is similar to a typical monocotyledonous atactostele, variation between different bamboos exists. Between the studied temperate and tropical species the main difference exists in the vascular bundle types. *Phyllostachys* species are characterized by type I vascular bundles while the species *Gigantochloa levis* and *Dendrocalamus asper* are distinguished by type III and IV vascular bundles. Each vascular bundle has early maturing fibres close to the vascular tissue and late maturing fibres. The late maturing fibres of *Phyllostachys* species are defined as fibres adjacent to the parenchyma cells and the late maturing fibres of *G. levis* and *D. asper* are defined as fibres of free fibre strands. The boundary between early and late maturing fibres is more difficult to indicate in *Phyllostachys* species than in *G. levis* and *D. asper* as they do not occur in a separate fibre cap.

The lignin distribution and structural variation was studied in detail for *P. viridiglaucescens* and *G. levis*. Nevertheless, the lignin distribution and topochemical variation of these species can be interpreted as representative for other species with type I and type III and IV vascular bundles as well.

The lignification starts at the outside of the culm wall. Lignin in the epidermis cell wall is deposited early during cell development and does not increase with age. The lignification of the hypodermis cell wall soon follows. In contrast to other cell types, the cell wall layers of the epidermis and hypodermis have a higher amount of hydroxycinnamic acids in *P. viridiglaucescens* than in *G. levis.* The lignification proceeds towards the inner part of the culm wall.

The fibres at the outer part of the culm wall, under the hypodermis are early maturing in both species. Their cell wall layers have UV-spectra with a pronounced guaiacyl peak and a shoulder at 310-312 nm indicating the presence of hydroxycinnamic acids, typical for grass lignin. The shoulder at 310-312 nm is clearer in *G. levis* culms.

Conclusions

The early maturing fibres close to the vascular tissue lignify earlier than the late maturing fibres being fibres adjacent to the parenchyma cells in *P. viridiglaucescens* and fibres of free fibre strands in *G. levis*. Higher content of hydroxycinnamic acids are observed in fibre wall layers of *G. levis* culm. This higher content is more conspicuous in late maturing fibres than in early maturing fibres.

The vessel wall has low lignin content in both bamboo species. The function of the early maturing fibres adjacent to the vessels is probably to reduce the susceptibility of the vascular tissue to collapse.

The lignin concentration in the cell corners and compound middle lamella is higher than in the layers of the secondary wall. From the lignification in young *P. viridiglaucescens* samples it is clear that lignification first takes place here and then proceeds into the secondary wall of the fibres.

All fibres have a lamellar structure with decreasing lignin content toward the cell lumen. The small layers have higher lignin content as is observed under the TEM but those could not be displayed in the scanning profiles.

Parenchyma cells lignify later than fibre cells. They also show a lamellar structure with an increasing lignin content from the lumen towards the middle lamella. Two types of parenchyma cells are present in bamboos: thick-walled and thin-walled cells. Some thin-walled parenchyma cells are filled with phenolic extractives. The cell corners of the thin-walled parenchyma cells are only slightly lignified in contrast to the cell wall adjacent to a cell wall of a thick parenchyma cell. In *G. levis* no difference in lignin content between parenchyma cells close to the lacuna and parenchyma cells still located in the inner culm wall but further away from the lacuna was observed. That was not expected as the parenchyma cells bordering the lacuna have a different cell shape, are often sclerified and must give support at the inner side of the culm wall.

A higher content of hydroxycinnamic acid esters is found in *G. levis* than in *P. viridiglaucescens*. The higher amount is mainly observed in the parenchyma wall layers at the inner part of the culm wall and the fibre wall layers of late maturing fibres (fibres of free fibre strands). Higher hydroxycinnamic acid esters are found in young (1- to 6-months old) compound middle lamella, young (3-months old) parenchyma cells and in young late maturing fibres of *P. viridiglaucescens* samples. In *P. viridiglaucescens*, in contrast to *G. levis*, the difference in hydroxycinnamic acid content between early and late maturing fibres disappears in culms of 6- to 12-months old. Hydroxycinnamic acid moieties cause the cross-linkage of cell wall polysaccharides and participate with lignin to generate polysaccharide-lignin complexes, which lead to an increase in wall rigidity (Ishii 1997; Morrison *et al.* 1998). They thus have an important effect on the wall mechanical properties. It is hypothized that culms of *P. viridiglaucescens* have to harden faster than culms of *G. levis*, the latter growing in a more stable climate.

The tropical bamboo species *G. levis* has higher lignin content than the temperate species *P. viridiglaucescens*.

No difference in lignin content between the different ages and between flowering and non-flowering culms of *G. levis* could be observed. In *P. viridiglaucescens* the lignin content of the fibre and parenchyma cell walls increases during the first year of growth but not during later years. An increase mainly in guaiacyl units in samples up to 6-months old was demonstrated. However, it is important to consider that the spectra represent only one of several layers of a cell wall. Possibly lignification is completed within one cell wall layer while the fibre retains the ability to form new layers and lignify them additionally.

The study of cell wall thickening was carried out on *P. nigra*, *P. viridiglaucescens*, *G. levis* and *D. asper*. The observed trends on cell wall thickening can be considered representative for other species as well. It has to be noted that in this study the variability between and within culms was taken into account. In most previous studies (e.g. Liese & Weiner 1996; Gritsch & Murphy 2005), the authors compared mean cell wall thicknesses.

Bamboo cell wall ultrastructure was studied in young *P. viridiglaucescens* samples and older *P. viridiglaucescens* and *G. levis* samples. An explanation for the difficulties with impregnation and sectioning is given. Due to the difference in structure between parenchyma cells and fibres folds are present in the sections. In this study the problem of incomplete impregnation was solved by dehydration of the samples with acetone and subsequent impregnation with the less viscous LR white™ resin. Probably, acetone removes the waxes from the cell walls and improves impregnation with LR white™ resin.

It is demonstrated that cell wall thickness does not significantly increase with age. Nevertheless, during the first year of growth a rising trend was observed. The high variability within one culm and between culms of the same age from one year on is partly masking a clear increased cell wall thickening at higher age. It is shown that culms of 44-months old (which represents almost 4 growing seasons) and older have thicker cell walls, suggesting that some kind of late cell wall maturing can take place within one culm. Gritsch *et al*. (2004b) demonstrated that mature 3-year old culms show an increased number of cells with nine or more layers in comparison with a 1-year old culm. So, thickening of the cell wall is due to deposition of additional cell wall layers as is also demonstrated in the TEM photographs.

Conclusions

The TEM photographs clearly show the high heterogeneity present as well in number as in thickness of cell wall layers. This variability is present in cell wall layers of both temperate bamboos with type I vascular bundles and tropical bamboos with type III and IV vascular bundles. This study is the first to reveal that the cell wall ultrastructure of early maturing fibres adjacent to the conductive tissues 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 type III an 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 type III an IV vascular bundles have a thinner cell wall with fewer cell wall layers.

The parenchyma cell wall is composed of different smaller layers with alternating lignin content. The number and thickness of layers is variable.

In summary, the main difference between bamboo species with type I vascular bundles and bamboo species with type III and IV vascular bundles is observed in the late maturing fibres. This seems logical as the early maturing fibres of both types have the same function: to protect the conductive tissue and the outer part of the culm wall as soon as possible. There is more opportunity for differences between the late maturing fibres. They provide strength to the bamboo culm in general, but less to the most important parts of the culm. The differences are reflected in the lignification and ultrastructure.

The overall silica content and distribution of bamboo culms from different ages both from temperate (*P. viridiglaucescens* and *P. nigra*) and tropical (*G. levis* and *D. asper*) species was analysed. The EDX-maps showed an unequal distribution of Si in the epidermis of *Phyllostachys* spp. and higher Si concentrations in the outer epidermal cell wall. *G. levis* and *D. asper* contain, in contrast to the studied *Phyllostachys* spp., also Si in the hypodermis cells. Regardless of diameter class, height and age, silica accounts for 0.04 to 0.11 % dry weight for *Phyllostachys* spp. and 0.08 to 0.11 % dry weight for *G. levis* and *D. asper* which is much lower than values mentioned in previous studies.

2. Comparison between the anatomy of the internode and the node

The node has always been neglected in the study of bamboo anatomy. This report is the first to present results on topochemical lignin composition, lignin distribution and cell wall thickening in bamboo nodes. Both lignification and cell wall thickening are studied for *Phyllostachys* species. Because of the different structure of the node it can not be divided into an outer, middle and inner part of the culm wall, contrary to the internode. The outer part of the nodal structure can be compared to the outer part of the culm wall of the internode. The middle and inner part of the culm wall of the internode are not present but instead a diaphragm is observed in the nodal structure. This diaphragm originates from the central cells and provides the transversal connection of the conductive tissue.

The lignification in the nodes proceeds as in the internodes: first at the outside of the culm (epidermis, hypodermis and fibre wall layers at the outer part of the culm wall). In the diaphragm the lignification of the fibre wall layers precedes that of the parenchyma wall layers.

The absorbance values at 280 nm of fibre wall layers at the outer part of the culm wall are comparable in nodes and internodes. However, the spectra of fibre wall layers at the outer part of the node have higher absorbance values at 310-312 nm, indicating higher content of hydroxycinnamic acids. The absorbance values at 280 and 310 nm are higher in cell wall layers of fibres and parenchyma cells of the diaphragm compared to absorbance values of early and late maturing fibres at the middle and inner part of the internode of the same culm. This indicates a higher lignin content in the node than in the internode.

The topochemical structure of the lignin is different in the diaphragm: more hydroxycinnamic acids, leading to an increase in wall rigidity are present in fibre and parenchyma cell wall layers.

The lignin content is only significantly lower in the nodal cell walls of shoots. This is in agreement with the conclusion that both lignification and cell wall thickening of fibre and parenchyma cell walls in the internode mainly take place during the first year of growth. It has to be noticed that further thickening and lignification probably can take place in cell walls of older bamboo culms by further deposition and subsequently lignification of cell wall layers.

In the diaphragm, thinner cell walls and cells with wider diameter are found compared to the cell walls and cell diameters of the corresponding cells in the internodal structure. The combination of higher diameters and thinner cell walls indicate that the density will be lower in the nodal diaphragm. This makes them probably less stiff than internodal structures. The fibre and parenchyma cell wall thickness in the node does not significantly increase with age.

It is hypothized that the combination of lower density and higher lignin content with more hydroxycinnamic acids induces the nodal diaphragm to become a flexible but strong structure which can resist to high bending stresses.

A significant difference between the Si content in the nodes and that of the internodes was not found but usually the content was somewhat higher in the nodes. Only Liese (1998) mentioned that the culm tissue itself contains hardly any silica and the nodes only small amounts. However, it is not clear whether the author means the whole node or only the nodal tissue without the epidermis. The latter would explain the somewhat higher Si content of the nodes as observed here.

3. Effect of these results for industrial use

It can be concluded that both lignification and cell wall thickening of fibre and parenchyma walls of internodes and nodes mainly take place during the first year of growth. Probably, further thickening and lignification can take place in cell walls of older bamboo culms but it was impossible to detect a significant increase for a population of culms. This can be attributed to the high variability present within one culm and even within one fibre cap. It is possible that cells, which still have a cell lumen further deposit and subsequently lignify cell wall layers. It is, however, unlikely that this will have a major impact on the overall proportion of cell walls or lignin content.

This conclusion has an impact on the use of bamboo for the panel industry: not only culms of 2- to 3-years old or older can be used (Lee *et al*. 1994; Bath 2003), but also younger culms. As harvesting of bamboo stands in Europe can take place covering the end of the growing season to early spring, from October to March (Gielis 2001), culms of at least 6-months old for which we demonstrated to be almost completely lignified are harvested. If, in addition, bamboo stands of several years old are harvested, a mix of younger and older culms would be offered and applied in the wood panel industry still reducing the possible effect of the younger culms. The conclusions formulated here relate to and are in agreement with the conclusions of Van Acker *et al*. (2000) that the impact factor of age on density and as a consequence on use is not present. These findings are especially true for the studies *Phyllostachys* species grown in Europe. As the sampled tropical culms came from a 4-years old plantation with non-mature bamboo plants, these results cannot be extrapolated to tropical bamboo species grown in the wild or in mature plantations. Furthermore, the findings relate to the use of bamboo species in the panel industry.

So, it is possible that the quality of the raw material is adequate for the use in the panel industry but is less favourable for the use as e.g. furniture. More studies on quality of accurately dated bamboo raw material with focus on end products are needed to validate the findings for different industrial uses.

Liese (1998) mentions that in bamboo culms the silica content amounts up to 5% and more depending on species thus affecting the cutting and pulping properties making bamboo less suitable for the wood processing industry. Haygreen & Bowyer (1987) state that silica content higher than 0.3 % can reduce the production rate due to the continuous replacement of cutting tools. However, Thibaut *et al*. (2004) give much lower values of 0.014 % silica by which there is no influence of Si on tool wears. This study concludes that the Si content is less than 0.3 % (0.04 to 0.11 % Si for *Phyllostachys* spp. and 0.08 to 0.11 % Si for the tropical species *G. levis* and *D. asper*). In terms of utilization, the Si should have little or no influence on the processing and tool wear for the species studied, especially because the Si is not present as crystals. Furthermore, as it is only located in the epidermis and hypodermis, removing the outer layers should also solve the problem. However, much more work is required on the Si content in bamboo species to understand the great differences between the Si content as measured in this study and in other studies.

4. Future research

In *P. viridiglaucescens* a difference in topochemical lignin composition between very young samples and older samples is present, with more hydroxycinnamic acids in young samples. From the literature (He & Terashima 1991; Azuma *et al.* 1996), it is known that immature stems are richer in ferulic acid. A shift from 310-320 nm toward shorter wavelengths related with these higher amount of ferulic acid was not observed in the UV-spectra of *P. viridiglaucescens* culms. Perhaps still younger more immature samples still surrounded by culm sheaths should be studied to observe this shift. Young, immature samples of other species with type I vascular bundles as well as species with other vascular bundle types should be studied to examine this difference in hydroxycinnamic acid content between young and older bamboo culms.

Nodes of species with other vascular bundle types should also be investigated to have a more complete picture on lignin distribution and composition in bamboo nodes.

More knowledge on lignin could not only be used to determine the optimal age for harvesting bamboo as raw material for the industry, but also to establish the optimal age for harvesting young edible shoots.

Furthermore, the actual lignin of bamboo internodes and nodes should be measured to obtain a complete view on lignin content in relation to age. A reliable and fast technique is NIR (Near Infrared Reflectance).

Suzuki & Itoh (2001) studied the lignification process in *Phyllostachys aurea* using rapid-freeze deep-etching electron microscopy to provide a 3D perspective on lignin deposition. They characterized the primary wall by having narrow spacing between the cellulose microfibrils in fibres. The secondary wall largely consisted of dense cellulose microfibrils also with narrow spacing. They suppose that the deposition of lignin proceeds in the pores between the cellulose microfibrils during maturation. The more porous carbohydrate matrix in the middle lamella and the primary wall allows greater lignin deposition by space filling than the more densely packed secondary cell wall. Whether the uneven distribution of lignin results entirely from differences in porosity of the carbohydrate matrix requires further investigation. Furthermore, the study of Suzuki & Itoh (2001) does not take into account the lamellar structure of the bamboo cell wall. As the narrow layers have higher lignin content than the broad layers, a possible more porous carbohydrate matrix in the narrow layers should be studied.

Donaldson (1991) studied the seasonal variations in lignification during one growth season in radiata pine. The number of lignifying cells reached a maximum during the summer suggesting that lignification is quite sensitive to environmental conditions such as temperature. Donaldson (2001) also mention that light and nutrient deficiency can lead to reduced lignification. To what extent environmental conditions have influence on the lignification and lignin content in bamboos has not been studied. In chapter II (lignification in bamboo internodes) it is stated that In *P. viridiglaucescens* the difference between UV-spectra of early and late maturing fibres disappears in culms of 6- to 12-months old whereas the difference in absorbance behaviour between early and late maturing fibres is still present in culms of *G. levis* of 40-months old. A possible explanation could be that culms of *P. viridiglaucescens* have to harden more quickly than culms of *G. levis*, growing in a more stable climate. However, more temperate as well as tropical species should be studied. The effect of environmental factors on the formation of cell wall layers should also be studied.

Bamboo culms and rhizomes respond to wounds in order to protect the surrounding tissues against damaging influences through the wound surfaces. The defence arsenal consists of a number of cellular reactions such as closure of sieve tubes by callose, formation of slime and tyloses, phenolics, suberised cell walls, wall lignification and also septa development in fibres. There is also accumulation and mobilization of starch around the wound, development of additional layers of the cell wall in parenchyma cells and fibres, and the formation of a suberin layer in vascular parenchyma cells (Weiner & Liese 1997). The UV-micrographs demonstrated that phenolic compounds are present in some short parenchyma cells. The technique could be used to study the wounding response of bamboo more profoundly, not only the formation of phenolic compounds but also the wall lignification during wounding.

Fibres of free fibre strands have a different ultrastructure and lignin composition than fibres of fibre caps adjacent to the vascular tissue. A study on the origin and early development of these free fibre strands in bamboos with bundle types III and IV is necessary to explain the observed differences between late maturing fibres of type I vascular bundles and late maturing fibres of type III and IV vascular bundles. Gritsch *et al.* (2004b) observed a degree of 'order' in the distribution of multilayered fibres within phloem caps of *Dendrocalamus asper* with multilayered cell walls common in fibres adjacent to the phloem elements and around the edge of the fibre cap. A similar study on fibres of free fibre strands would be useful to follow the pattern of cell wall layer deposition in these fibres.

Silica distribution is different in different species. Gritsch *et al.* (2004b) observed silica bodies embedded amongst fibres in the periphery of the vascular bundles and in the intercellular spaces between parenchyma cells in the ground tissue in *Guadua angustifolia*, indicating that much more species should be studied to obtain insight in the Si distributional variation present in bamboo culms. It has been shown that SEM-EDX is a powerful tool to study the silica distribution in bamboo culms.

More work is required on the Si content in bamboo species to understand the great differences between the values measured in this study and as known from the literature. It would be helpful to cross-check the values by using other methods (e.g. ICP-MS (Inductively coupled plasma atomic emission spectrometry)). Furthermore, environmental condition (e.g. soil) should be taken into account when comparing the Si content.

Next to these suggestions for further fundamental research, investigations to culm quality e.g. strength measurements should be carried out, not only in respect to bamboo as raw material for the panel industry but also in respect to the use of bamboo as solid material. Further studies on percentages of the different tissue is also of importance, not only in one part of the culm but in the whole culm. Furthermore, the mechanical properties of bamboo nodes, with a probable lower density in the diaphragm, should be evaluated.

SUMMARY

SAMENVATTING

SUMMARY

AGE-RELATED ANATOMICAL ASPECTS OF SOME TEMPERATE AND TROPICAL BAMBOO CULMS (POACEAE: BAMBUSOIDEAE)

Bamboo is a self-regenerating very fast growing plant with numerous traditional uses and many new products. In the "Bamboo for Europe" project (FAIR-CT96-1747, 1997-2000) it was shown that bamboo can be used as additional or alternative raw material for the wood processing industry and especially for the panel industry in Europe (Van Acker *et al*. 2000). As in wood, anatomical structures determine the quality of the product. Because fibre and parenchyma cells retain their living protoplasts and connections with neighbouring cells, structural modifications are possible during ageing of the culm (Liese & Weiner 1997; Murphy & Alvin 1997a; Liese 1998). The anatomical changes are cell wall thickening by deposition of additional layers (Alvin & Murphy 1988; Liese & Weiner 1996; Liese & Weiner 1997; Liese 1998) and progressive lignification (Fujii 1985; Kawase *et al*. 1986; Yoshizawa *et al*. 1991; Murphy & Alvin 1997) in fibre and parenchyma cells over several years. In contrast to these authors, Van Acker *et al.* (2000) could not clearly show an impact pattern of age on density. As culm density is correlated with cell wall thickness, no significant cell wall thickening should take place during later years. Similarly, Itoh (1990) and Abd. Latif *et al.* (1996) found that lignification is completed at the end of one growing season. These discussed structural modifications are very important for the strength of the culm (Murphy & Alvin 1992). They have a major impact on the applicability of different bamboo species and on the most suitable age for harvesting.

The present study concentrates upon these age-related changes in bamboo culms in consideration of industrial use. Aspects of lignification and cell wall thickening for which contradicting results are reported in the literature of internodal and nodal fibre and parenchyma cells are investigated. Furthermore, silica distribution and content was studied because more knowledge on the content and location of the silica cells could be useful for the application (Van Acker *et al*. 2000; Hamdan & Abd. Latif 1992).

Summary

A first objective was getting further insight into the deposition sequence and distribution of lignin structural units in different anatomical regions of bamboo culms during ageing. This was studied topochemically and semiquantitatively by means of UV-microspectrophotometry in *Phyllostachys viridiglaucescens* (Carrière) A. et C. Rivière and *Gigantochloa levis* (Blanco) Merrill. The study revealed that *p* - coumaric and ferulic acids are widely distributed in *P. viridiglaucescens* and that their content is dependent on the anatomical location and the differentiation phase. Hydroxycinnamic acids are even more widely distributed in *G. levis*. The early maturing fibres at the outer part of the culm wall and adjacent to the vascular tissue reveal a maximum absorbance at 280 nm (guaiacyl peak) whereas the late maturing fibres display a clearer shoulder at 310-320 nm (due to hydroxycinnamic acids). This is in contrast to *P. viridiglaucescens* where the late maturing fibres display a clear shoulder at 310-320 nm in young culms and a maximum guaiacyl peak in older culms. The compound middle lamellae show higher absorbance values and are richer in *p*-coumaric and ferulic acid esters in comparison to the layers of the secondary wall. The lignin in the epidermis cell wall is deposited early in the development and does not increase with age. This is in contrast with the fibres and the ground parenchyma cells where an increasing trend in lignification during the first year is shown. However, no difference in lignin content between 1-year old and older samples and between flowering and non-flowering culms could be observed. The secondary fibre wall has a lamellar structure with an increasing lignin content from the centre towards the compound middle lamella. The vessel walls have a low lignin content.

Second, 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 culms 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 one 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.

Summary

Because the anatomical structure and mechanical properties of the culm node is poorly documented in contrast to the culm internode, a third aim of this thesis was to investigate the lignification and cell wall thickening in developing and mature bamboo nodes. It deals with the deposition sequence and distribution of lignin structural units and cell wall thickening in the outer part of the node and in the diaphragm of *P. viridiglaucescens* and *P. nigra* samples. The lignification during ageing was studied topochemically by means of UV-microspectrophotometry.

A combination of light microscopy and image analysis was used to study the nodal structure and to measure cell wall thickness. The lignification of the node starts at the outside of the culm wall. The lignin content in the epidermis cell wall of young shoots is as high as in epidermis cell walls of 9-years old culms. In shoots, the lignin content of hypodermis and fibre cell walls at the outer part of the node are higher than the lignin content of fibre and parenchyma cells of the diaphragm. In older nodes, the lignin content values of the outer part of the node and of the diaphragm are more similar but the fibre and parenchyma cells of the diaphragm have higher values of *p*-coumaric and ferulic acids. The fibre and parenchyma cell wall thickness does not significantly increase during ageing. In the diaphragm, the cell walls are thinner and the cell diameter is higher than in the outer part of the node. It is hypothesized that thinner cell walls in combination with higher cell diameters and more hydroxycinnamic acids in the diaphragm play an important role in the biomechanical function of the node to act as a spring-like joint to support the culm by bending forces.

Finally, the silica distribution and content was studied in some both temperate (*Phyllostachys viridiglaucescens* and *Phyllostachys nigra*) and tropical (*Gigantochloa levis* and *Dendrocalamus asper*) bamboo species. Regardless of diameter class, height and age, silica accounts for 0.04 to 0.11 % dry weight for *Phyllostachys* spp. and 0.08 to 0.11 % dry weight for *G. levis* and *D. asper* which is much lower than values mentioned in previous studies. *G. levis* and *D. asper* also contain, in contrast to the studied *Phyllostachys* spp., Si in the hypodermis cells. The EDX-maps showed an unequal distribution of Si in the epidermis of *Phyllostachys* spp. and higher Si concentrations in the outer epidermal cell wall.

SAMENVATTING

LEEFTIJDSGERELATEERDE ANATOMISCHE ASPECTEN VAN ENKELE GEMATIGDE EN TROPISCHE BAMBOEHALMEN (POACEAE: BAMBUSOIDEAE)

Bamboes zijn zelf-regenererende zeer snel groeiende planten met talrijke traditionele gebruiken en geschikt voor allerlei nieuwe producten. In het '*Bamboo for Europe*' project (FAIR-CT96-1747, 1997-2000) werd aangetoond dat bamboe als bijkomende of alternatieve grondstof kan worden gebruikt in de Europese houtverwerkende industrie en meer bepaald in de plaatindustrie (Van Acker *et al*. 2000). Zoals in hout bepalen de anatomische structuren de kwaliteit van het afgewerkte product. Omdat vezels en parenchymcellen hun levende protoplast en hun verbinding met naburige cellen behouden, zijn structurele veranderingen tijdens het verouderingsproces van de halm mogelijk (Liese & Weiner 1997; Murphy & Alvin 1997a; Liese 1998). Deze anatomische veranderingen zijn celwandverdikking door afzetting van bijkomende lagen (Alvin & Murphy 1988; Liese & Weiner 1996; Liese & Weiner 1997; Liese 1998) en progressieve lignificatie in vezels en parenchymcellen (Fujii 1985; Kawase *et al*. 1986; Yoshizawa *et al*. 1991; Murphy & Alvin 1997). In tegenstelling tot deze auteurs konden Van Acker *et al*. (2000) geen duidelijke impact van de leeftijd op de densiteit aantonen. Aangezien densiteit gecorreleerd is met celwanddikte, zou er geen significante celwandverdikking plaatsvinden tijdens latere jaren. In overeenstemming hiermee, vonden Itoh (1990) en Abd. Latif *et al.* (1996) dat de lignificatie voltooid is in één groeiseizoen. Deze bediscussieerde structurele veranderingen hebben een grote invloed op de bruikbaarheid van de verschillende bamboesoorten (Murphy & Alvin 1992) en hun geschikte oogstleeftijd in functie van de toepassing.

Deze studie concentreert zich op leeftijdsgerelateerde veranderingen in bamboehalmen met het oog op gebruik ervan in de industrie. Aspecten van lignificatie en celwandverdikking, waarvoor tegenstrijdige resultaten gepubliceerd werden in de literatuur, van zowel internodale als nodale vezels en parenchymcellen werden onderzocht. Bovendien werd de verspreiding van en de hoeveelheid silicium bestudeerd. Meer kennis van de hoeveelheid silicium en de locatie van de silica cellen kan nuttig zijn voor het gebruik (Van Acker *et al*. 2000; Hamdan & Abd. Latif 1992).

Samenvatting

Een eerste doelstelling was beter inzicht krijgen in de afzettingsvolgorde en distributie van lignine-eenheden tijdens het verouderen in verschillende anatomische zones van bamboehalmen. Dit aspect werd topochemisch en semikwantitatief bestudeerd aan de hand van UV-microspectrofotometrie in *Phyllostachys viridiglaucescens* (Carrière) A. et C. Rivière en *Gigantochloa levis* (Blanco) Merrill. De studie toonde aan dat *p*-coumarine- en ferulazuur wijd verspeid zijn in *P. viridiglaucescens* en dat de hoeveelheid afhankelijk is van de anatomische locatie en differentiatiefase. Hydroxykaneelzuren zijn zelfs in hogere mate aanwezig in *G. levis*. De vroegrijpende vezels aan de buitenzijde van de halmwand en grenzend aan het vasculair weefsel tonen een maximum absorptie op 280 nm (guaiacyl piek) terwijl laatrijpende vezels een duidelijker schouder vertonen op 310-320 nm. Dit is in contrast met *P. viridiglaucescens* waar de laatrijpende vezels een duidelijke schouder hebben op 310-320 nm in jonge halmen en een maximum guaiacyl piek in oudere halmen. De samengestelde middenlamel heeft hogere absorptiewaarden en is rijker aan *p*-coumarine- en ferulazuuresters in vergelijking met de secundaire celwandlagen. De lignine in de epidermiscelwand wordt reeds vroeg in de ontwikkeling afgezet en de hoeveelheid stijgt niet met de leeftijd. In de vezels en het grondparenchym daarentegen is een stijgende trend in lignificatie tijdens het eerste jaar aangetoond. Een verschil in lignine-inhoud tussen één jaar oude halmen en halmen ouder dan 1 jaar en tussen bloeiende en niet-bloeiende halmen kon echter niet worden waargenomen. De secundaire vezelwand heeft een lamellaire opbouw met een stijgende lignine-inhoud van het centrum naar de samengestelde middenlamel. De vatwand heeft een lage lignine-inhoud.

Ten tweede probeerde deze studie de tegenstrijdige resultaten van vorige studies over celwandverdikking van bamboehalmen te verhelderen aan de hand van licht- en transmissie-electronenmicroscopie in combinatie met beeldanalyse. Zowel vezel- als parenchymwanddikte van gematigde (*Phyllostachys* spp.) en tropische (*Gigantochloa levis* en *Dendrocalamus asper*) bamboehalmen van verschillende leeftijd werden beschouwd met het oog op hun geschiktheid voor de houtindustrie. Een grote heterogeniteit in celwanddikte en celwandgelaagdheid van vezels in één halm werd waargenomen. '*Nested design ANOVA's*' toonden een stijgende trend in wanddikte van laatrijpende vezels en parenchymcellen tijdens het eerste jaar maar geen significante verdikking gedurende latere jaren. De hoge variabiliteit in één halm en tussen halmen van eenzelfde leeftijd maakt een duidelijke stijgende celwandverdikking op hogere leeftijd moeilijk vaststelbaar. Toch werden de hoogste waarden van vezelwanddikte gemeten in halmen van 44 maand oud of ouder wat suggereert dat een soort late rijping kan plaatsvinden in een halm.

Samenvatting

In vergelijking met het internodium zijn de anatomische structuur en mechanische eigenschappen van de knoop slecht gedocumenteerd. Een derde doelstelling was daarom de lignificatie en celwandverdikking in de knoop van ontwikkelende en rijpe bamboehalmen te onderzoeken. Dit deel handelt over afzettingsvolgorde en distributie van lignine-eenheden en celwandverdikking in het buitenste deel van de knoop en in het diafragma van *P. viridiglaucescens* en *P. nigra* stalen.

De lignificatie tijdens het verouderingsproces werd topochemisch onderzocht door gebruik te maken van UV-microspectrofotometrie. Een combinatie van lichtmicroscopie en beeldanalyse werd aangewend om de celwanddikte te meten. De lignificatie start aan de buitenzijde van de halm. De lignine-inhoud in de epidermiscelwand van jonge scheuten is even hoog als in 9 jaar oude halmen. In scheuten is de lignine-inhoud van de hypodermis en vezelcelwanden aan de buitenzijde van de knoop hoger dan de lignine-inhoud in vezel- en parenchymcellen van het diafragma. In oudere knopen is de lignine-inhoud van het buitenste deel van de knoop en het diafragma meer gelijk maar de vezel- en parenchymcellen van het diafragma hebben meer *p*-coumarine- en ferulazuuresters. De vezel- en parenchymcelwanddikte stijgt niet significant tijdens het verouderen. In het diafragma zijn de celwanden dunner en hebben de cellen een hogere celdiameter dan in het buitenste deel van de knoop. De hypothese wordt gesteld dat een combinatie van hogere celdiameter, lagere celwanddikte en meer hydroxykaneelzuren in het diafragma een belangrijke rol spelen in de biomechanische functie van de knoop om als op een veer lijkende verbindingsknoop te fungeren bij buigkrachten.

Als laatste doelstelling werd de silicadistributie en -inhoud van enkele gematigde (*Phyllostachys* spp.) en tropische (*G. levis* en *D. asper*) soorten bestudeerd. Onafhankelijk van diameterklasse, hoogte en leeftijd telt de silica voor 0.04 tot 0.11 % drooggewicht bij *Phyllostachys* spp. en voor 0.08 to 0.11 % drooggewicht bij *G. levis* and *D. asper* wat veel lager is dan waarden zoals vermeld in vorige studies. In tegenstelling tot de bestudeerde *Phyllostachys* spp. bevatten *G. levis* and *D. asper* Si in de hypodermiscellen. De EDX-kaarten tonen een ongelijke verdeling van Si in de epidermis van *Phyllostachys* spp. en hogere Si-concentraties in de buitenste epidermale lagen aan.

GLOSSARY

GLOSSARY

Adventitious root: Applied to roots arising from stem nodes to distinguish these from lateral roots.

Anastomosis: Cross-connection of vessels and sieve tubes in the diaphragm, union of one vascular bundle with another one.

Anticlinal division: Cell division at right angles, or perpendicular to the surface.

Apical meristem: Group of cells at the growing apices of shoot and root that divide to ultimately produce the primary plant body and maintain itself as a region of cell division.

Arm cell: Specialized leaf cell, characteristic of Bambusoideae and typically 'M'-shaped.

Atactostele: Stelar pattern characteristic of many monocotyledonous stems in which the vascular bundles appear scattered within the ground tissue.

Auricle: Small extensions of the leaf. Found at the junction of the blade and the sheath in grasses.

Bending strength: Characteristic of wood that enables it to sustain bending stresses without changing shape or breaking.

Bract: A (sometimes leaf-like) structure whose axillary bud is developed (e.g. into an inflorescence).

Bracteate: Bearing bracts.

Cell wall: The structurally compound enclosing a plant cell; in mature cells it consists ontogenetically of different superimposed layers.

Cellulose: A polymer composed of hundreds of glucose molecules linked in a linear chain and found in plant cell walls.

Chlorenchyma: Tissue composed of parenchyma cells containing chloroplasts.

Closed vascular bundle: Vascular bundle lacking a vascular cambium and thus lacking the potential for secondary growth.

Companion cell: Parenchymatous cell positioned next to a sieve tube element as a sister cell and arising by division of a sieve tube element mother cell.

Compound middle lamella: Compound layer between adjacent plant cells consisting of two primary walls and an intercellular layer (middle lamella).

Cortex: Outer part of a culm or rhizome, between the epidermis and the vascular tissue; composed predominantly of parenchyma.

Glossary

Compression strength: Characteristic of wood that enables it to sustain compression (occurs when wood is pushed inward along the grain at the ends to shorten the wood or reduce its volume) without changing shape or breaking.

Culm: The aerial axis emerging from buds of the subterranean system, divided into nodes and internodes.

Culm neck: The constricted basal part, characteristic of most of the segmented vegetative axes of a bamboo plant.

Culm sheath: A sheathing organ, which embraces the developing internode(s). The culm sheaths protect the plant parts that need mechanical protection. As soon as the growth has ended, they loose their function, dry and fall of or stay on the plant, depending on the species.

Cuticle: A thin, waxy layer of noncellular material that covers the outer surface of all parts of the primary shoot; consists primarily of cutin and wax.

Cutin: Biopolymer composed of fatty and hydroxy fatty acids that is a major chemical component of the cuticle.

Density: Expresses the weight (mass) of wood per cubic volume at a specific moisture content.

Diaphragm: A transversal tissue partition of the culm at the node, containing intensive interconnections of vessels and sieve tubes. A rigid structure that lends strength to the segmented axis of bamboos.

Distich: Parts arranged alternatively in two rows one on each opposite side as in grass leaves and the florets within the spikelet.

Epidermis: The outermost layer of a culm or rhizome, often with thickened and cutinised outer wall; usually consisting of one cell layer.

Fibre: A long, narrow usually thick-walled and lignified cell, providing mechanical support for the culm as fibre sheath or bundle.

Fibre bundle: See fibre sheath.

Fibre cap: See fibre sheath.

Fusoid cell: Large central cells in the leaves of Bambusoideae, on either side of the vascular bundle and surrounded by ‘arm cells’, the latter typically ‘M’-shaped.

Fibre sheath: A group of fibres that forms a part of the vascular bundle (free or attached to the protoxylem, metaxylem and phloem) (= fibre cap, fibre bundle, sclerenchyma sheath).

Glume: Bracts at the base of a spikelet (usually two) which do not themselves contain florets.

Gregarious flowering: Applied commonly to bamboos when many individuals of a species flower collectively after a long interval. In bamboos often associated with monocarpic flowering.

Ground tissue: The tissue that forms the bamboo culm; consists of parenchyma cells.

Hypodermis: The layer beneath the epidermis, comprising thick-walled sclerenchymatous cells in bamboo culms.

Intercalary meristem: Meristematic tissue separated from the apical meristem in the primary body by more or less mature tissues.

Intercellular space: Space between adjacent cells.

Internode: The part of a culm or rhizome that lies between two nodes.

Lacuna: Inner space of hollow culm (= pith cavity).

Lamella: A thin cell wall layer.

Lemma: The outer bract, which together with the palea, encloses the flower

Leptomorph: A term coined especially to designate a slender, elongate type of rhizome proper. Most of the lateral buds are dormant. The majority of those that develop produce culms directly; a few produce other rhizomes. In certain species the leptomorph rhizome can turn upward to form a culm.

Lignification: Formation of a 3D polymer (lignin) within the cell wall that provides strength to the culm.

Ligule: A thin, apical extension of a sheath proper, adaxial to the locus of insertion of the sheath blade.

Lumen: A hollow space that exists in a single cell

Maturation: The process of lignification and cell wall thickening of the cell wall that strengthens the culm.

Meristem: Group of cells capable of active cell division, thereby adding new cells to the plant body and maintaining itself as a dividing cell system.

Microfibril: A threadlike component of the cell wall consisting of many parallel cellulose molecules.

Middle lamella: Thin, usually pectic wall layer between adjacent cells. In woody tissues, pectin is replaced by lignin.

Modulus of elasticity (MOE): Measure to characterize the stiffness (ability of a material to resist bending) of wood.

Modulus of rupture (MOR): The maximum load that can be applied, in bending, to a material before it fails.

Monocarpic flowering: Having but a single reproductive cycle within the lifetime of a plant; characterizes bamboos that flower but once and die.

Monophyletic: A group of all organisms that share a single common ancestor.

Monopodial: In bamboos applied to describe an elongated type of rhizome which runs indefinitely, producing culms from lateral buds, resulting in single-stemmed culms rhizome.

Glossary

Node: A segmentation of the culm or rhizome from where branches, leaves or roots originate. At the nodes, a diaphragm divides the culm.

Oral setae: Conspicuous bristles on the sheath near its junction with the leaf. Cf. Auricle.

Pachymorph: A term coined especially to designate a short thick type of rhizome proper. Lateral buds of a pachymorph rhizome procuce only rhizomes; a culm can arise directly only from the apex of such an axis.

Parenchyma: More or less isodiametric cells with simple pits that store and distribute food material.

Pectin: A polymer of galacturonic acid found in the middle lamella and the primary wall of plants.

Periclinal division: Cell division parallel to the organ surface; division of a cambial initial in the tangential longitudinal plane.

Petiole: The leaf stalk.

Phloem: Conducting tissue for the downward transportation of assimilates, consists of sieve tubes and companion cells.

Pith cavity: See lacuna.

Polyphyletic: An individual taxon to which different direct ancestors contributed. So, not sharing a common direct ancestor.

Primary wall: The first-formed cell wall layer with a typical criss-cross orientation of microfibrils.

Pseudopetiole: A false petiole i.e. the narrowed basal part of a leaf lamina in some bamboos.

Pseudospikelet: A spikelet-like inflorescence unit, its glumes with functionally axillary buds.

Rhizome: The segmented subterranean stem system.

Root: In grasses, as in most monocots, the primary root system is very short lived, being replaced by adventitious roots arising from shoot nodes.

Runkel ratio: The indicator for the pulping quality of fibres, expressed as two times the fibre wall thickness divided by the fibre lumen diameter.

Sclereid: A cell with thick, lignified walls as a strengthening element.

Sclerenchyma: A tissue composed of sclerenchyma cells. These are cells with thick, lignified secondary walls.

Secondary wall: Layers laid down on top of the primary wall during the differentiation and further ageing of a cell. The fact that the cell wall structure of bamboo fibres and parenchyma cells comprises several layers in the secondary has significant implications for the nomenclature of the secondary wall in bamboo. In this work the term '*secondary wall*' will be used when all cell wall layers (except the compound middle lamella) are indicated. When a specific layer is meant, the terms L_x will be used (Figure 1-14, p. 24).

SEM-EDX: Scanning electron microscopy with energy dispersive x-ray analyser.

Shear strength: The maximum shear stress that can be sustained by a material before rupture.

Sheath scar: The mark left on the culm after abscission of the culm sheath.

Shoot: A young culm in any stage of its development short of maturity in height.

Shrinkage: The dimensional change in wood caused by a decrease in the moisture content below the fibre saturation point.

Silica: Silicon dioxide, SiO_2

Silica body: Silicon dioxide laid down in appreciable amounts in many grass leaves. The crystals form characteristic shapes for groups of grasses.

Silica cell: A cell filled by a single silica body.

Silicon: The element Si.

Specific gravity: Ratio of the oven-dried wood to the weight of an equal volume of displaced water at 4°C. Specific gravity increases as the moisture content decreases. As a result, specific gravity must be computed for different moisture contents. Specific gravity is the density of a substance divided by the density of water. Since water has a density of 1 gram/cm^3, and since all of the units cancel, specific gravity is the same number as density but without any units.

Spikelet: A basic structural component of every normal gramineous inflorescence, comprising a segmented axis (rachilla) with usually 2 empty glumes, lemmas and paleas and flowers.

Stiffness: ability of a material to resist bending

Sympodial: In bamboos applied to describe a rhizome where the apex turns upward to produce a culm and the rhizome is continued by a lateral branch.

Synflorescence: The whole flower aggregation, i.e. the system of the main florescence with its co-florescences.

TEM: Transmission electron microscopy.

Tensile strength: Characteristic of wood that enables it to sustain tension (pulling or stretching forces) without changing shape or breaking.

Transfer cell: Cell with irregular ingrowths from the cell wall, providing an extensive interface between the wall and the protoplast to facilitate a more efficient movement of solutes.

Glossary

Trimerous: Arranged in threes. In grasses the floral parts show modifications of this, being more strongly trimerous in bamboos, less elsewhere.

Vascular bundle: Consists in bamboo of two metaxylem vessels, protoxylem and phloem, surrounded by fibre sheaths and in sympodial taxa accompanied by fibre bundles.

Vessel: An axial series of cells (vessel element) arranged in axial direction for water conduction.

Xylem: The water-conducting tissue in plants, serving also as supporting tissue, characterized by the presence of tracheary elements.

References: McClure 1966; Chapman 1997; Liese 1998; Dickinson 2000

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APPENDIX 1

SAMPLES DATA

Code	Species	Age (in months)	Origin	Cell wall measurements	Lignin distribution	Silica content	Nodes	Wall thickness nodes
BL-031	*P. nigra*	1	Ghent, Belgium	X	-	X	-	-
BL-039	*P. nigra*	1	Ghent, Belgium	X	-	-	-	X
BL-040	*P. nigra*	3	Ghent, Belgium	X	-	X	-	X
BL-041	*P. nigra*	3	Ghent, Belgium	X	-	-	-	-
BL-057	*P. nigra*	6	Ghent, Belgium	X	-	-	-	-
BL-058	*P. nigra*	6	Ghent, Belgium	X	-	-	-	X
BL-080	*P. nigra*	8	Prafrance, France	X	-	-	-	-
BL-084	*P. nigra*	8	Prafrance, France	X	-	X	-	X
BL-143	*P. nigra*	9	Ghent, Belgium	X	-	X	-	X
BL-144	*P. nigra*	9	Ghent, Belgium	X	-	-	-	-
BL-145	*P. nigra*	12	Ghent, Belgium	X	-	X	-	X
BL-146	*P. nigra*	12	Ghent, Belgium	X	-	-	-	-
BL-076	*P. nigra*	20	Prafrance, France	X	-	-	-	X
BL-083	*P. nigra*	20	Prafrance, France	X	-	-	-	-
BL-153	*P. nigra*	24	Ghent, Belgium	-	-	X	-	-
BL-154	*P. nigra*	24	Ghent, Belgium	-	-	X	-	X
BL-087	*P. nigra*	32	Prafrance, France	X	-	X	-	X
BL-078	*P. nigra*	44	Prafrance, France	X	-	-	-	-
BL-088	*P. nigra*	44	Prafrance, France	X	-	-	-	X
BL-077	*P. nigra*	56	Prafrance, France	X	-	-	-	-
BL-089	*P. nigra*	56	Prafrance, France	X	-	X	-	X
BL-079	*P. nigra*	68	Prafrance, France	X	-	-	-	X
BL-075	*P. nigra*	80	Prafrance, France	X	-	-	-	-
BL-081	*P. nigra*	80	Prafrance, France	X	-	-	-	X
BL-074	*P. nigra*	92	Prafrance, France	X	-	-	-	X
BL-073	*P. nigra*	92	Prafrance, France	X	-	-	-	-
BL-082	*P. nigra*	104	Prafrance, France	X	-	-	-	-
BL-086	*P. nigra*	104	Prafrance, France	X	-	X	-	X

Code	Species	Age (in months)	Origin	Cell wall measurements	Lignin distribution	Silica content	Nodes	Wall thickness nodes
BL-150	*P. nigra*	shoot	Ghent, Belgium	-	-	-	X	-
BL-151	*P. nigra*	shoot	Ghent, Belgium	-	-	-	X	-
BL-152	*P. nigra*	shoot	Ghent, Belgium	-	-	-	X	-
BL-032	*P. viridiglaucescens*	1	Meise, Belgium	X	-	-	-	-
BL-033	*P. viridiglaucescens*	1	Meise, Belgium	X	-	-	-	-
BL-034	*P. viridiglaucescens*	1	Meise, Belgium	X	X	X	-	X
BL-037	*P. viridiglaucescens*	1	Meise, Belgium	X	-	-	-	-
BL-042	*P. viridiglaucescens*	3	Meise, Belgium	X	X	X	-	X
BL-043	*P. viridiglaucescens*	3	Meise, Belgium	X	-	-	-	-
BL-044	*P. viridiglaucescens*	3	Meise, Belgium	X	-	-	-	-
BL-059	*P. viridiglaucescens*	6	Meise, Belgium	X	-	-	-	-
BL-060	*P. viridiglaucescens*	6	Meise, Belgium	X	-	-	-	-
BL-061	*P. viridiglaucescens*	6	Meise, Belgium	X	X	-	X	X
BL-100	*P. viridiglaucescens*	8	Prafrance, France	X	-	-	-	-
BL-102	*P. viridiglaucescens*	8	Prafrance, France	X	X	X	-	X
BL-140	*P. viridiglaucescens*	9	Meise, Belgium	X	-	-	-	-
BL-141	*P. viridiglaucescens*	9	Meise, Belgium	X	X	X	-	X
BL-142	*P. viridiglaucescens*	9	Meise, Belgium	X	-	-	-	-
BL-147	*P. viridiglaucescens*	12	Meise, Belgium	X	-	-	-	-
BL-148	*P. viridiglaucescens*	12	Meise, Belgium	X	-	-	-	-
BL-149	*P. viridiglaucescens*	12	Meise, Belgium	X	-	-	-	X
BL-099	*P. viridiglaucescens*	20	Prafrance, France	X	-	-	-	-
BL-104	*P. viridiglaucescens*	20	Prafrance, France	X	-	-	-	X
BL-155	*P. viridiglaucescens*	24	Meise, Belgium	-	-	X	-	-
BL-156	*P. viridiglaucescens*	24	Meise, Belgium	-	-	X	-	X
BL-095	*P. viridiglaucescens*	32	Prafrance, France	X	-	-	-	-
BL-096	*P. viridiglaucescens*	32	Prafrance, France	X	X	X	X	X
BL-094	*P. viridiglaucescens*	44	Prafrance, France	X	-	-	-	X

Code	Species	Age (in months)	Origin	Cell wall measurements	Lignin distribution	Silica content	Nodes	Wall thickness nodes
BL-103	*P. viridiglaucescens*	44	Prafrance, France	X	-	-	-	-
BL-090	*P. viridiglaucescens*	56	Prafrance, France	X	-	-	-	-
BL-092	*P. viridiglaucescens*	56	Prafrance, France	X	X	X	-	X
BL-105	*P. viridiglaucescens*	68	Prafrance, France	X	-	-	-	X
BL-106	*P. viridiglaucescens*	68	Prafrance, France	X	-	-	-	-
BL-097	*P. viridiglaucescens*	80	Prafrance, France	X	-	-	-	X
BL-101	*P. viridiglaucescens*	80	Prafrance, France	X	-	-	-	-
BL-091	*P. viridiglaucescens*	92	Prafrance, France	X	-	-	-	-
BL-093	*P. viridiglaucescens*	92	Prafrance, France	X	-	-	-	X
BL-098	*P. viridiglaucescens*	104	Prafrance, France	X	X	X	X	X
BL-107	*P. viridiglaucescens*	104	Prafrance, France	X	-	-	-	-
BL-027	*P. viridiglaucescens*	12 (?)	Meise, Belgium	-	X	-	X	-
BL-030	*P. viridiglaucescens*	12 (?)	Meise, Belgium	X	-	-	-	-
BL-028	*P. viridiglaucescens*	> 12	Meise, Belgium	-	X	X	-	-
BL-063	*P. viridis*	8	Prafrance, France	X	-	-	-	-
BL-068	*P. viridis*	20	Prafrance, France	X	-	-	-	-
BL-066	*P. viridis*	32	Prafrance, France	X	-	-	-	-
BL-062	*P. viridis*	44	Prafrance, France	X	-	-	-	-
BL-069	*P. viridis*	56	Prafrance, France	X	-	-	-	-
BL-072	*P. viridis*	68	Prafrance, France	X	-	-	-	-
BL-065	*P. viridis*	80	Prafrance, France	X	-	-	-	-
BL-067	*P. viridis*	92	Prafrance, France	X	-	-	-	-
BL-064	*P. viridis*	104	Prafrance, France	X	-	-	-	-
BL-071	*P. viridis*	128	Prafrance, France	X	-	-	-	-
BL-070	*P. viridis*	152	Prafrance, France	X	-	-	-	-
BL-127	*D. asper*	8	Real Quezon, Philippines	-	-	X	-	-
BL-129	*D. asper*	8	Real Quezon, Philippines	X	-	-	-	-
BL-135	*D. asper*	8	Real Quezon, Philippines	X	-	-	-	-

Code	Species	Age (in months)	Origin	Cell wall measurements	Lignin distribution	Silica content	Nodes	Wall thickness nodes
BL-137	*D. asper*	8	Real Quezon, Philippines	X	-	-	-	-
BL-124	*D. asper*	21	Real Quezon, Philippines	X	-	X	-	-
BL-128	*D. asper*	21	Real Quezon, Philippines	X	-	-	-	-
BL-134	*D. asper*	21	Real Quezon, Philippines	X	-	-	-	-
BL-138	*D. asper*	21	Real Quezon, Philippines	X	-	-	-	-
BL-131	*D. asper*	29	Real Quezon, Philippines	X	-	-	-	-
BL-139	*D. asper*	32	Real Quezon, Philippines	X	-	-	-	-
BL-133	*D. asper*	35	Real Quezon, Philippines	X	-	-	-	-
BL-125	*D. asper*	41	Real Quezon, Philippines	X	-	-	-	-
BL-132	*D. asper*	42	Real Quezon, Philippines	X	-	-	-	-
BL-130	*D. asper*	43	Real Quezon, Philippines	X	-	X	-	-
BL-136	*D. asper*	45	Real Quezon, Philippines	X	-	-	-	-
BL-110	*G. levis*	8	Real Quezon, Philippines	X	X	-	-	-
BL-114	*G. levis*	8	Real Quezon, Philippines	X	X	X	-	-
BL-117	*G. levis*	8	Real Quezon, Philippines	X	-	-	-	-
BL-120	*G. levis*	8	Real Quezon, Philippines	X	-	-	-	-
BL-116	*G. levis*	19	Real Quezon, Philippines	X	-	-	-	-
BL-113	*G. levis*	21	Real Quezon, Philippines	X	X	X	-	-
BL-122	*G. levis*	21	Real Quezon, Philippines	X	-	-	-	-
BL-109	*G. levis*	22	Real Quezon, Philippines	X	X	-	-	-
BL-119	*G. levis*	29	Real Quezon, Philippines	X	-	-	-	-
BL-115	*G. levis*	31	Real Quezon, Philippines	X	-	-	-	-
BL-108	*G. levis*	32	Real Quezon, Philippines	X	-	-	-	-
BL-123	*G. levis*	32	Real Quezon, Philippines	X	-	-	-	-
BL-118	*G. levis*	39	Real Quezon, Philippines	X	-	-	-	-
BL-121	*G. levis*	39	Real Quezon, Philippines	X	-	-	-	-
BL-111	*G. levis*	40	Real Quezon, Philippines	X	X	-	-	-
BL-112	*G. levis*	40	Real Quezon, Philippines	X	X	X	-	-

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-031	*P. nigra*	1	6	208	17	3	Culm length: 4 m 20
			12	217	13	2	
BL-039	*P. nigra*	1	6	234	17	4	Culm length: 4 m 53
			12	211	14.5	3.5	
BL-040	*P. nigra*	3	6	190	18	4	Culm green
			12	210	19	2.5	
BL-041	*P. nigra*	3	6	185	21.5	3.5	Culm green
			12	245	15	3	
BL-057	*P. nigra*	6	6	205	11.5	3	Culm green with black spots
			12	193	9	2	
BL-058	*P. nigra*	6	6	213	24	4	
			12	229	17.5	3	
BL-080	*P. nigra*	8	6	220	26	3	
			12	270	20	2.5	
BL-084	*P. nigra*	8	6	220	31	4	
			12	295	27	3.5	
BL-143	*P. nigra*	9	6	211	22	3	
			12	250	14	2	
BL-144	*P. nigra*	9	6	247	19.5	2	
			12	258	11	1.5	
BL-145	*P. nigra*	12	6	230	16	3	
			12	267	13.5	2.5	
BL-146	*P. nigra*	12	6	221	12.5	2.5	
			12	182.5	8	1.5	
BL-076	*P. nigra*	20	6	345	30	4	Culm length: ± 9 m
			12	415	24	3	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-083	*P. nigra*	20	6	190	33	3.5	
			12	270	27	3	
BL-087	*P. nigra*	32	6	270	31.5	3	
			12	365	28.5	3	
BL-078	*P. nigra*	44	6	325	27	4	Culm length: ± 7.5 m
			12	415	23	3	
BL-088	*P. nigra*	44	6	270	41	4.5	
			12	400	40	4	
BL-077	*P. nigra*	56	6	220	37	4	Culm length: ± 7.6 m
			12	300	33	3	
BL-089	*P. nigra*	56	6	280	36	4.5	
			12	420	31.5	4	
BL-079	*P. nigra*	68	6	220	36	3.5	
			12	295	30	3	
BL-075	*P. nigra*	80	6	255	21	3	
			12	300	29	3	
BL-081	*P. nigra*	80	6	195	36	4.5	
			12	250	33	3.5	
BL-073	*P. nigra*	92	6	200	31.5	4	
			12	300	26	3	
BL-074	*P. nigra*	92	6	180	35	4	Culm length: ± 7.3 m
			12	300	32	3.5	
BL-082	*P. nigra*	104	6	280	25	3.5	
			12	365	18	2	
BL-086	*P. nigra*	104	6	265	34	4	
			12	375	31	3.5	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-032	*P. viridiglaucescens*	1	6	208	17	3	Culm length: ± 4.2 m, not completely elongated
				217	13	2	
BL-033	*P. viridiglaucescens*	1	6	280	16.5	4	
			12	214	12	3	
BL-034	*P. viridiglaucescens*	1	6	230	17.5	4	
			12	266	14.5	2.5	
BL-037	*P. viridiglaucescens*	1	6	238	9.5	4.5	
			12	224	7.5	2.5	
BL-042	*P. viridiglaucescens*	3	6	250	22	5.5	
			12	273	16.5	3.5	
BL-043	*P. viridiglaucescens*	3	6	211	23	5.5	
			12	281	19.5	4	
BL-044	*P. viridiglaucescens*	3	6	192	15	5	
			12	186	13	4	
BL-059	*P. viridiglaucescens*	6	6	215	10	2	
			12	158	8	2.5	
BL-060	*P. viridiglaucescens*	6	6	246	13	3.5	
			12	218	9	2	
BL-061	*P. viridiglaucescens*	6	6	218	17	4	
			12	263	14	2.5	
BL-100	*P. viridiglaucescens*	8	6	295	55	7	Culm length: ± 11 m
			12	370		5	
BL-102	*P. viridiglaucescens*	8	6	280	57	7	
			12	375	58	5	
BL-140	*P. viridiglaucescens*	9	6	231	23	5	
			12	250	21	3.5	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-141	*P. viridiglaucescens*	9	6	232	21.5	5.5	
			12	288	19	4	
BL-142	*P. viridiglaucescens*	9	6	260	21	4	
			12	281	18.5	2.5	
BL-147	*P. viridiglaucescens*	12	6	300	23	5	
			12	331	20	4	
BL-148	*P. viridiglaucescens*	12	6	263	23	5	
			12	292	21	4	
BL-149	*P. viridiglaucescens*	12	6	210	14	3.5	
			12	239	11	3	
BL-099	*P. viridiglaucescens*	20	6	240	49.5	6.5	
			12	340	51	5	
BL-104	*P. viridiglaucescens*	20	6	285	35	4.5	
			12	320	30.5	3.5	
BL-095	*P. viridiglaucescens*	32	6	165	53	7	
			12	345	53	4.5	
BL-096	*P. viridiglaucescens*	32	6	305	53	6	
			12	365	51	4.5	
BL-094	*P. viridiglaucescens*	44	6	165	45	5.5	
			12	180	40	4	
BL-103	*P. viridiglaucescens*	44	6	325	37	6.5	
			12	400	36	5	
BL-090	*P. viridiglaucescens*	56	6	270	39	5.5	Culm length: ± 10.1 m
			12	335	38	4.5	
BL-092	*P. viridiglaucescens*	56	6	320	49	6.5	Culm length: ± 9.6 m
			12	355	47	5.5	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-105	*P. viridiglaucescens*	68	6	275	37	6.5	
			12	405	38.5	5	
BL-106	*P. viridiglaucescens*	68	6	265	41	7	
			12	300	41	5	
BL-097	*P. viridiglaucescens*	80	6	320	41.5	5.5	
			12	370	41	4.5	
BL-101	*P. viridiglaucescens*	80	6	270	47	6.5	
			12	365			
BL-091	*P. viridiglaucescens*	92	6	200	37	6.5	Culm length: ± 10.3 m
			12	265	38	5	
BL-093	*P. viridiglaucescens*	92	6	215	43.5	7	
			12	260	42	5.5	
BL-098	*P. viridiglaucescens*	104	6	220	46	6.5	
			12	265	45	5	
BL-107	*P. viridiglaucescens*	104	6	310	37.5	6	
			12	385	35	4	
BL-027	*P. viridiglaucescens*	12 (?)	6	260	19	3.5	
			12	275	15	2	
BL-030	*P. viridiglaucescens*	12 (?)	6	190	16	3.5	
			12	95	13	2.5	
BL-028	*P. viridiglaucescens*	>12	6	270	11	3	
			12	190	10	2	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-105	*P. viridiglaucescens*	68	6	275	37	6.5	
			12	405	38.5	5	
BL-106	*P. viridiglaucescens*	68	6	265	41	7	
			12	300	41	5	
BL-097	*P. viridiglaucescens*	80	6	320	41.5	5.5	
			12	370	41	4.5	
BL-101	*P. viridiglaucescens*	80	6	270	47	6.5	
			12	365			
BL-091	*P. viridiglaucescens*	92	6	200	37	6.5	Culm length: ± 10.3 m
			12	265	38	5	
BL-093	*P. viridiglaucescens*	92	6	215	43.5	7	
			12	260	42	5.5	
BL-098	*P. viridiglaucescens*	104	6	220	46	6.5	
			12	265	45	5	
BL-107	*P. viridiglaucescens*	104	6	310	37.5	6	
			12	385	35	4	
BL-027	*P. viridiglaucescens*	12 (?)	6	260	19	3.5	
			12	275	15	2	
BL-030	*P. viridiglaucescens*	12 (?)	6	190	16	3.5	
			12	95	13	2.5	
BL-028	*P. viridiglaucescens*	>12	6	270	11	3	
			12	190	10	2	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-063	*P. viridis*	8	6	320	75.5	7	Culm length: ± 15 m
			12	450	57	5.5	
BL-068	*P. viridis*	20	6	380	85	7	
			12	535		6	
BL-066	*P. viridis*	32	6	340	77	7	
			12	430	74	5.5	
BL-062	*P. viridis*	44	6	340	66	5.5	Culm length: ± 14.5 m
			12	445	56	5	
BL-069	*P. viridis*	56	6	385	70	6.5	
			12	495	64	5	
BL-072	*P. viridis*	68	6	315	31	6.5	
			12	450		5.5	
BL-065	*P. viridis*	80	6	380	63	6.5	
			12	500	58	5	
BL-067	*P. viridis*	92	6	325	79	7	
			12	410	69	5	
BL-064	*P. viridis*	104	6	325	83	8.5	
			12	500	77	6	
BL-071	*P. viridis*	128	6	310	73	6	
			12	440	73	5.5	
BL-070	*P. viridis*	152	6	365	62	6	
			12	515	57	5	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-127	*D. asper*	8	6	300	40	13	Culm length: ± 8.06 m; fertilizer: cow dung; no flowering
			12	385	29.5	7.5	
BL-129	*D. asper*	8	6	300	39	13.5	Culm length: ± 6.28 m; fertilizer: cow dung; no flowering
			12	337	27	7	
BL-135	*D. asper*	8	6	375	45.5	10	Culm length: ± 8.53 m; fertilizer: chicken manure, no flowering
			12	479	39	6	
BL-137	*D. asper*	8	6	277	59	13	Culm length: ± 11.5 m; fertilizer: chicken manure, no flowering
			12	395	48	7	
BL-124	*D. asper*	21	6	348	34.5	12	Culm length: ± 6.28 m; fertilizer: cow dung; no flowering
			12	338	24	5.5	
BL-128	*D. asper*	21	6	270	40.5	13	Culm length: ± 6.43 m; fertilizer: cow dung; no flowering
			12	330	29.5	8	
BL-134	*D. asper*	21	6	333	45	14.5	Culm length: ± 8.68 m; fertilizer: chicken manure, no flowering
			12	392	37	7.5	
BL-138	*D. asper*	21	6	305	49	14	Culm length: ± 7.62 m; fertilizer: chicken manure, no flowering
			12	419	39.5	9	
BL-131	*D. asper*	29	6	244	34.5	11	Culm length: ± 4.99 m; fertilizer: cow dung; no flowering
			12	292	22	6	
BL-139	*D. asper*	32	6	335	53	12.5	Culm length: ± 9.58 m; fertilizer: chicken manure, no flowering
			12	400	48	10	
BL-133	*D. asper*	35	6	333	32	9.5	Culm length: ± 6.37 m; fertilizer: chicken manure, no flowering
			12	380	23	4.5	
BL-125	*D. asper*	41	6	300	21	7	Culm length: ± 3.97 m; fertilizer: cow dung; no flowering
			12	244	14.5	3.5	
BL-132	*D. asper*	42	6	322	26	7	Culm length: ± 5.06 m; fertilizer: chicken manure, no flowering
			12	269	15	3	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-130	*D. asper*	43	6	252	19	6.5	Culm length: ± 3.15 m; fertilizer: cow dung; no flowering
			12	173	11.5	3	
BL-136	*D. asper*	45	6	395	25	6	Culm length: ± 5.12 m; fertilizer: chicken manure, no flowering
			12	248	17	4	
BL-110	*G. levis*	8	6	330	57	8	Culm length: ± 8.83 m; no fertilizer; flowering
			12	340	48	5.5	
BL-114	*G. levis*	8	6	401	56	9	Culm length: ± 8.93 m; no fertilizer; no flowering
			12	450	47	7	
BL-117	*G. levis*	8	6	278	40	9	Culm length: ± 6.99 m; fertilizer: cow dung; flowering
			12	453	34	4	
BL-120	*G. levis*	8	6	255	44	8.5	Culm length: ± 7.71 m; fertilizer: cow dung; no flowering
			12	367	36	5	
BL-116	*G. levis*	19	6	273	33	8	Culm length: ± 5.17 m; fertilizer: cow dung; flowering
			12	344	26.5	4	
BL-113	*G. levis*	21	6	347	40	8	Culm length: ± 8.85 m; no fertilizer; no flowering
			12	487	31	4.5	
BL-122	*G. levis*	21	6	293	25.5	7	Culm length: ± 4.33 m; fertilizer: cow dung; no flowering
			12	292	16.5	4	
BL-109	*G. levis*	22	6	285	50	8	Culm length: ± 8.2 m; no fertilizer; flowering
			12	389	46	6	
BL-119	*G. levis*	29	6	272	26.5	7	Culm length: ± 4.31 m; fertilizer: cow dung; flowering
			12	292	16.5	4	
BL-115	*G. levis*	31	6	310	41	9	Culm length: ± 7.42 m; no fertilizer; no flowering
			12	419	33.5	4.5	

Code	Species	Age (in months)	Internode number	Internode length (in mm)	Internode diameter (in mm)	Internode wall thickness (in mm)	Remarks
BL-108	*G. levis*	32	6	326	47	9	Culm length: ± 7.36 m; no fertilizer; flowering
			12	432	38	6.5	
BL-123	*G. levis*	32	6	325	26	6.5	Culm length: ± 4.11 m; fertilizer: cow dung; no flowering
			12	179	19	4	
BL-118	*G. levis*	39	6	290	21.5	5.5	Culm length: ± 3.88 m; fertilizer: cow dung; flowering
			12	271	13.5	3	
BL-121	*G. levis*	39	6	245	19	6.5	Culm length: ± 3.56 m; fertilizer: cow dung; no flowering
			12	220	10	2.5	
BL-111	*G. levis*	40	6	253	28	7	Culm length: ± 5 m; no fertilizer; flowering
			12	295	22	3.5	
BL-112	*G. levis*	40	6	266	26	6.5	Culm length: ± 3.80 m; no fertilizer; no flowering
			12	315	19.5	4	

APPENDIX 2

DATA UV-SPECTRA AND CELL WALL MEASUREMENTS

Folder Cell wall measurements: matrix with data of the cell wall measurements (Chapter III, p. 77)

wall xyin: fibre wall thickness at position xylem inner side

diam xyin: fibre diameter at position xylem inner side

wall xyout: fibre wall thickness at position xylem outer side

diam xyout: fibre diameter at position xylem outer side

wall phlin: fibre wall thickness at position phloem inner side

diam phlin: fibre diameter at position phloem inner side

wall phlout: fibre wall thickness at position phloem outer side

diam phlout: fibre diameter at position phloem outer side

Folder Nodes: data UV-spectra of the nodes (Chapter IV, p. 101)

first row = age of the sample; first column: wavelength

CW: layer of the secondary wall

CML: compound middle lamella

c.s.: culm sheath

Folder G. levis: data UV-spectra of *G. levis* samples (Chapter II, p. 56)

first row = age of the sample; first column: wavelength

CW: layer of the secondary wall

CML: compound middle lamella

Folder P. viridiglaucescens: data UV-spectra of *P. viridiglaucescens* samples (Chapter II, p. 44)

first row = age of the sample; first column: wavelength

CW: layer of the secondary wall

CML: compound middle lamella

APPENDIX 3

BAMBOO AS RAW MATERIAL FOR THE WOOD PROCESSING INDUSTRY IN EUROPE.

SIMILARITIES BETWEEN BAMBOO AND WOOD AS LIGNOCELLULOSIC MATERIALS.

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SUBMITTED FOR PUBLICATION IN HOLZ ALS ROH UND WERKSTOF.

BAMBOO AS RAW MATERIAL FOR THE WOOD PROCESSING INDUSTRY IN EUROPE

SIMILARITIES BETWEEN BAMBOO AND WOOD AS LIGNOCELLULOSIC MATERIALS[6]

Summary

Coordinated by the industry a group of research institutes and universities have been working within an EU funded research project on the evaluation of the usability of bamboo as an alternative raw material for the wood processing industry in Europe. Some 10 different bamboo species, mainly of the genus *Phyllostachys* were selected and valued for their production and adaptation parameters in plantations representative for western and southern Europe. From additional research work on harvesting techniques and corresponding crop management systems it seemed that bamboo has potential as an alternative crop for the agricultural sector in Europe.

Contrary to agricultural crops, bamboo belongs to the forest ecosystem. As such bamboo has a role in forestry mainly of the tropical region. Introducing bamboo in e.g. Europe for purposes other than ornamental garden uses should emphasize on the forest product utilization of this material source. The overall objective of the collaborative European research program in line with this strategy was the production of industrial bamboo products using existing wood transformation technology.

The *Phyllostachys* bamboo species studied proved to be very similar and showed densities close to many wood species. The two main anatomical differences with importance for processing alongside wood are the absence of a radial anatomical component like the rays in wood and the presence of vascular bundles in a ground parenchyma tissue contrary to the xylem origin of wood. Fibre length of bamboo is shorter than in softwood but longer than observed for hardwood species. In summary it can be concluded that bamboo plantations could provide in suitable lignocellulosic resource supply for the wood industry.

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[6] Adapted from:

Joris Van Acker, Bieke Lybeer , Marc Stevens, Johan Gielis , Joris De Vos. Bamboo as raw material for the wood processing industry in Europe. Similarities between bamboo and wood as lignocellulosic materials.

1. Introduction

Bamboo is endemic in all parts of the world except in Europe where it did not survive the last glacial period. The northernmost natural distribution limit is the North of China, corresponding to the latitude of southern Europe. Since the first introduction of bamboo in Europe (1827), about 400 different genotypes have been imported. The actual annual production of bamboo plants in Europe is estimated in millions units that are nearly exclusively used for ornamental purposes. Europe is technologically more advanced in micro-propagation and the selection of superior genotypes of bamboo than in industrial validation of this raw material. The bamboo plant producers envisage however in the long term the industrial transformation of bamboo.

The present paper intends to summarize the current literature on bamboo characteristics relevant to its utilization as an alternative raw material to wood in industrial processing and provides in some relevant extra experimental data.

2. Characterisation of bamboo as a material source

2.1. Bamboo culm structure and anatomy

The structure of a bamboo culm is fairly different to that of a wooden trunk. Liese (1998a) compiled the state of the art on bamboo anatomy showing links to utilization and his publication was used as a basis for the description of relevant parameters in this paper. Earlier publications of Liese (1985, 1987) already emphasized the importance of research on bamboo and the diversity of its applications.

Bamboo culms develop their tissue within only a few months (Liese 1998b). This is faster than any other plant. The culm is characterized by nodes. These provide the transversal interconnection in the culm by their solid cross wall, the diaphragm. In the internodes the cells are strongly oriented axially. In the nodes a more complex structure is found (Ding & Liese 1997; André 1998). The internodes have a culm wall surrounding a large cavity, the lacune. Contrary to wood no radial cell elements exist which greatly hinders lateral movements of nutrients or liquids. Wood consists basically of xylem, being the water transport system that gradually looses its function with age. Bamboo on the other hand remains functional throughout the life span of a culm. The life expectancy of a bamboo culm is about 15 years.

A transversal section of a culm internode shows the parenchymatous ground tissue in which collateral vascular bundles are embedded. The vascular bundles are clearly contrasted macroscopically by their dark sclerenchymatous cells. The culm of e.g. *Phyllostachys edulis* consists of about 54 % parenchyma and 38 % fibres. The remaining part is the conducting system, which includes xylem (vessels) and phloem (sieve tubes and companion cells), accounting for about 8 % of the total culm, or much less in comparison with wood species. The lumen area of softwood tracheids is 60-70 % and of hardwood vessels 15-30 % (Liese 1994). Access to the vessels gets reduced as soon as the culm is harvested and seasoned. As a wound reaction, slime and tyloses develop from the neighboring parenchyma cells, and move into the vessels blocking the lumina.

The younger stage of a culm is marked by certain external characteristics which can be used as indicators to avoid premature harvesting: presence of culm sheaths, bud break, branching pattern, number of leaf scars, colour change of the stem (fresh green becomes yellow-grey) and a hairy culm (glabrous at maturity). The flowering of culms is mostly followed by their widespread death. Dying culms become brittle and often bend down and break. This phenomenon is not associated with fungal degradation. However, the process of chemical/structural changes is still inadequately understood. A complete exhaustion of starch precedes the flowering of culms. Bamboo, in general, is vulnerable to attack by fungi and insects as it has low resistance to such organisms. The culm tissue does not contain phytotoxic substances. Susceptibility of bamboo to fungal decay has only recently been investigated in more detail (Liese 1985; Murphy *et al.* 1991, 1997b; Kleist *et al.* 2002) showing that mature bamboo is comparable to many wood species with low to intermediate durability. Bamboo culms as well as bamboo products are very vulnerable to powder post beetles, e.g. *Lyctus* spp., related to the presence of starch in the parenchyma.

The species of the genus *Phyllostachys* have monopodial rhizomes and as such have vascular bundles of Type I (Grosser & Liese 1971). A vascular bundle Type I consists of only one part: the central vascular bundle, surrounded by a supporting tissue of four sclerenchyma sheaths (Fig.1). They are symmetrically located and nearly of the same size. This type is also called "open-type". Liese & Grosser (2000) call this type subtype Ia. The tissue arrangement does not change longitudinally within an internode as the bundles are strictly axially oriented. The elongation of a culm results from the expansion of individual internodes, already present in the bud. Differentiation starts at the upper part of an internode with the elongation of different cell types.

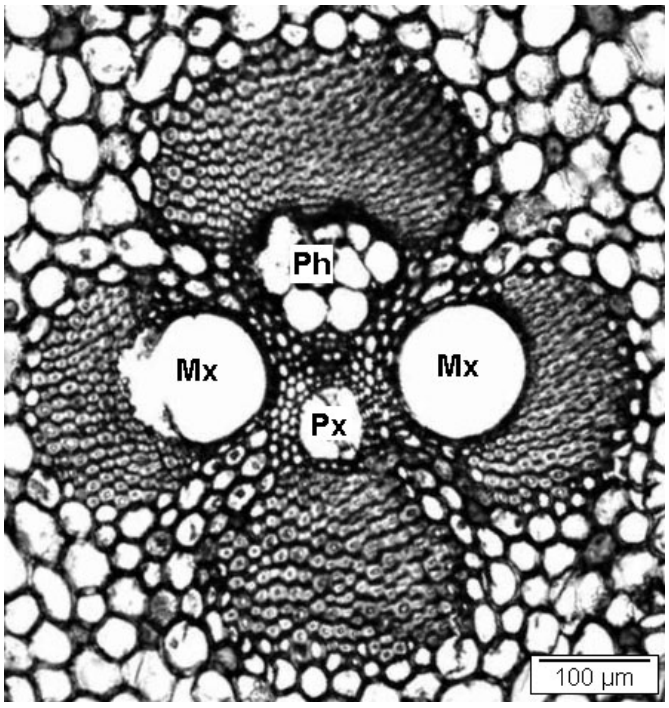

Figure 1. A vascular bundle Type I of *P. viridis* consisting of central xylem (Px and Mx) and phloem (Ph) surrounded by a supporting tissue of four sclerenchyma sheaths (fibre caps)

The protoxylem consists of one or more tracheal elements located between two large metaxylem vessels towards the pith cavity. Often, the protoxylem of monopodial species shows tyloses in the cells, developed from the surrounding parenchyma. The two large vessels of the metaxylem are separated by parenchyma. One or two layers of lignified parenchyma cells, which are generally smaller than the ground parenchyma cells, surround them. Individual vessel members have a length of about 200-600 µm, with considerable variation both across and along the culm. The secondary wall of a metaxylem vessel shows zonation into two distinct parts: S_1 (about 0.2 µm broad, fibrils arranged in flat spiral towards the cell axis at an angle of 80-90°) and S_2 (about 2.2 µm, microfibril orientation with an angle of 30°-90°). The phloem or metaphloem consists of large, thin-walled sieve tubes and smaller companion cells originating from the same mother cells.

As the bamboo culm tapers from base to tip, the walls of the internodes become smaller with increasing height, e.g. for *Phyllostachys makinoi* 11 mm at internode 2 and 2 mm at internode 34 (Grosser & Liese 1974). The total number of vascular bundles decreases with height while their density increases, e.g. for *Phyllostachys makinoi* at respectively internodes 2 and 34 increasing from 200 up to 700 per cm². Therefore, in the vertical direction, the narrowing of the culm wall results in a reduction of the inner portion that has more parenchyma and less vascular bundles. Thus the upper part of a culm contains as much or more fibres per unit area, whereas the basal part has a higher parenchyma content. The size of vascular bundles decreases from the base to the top of the culm. As their radial diameter reduces much more than the tangential diameter, the shape of vascular bundles changes from a radial elongated form to a roundish or oval form near the top. Nearer the periphery, the bundles are smaller and numerous with only a few parenchyma cells in between (Fig. 2). The three xylem fibre sheaths are often amalgamated into an ellipsoid, making the xylem sheath larger than the phloem sheath. However the variation of vascular bundles along culm length (height) is more significant than the radial one. Vessels towards the outside of the culm are smaller than in the middle and inner parts. Along the culm height, they are larger in the middle than at the base and top.

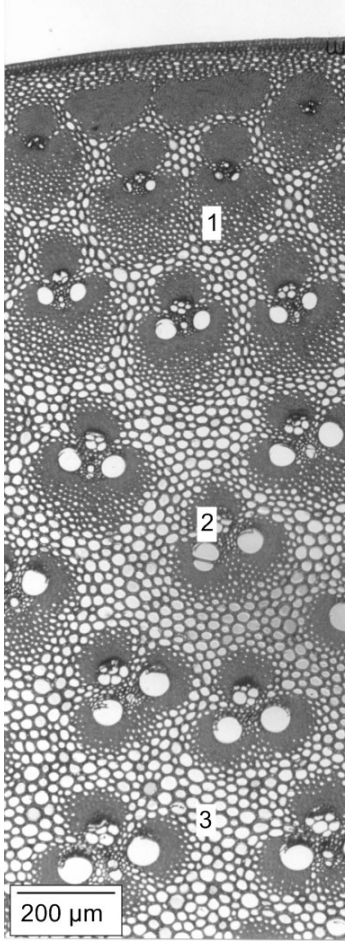

Figure 2. Overview of a transversal section of internode 2 of a *P. vivax* culm. The vascular bundles differ in radial direction and have their typical form in the middle of the culm wall (2). Close to the lacune or center part (3) the vascular bundles are surrounded by smaller sclerenhyma sheats and mainly ground parenchyma while near the periphery (1) the bundles are smaller and numerous with only a few parenchyma cells in between.

2.2. Maturation of parenchyma and fibres as main anatomical tissues

The ground tissue surrounding the vascular bundles is made up of parenchyma. Parenchyma cells are small near the outer culm wall and become larger, especially in length, towards the inner part, and get smaller again near the pith cavity. They are of two types: vertically elongated cells and short cube-like ones interspersed among the former. He *et al.* (2002) showed a difference in lignification and in hemicellulose distribution between the two parenchyma cell types, concluding that the short cells remain in the stage before the onset of cell elongation. The elongated cells have a length of 20-80 µm and a width of 25-40 µm, becoming shorter near the nodes. The secondary wall of the elongated cells has a polylamellate structure consisting of up to 20 lamellae with alternate orientation. Recently Murphy *et al.* (1997a) and Crow & Murphy (2000) obtained evidence of a helicoidal microfibrillar orientation in the parenchyma cell walls. The direction of the microfibrils in the broad lamellae (thickness 0.2 µm) follows slightly a bow-like pattern and merges into the narrow lamellae (0.04 µm). This fibrillar orientation gives the impression of a herringbone pattern. During the maturation of a culm, the number of wall lamellae increases.

The short cells are characterized by denser cytoplasm and thin walls without lignification, even in older culms. As parenchyma cells are the storage tissue for the plant, abundant starch granules can fill up the cells. Starch is abundant in elongated parenchyma of the ground tissue, vascular parenchyma sheaths, parenchyma cells around the nodes and in the diaphragm. Fibres may also contain starch. Starch granules are not present in the shorter intersepted parenchyma and the cortex parenchyma. Parenchyma cells remain active as long as the individual culm remains alive. The starch content of a culm is of considerable importance in terms of utilization. Starch accounts for the susceptibility of harvested culms to beetle attack and accelerates fungal deterioration. The age of the culm influences the starch content. Alvin & Murphy (1988) investigated culms up to three years and found virtually no starch during the first year of growth but many in older culms. Abd. Latif *et al.* (1991) found significant correlation of starch content with age of 1 to 3 year old culms while sugars were higher in the culm top. Relationship with actual local impact of photosynthesis was supposed. Research on *Phyllostachys viridiglaucescens* (Weiner & Liese 1997) has shown the lack of starch granules in young, growing culms. Liese & Abd. Latif (2000) showed that starch content differs significantly between species, site, age, culm height and harvesting month.

In addition to its importance for the storage and strength of a culm, the parenchyma has influence also on moisture content. The higher water content of a culm at the base compared to the top has been attributed to the different amount of parenchyma present (Liese & Grover 1961). The water-holding capacity of a culm reveals a close correlation between the parenchyma contents at the bottom and top parts. The moisture content of green bamboo also varies between innermost layers (155 %) and peripheral layers (70 %) resulting in a mean value of 100 % (Kumar & Dobriyal 1990). The bamboo culm moisture content varies also widely throughout the year, with a maximum during the rainy season and a minimum during dry period. Seasoning is very slow because of the impermeable 'skin' and hence the moisture content at the time of harvest can be of importance. The high amount of parenchyma in bamboo also reflects in considerable amounts of dust when processed.

Fibres constitute about 40 % of sclerenchymatous tissue volume of the culm. They occur as fibre caps (sheaths) surrounding the conducting elements. The genus *Phyllostachys* has only sclerenchyma sheaths as supporting tissue belonging to the vascular bundles of Type I. These vascular bundles have an outer (phloem side) and inner (protoxylem side) fibre sheath as well as two lateral sheaths. The former two decrease in size across the culm from the inner to the outer part, where they tend to fuse together to form a massive supporting tissue. Axially their size decreases from the bottom to the top. The lateral caps do not show much size difference. The caps in some cases like *Phyllostachys edulis* join together embracing the conducting cells.

Fibres are characterized by their slender form. Their length influences the strength of the culm and its pulping properties. Different sources indicate that *Phyllostachys nigra* has the shortest fibre length of only 1.04 mm (Liese & Grosser 1972). While the fibre length for different *Phyllostachys* species ranges from 1 to over 2 mm the corresponding width varies from 10 to 15 µm. Fibres of bamboo are longer than those of hardwood (approximately 1 mm), but shorter than softwood fibres (3-4 mm). Often, shorter and smaller fibres occur at the peripheral layer, their length increasing to a maximum in the outer third of the culm wall and decreasing again towards the inner wall. The length differences across the culm wall amount to about 20-50 %.

Longitudinal fibre length variations of more than 100 % exist within one internode. The shortest fibres are always located near the nodes and the longest are to be found in the middle of the internode. The mean fibre length of an internode is correlated with the internode length. Both longer fibres and the longest internodes are found in the middle region of a culm. The fibres are generally thicker in the bottom portion than at the top. The fibre length-to-width ratio varies across the culm wall from 70:1 to 150:1. The fibre length is strongly correlated with the fibre diameter (10-40 µm), the cell wall thickness and the internode diameter. The Runkel ratio or the ratio between 2x cell wall thickness (4-10 µm) and the lumen diameter (2-20 µm) ranges from 1 to 4. These values are influenced by fibre maturation, which leads to an increase in wall thickness. The smaller fibre wall thickness of immature culms gives a lower Runkel ratio that makes them unsuitable for pulping. Abd. Latif & Liese (2001) could correlate vascular bundle distribution and size, metaxylem diameter, percentages of metaxylem vessel and fibre sheath, fibre length and lumen diameter, fibre wall thickness and Runkel ratio with age and culm height, while nearly all properties differed insignificantly only with site.

Numerous layers constitute the fibre cell wall. Results on the number of lamellae in the fibre cell wall have been reported up to a maximum of 18. Gritsch *et al.* (2004) concluded that the multilayered structure of fibre cell walls was formed mainly 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 layering was not found to be specifically related to the thickness of the cell wall. The number is highest in the fibres either adjacent to vascular bundle elements or at the periphery of fibre bundles close to the ground tissue. In transverse sections, the fibre wall is characterized by a regular alternation of broad and narrow lamellae. Near to the middle lamella, the first lamella of the secondary wall shows fibrils oriented at an angle of 50° to the cell axis. This can be considered as a transition lamella, but it is not a constant feature within all fibre cells. In the broad lamellae the microfibrils are oriented at an angle of 2-5°, which increases up to 10-20° towards the inner part of the wall. The narrow lamellae show mostly fibrils oriented horizontally at an angle of 85°-90°, which remains constant over the whole width of the wall. The innermost lamellae near the lumen are thinner than the more peripheral ones. The broad layers with axial orientation and occasionally more transversally oriented thin layers were also found through observation of the oriented growth of microhyphae of soft rot fungi (Murphy *et al.* 1997a). The lamella at the lumen boundary has no resemblance in fibrillar texture to the tertiary wall typical of wood fibres.

Generally, fibres at the outer culm wall have a thicker wall that is more lamellated than the inner fibres. Cell wall thickening of bundle sheath fibres starts from the inner vascular side and proceeds to the outer parenchyma side. Fibre maturation can be a process prolonged over many growing seasons. Cell wall development from a young shoot to a mature culm and during further ageing was analyzed only recently (Murphy & Alvin 1992, 1997a, 1997b; Liese & Weiner 1996; Gritsch *et al.* 2004; Lybeer & Koch 2005). Liese & Weiner (1996, 1997) investigated in detail culms of *Phyllostachys viridiglaucescens* aged up to 12 years. During the first month of the growing period the cell wall thickness at the 20th internode was 1.5-1.7 µm and most fibres were still unlignified. At 3 months they were fully elongated (2.3 µm) and had three lignified lamellae.

The wall thickness evolved from 2.6 µm after 1 year, over ± 6 µm after 6 years to 8 µm after 12 years containing eight lamellae. The increase in wall thickness was caused by the deposition of additional lamellae. The lignification of *Phyllostachys heterocycla* was studied by Itoh (1990), revealing full lignification of the component cells within one growing season. The lignin content in culms aged 2 to 4 years determined at internodes 10, 20 and 30 only varied from 23.2 to 26.4 %. Fengel & Shao (1984, 1985) determined as chemical composition for *Phyllostachys makinoi* 25.5 % lignin, 45.3 % alpha-cellulose, 24.3 % polyoses and 2.6 % extractives. The main polyose is arabinoxylan and the bamboo lignin is rich in syringyl units. Murphy & Alvin (1997b) showed that *Phyllostachys viridiglaucescens* bamboo culms were only half lignified at year 1 (approximately 13 %) compared to 3 year old culms (over 26 %) having lignin content comparable to wood. Lybeer & Koch (2005) revealed that *p* - coumaric and ferulic acids are widely distributed in *Phyllostachys viridiglaucescens* and that their content is dependent on the anatomical location and the differentiation phase. The lignin in the epidermis cell wall is deposited early in the development and does not increase with age which is in contrast with the fibres and the ground parenchyma cells where an increasing trend in lignification during the first year is shown. The S_2 fibre wall has a lamellar structure with increasing lignin content from the centre towards the compound middle lamella. Lin *et al.* (2002) showed that fibre walls are rich in guaiacyl lignin in the early stage of lignification, and lignin rich in syringyl units is deposited in the later stage. The lignification process may last even up to 7 years. An important feature of a lignified fibre is its liveliness. Whereas the fibres in hardwood normally die after cell wall differentiation with the simultaneous degeneration of their cytoplasm, bamboo fibres retain their cytoplasmatic activity long after cell wall lignification. The fibres retain a living protoplast and appear to undergo progressive septation (Murphy & Alvin 1997a). Contrary to angiosperms, the septate fibres show secondary wall apposition (Parameswaran & Liese 1977). In a similar way, thickening of parenchyma cell walls was noted in older culms (Alvin & Murphy 1988) up to 3 years. Cells with small walls may represent still immature fibres, but often they are parenchyma cells, recognizable by their square ends in the longitudinal section (Murphy & Alvin 1992). Bath (2003) found that for two Indian bamboos cell-wall thickening of fibres was accomplished after 3 years leading to a polylamellate cell-wall structure. In ground parenchyma, although the wall thickening was evident, lamellation was not distinct.

The shortest fibres occur in the diaphragm at all culm levels. Being about 340 µm long at the nodal level, the fibres are only one-third their normal length in an internode. The changes in width and shape of the fibres at the node lead to a length-to-width ratio of 40:1 to 60:1. Owing to the shortening of the fibres in the nodal part and their simultaneous wall thickening, bamboo culms often break at the nodes when under tension.

2.3. Physical-mechanical and chemical properties of bamboo

The specific gravity of bamboo varies approximately from 0.5 to 0.9 g/cm³ but can differ considerably within the culm and between species (Zhou 1981; Janssen 1991). It increases during maturation of the culm from 1 to 3 years owing to the thickening of the fibre walls, but slightly also during later years. Cell wall thickening leads to an increase in basic density of the culm material. The increase in density is dramatic during the first two years but become more gradual during the third year and stabilized thereafter (Bath 2003). At the base, the bending strength of the outer part is 2-3 times higher than that of the inner part. Older culms are more dimensionally stable than younger ones. Both the radial and tangential shrinkage decrease with the height of the culm since the top portion has a higher number of vascular bundles and lower initial moisture content. Older bamboos were also found to shrink less than younger ones (Abd. Latif *et al.* 1995). *Bambusa heterostachya* culms of 1, 2 and 3 year and old showed similar variation in shrinkage ranging from 17 to 24 % in radial and 5 to 11 % in tangential direction. Lee *et al.* (1994) measured average shrinkages from green to air-dry conditions of 0.02, 9.25 and 18.21 percent in longitudinal, tangential and radial directions respectively. Radial shrinkage was about twice as big as shrinkage in tangential direction. In order to obtain medium to high quality products and recovery rates of more than 50 %, usage of at least 2-year old culms is suggested in order to avoid less lignified fibres and parenchyma walls. Culms of higher age are preferred for bamboo furniture since they show less shrinkage and splitting. Immature culms are prone to splitting, shrinkage, breakage and biological attack.

Fibre length shows a positive correlation with the modulus of elasticity (MOE) and the compression strength. The fibre wall thickness correlates positively with compression strength limit and with the modulus of elasticity (MOE), but negatively with the modulus of rupture (MOR) (Abd. Latif *et al.* 1990, 1993). Janssen (1991) compiled mainly unknown publications on mechanical properties showing increased strength with age, with decreasing culm diameter, with increasing height in the culm, with lower moisture content (below the fibre saturation of approx. 30 %), with higher ratio outer to inner layer, with higher ratio of vascular bundles to parenchyma and with increased fibre length. The ratio between the ultimate compression strength and the mass per volume is slightly higher for dry bamboo (0.094) as compared with 0.084 for dry wood. According to Janssen (1990) this might be due to the higher cellulosic content of 55 percent of bamboo compared with 50 percent in wood. Bamboo culms for weaving are chosen from species that are easy to split and are not old enough to become brittle (preferably below two years). Especially the node causes a serious problem of reduced elasticity and uniformity of bamboo strips. The nodal portion of a culm has shorter fibres and a lower holocellulose content but a higher content of extractives, pentosans, lignin and ash than the internodal portion. Nodes have a major influence on the culm's mechanical strength. They show a higher specific gravity, a lower volume shrinkage and a lower tensile strength than the internodes mainly because of shorter fibres and distorted vascular bundles (Kabir *et al.* 1996).

The fractural behaviour of a culm is different from that of wood. Breaking modes differ for fibres and parenchyma. Cracks are deflected in the direction of the fibres. Ruptured fibre walls can show both splintering and cross fracture types. The cross fracture could be classified as having a flat, smooth face or a ridged face. The flat face fracture occurs predominantly in the broad lamellae zones. The ridged fracture appears only occasionally, showing a tendency for spiral arrangement of the fibrils. Under tension the lamellae can separate with the weakest regions corresponding to narrow lamellae. The alternating fibrillar orientation in the two lamellae types contributes partially to a nullification of the anisotropic properties. Because of the presence of such polylamellate fibres at the statically efficient sites, the peripheral culm zone presents a highly reinforced area (Parameswaran & Liese 1976, 1981).

The ground tissue discussed up to now lies between two terminal layers: a cortex at the outside of the culm and a pith ring at the inside towards the lacune. At the inner side of the culm wall, cellular layers surround the pith cavity, becoming more pronounced as the culm ages. This pith ring, pith periphery or 'bamboo yellow' is a non-vascular tissue composed of layers of parenchyma cells, which are often heavily thickened and lignified. The epidermis, which is the outermost layer of the cortex, contains axially elongated cells, shorter cork and silica cells, and stomata. Cork and silica cells are small and may appear in pairs between the long cells. Often the longer one of the pair is a cork cell and the smaller one is a silica cell. The silica cells may contain a high amount of silica (silicon dioxide), which strengthens the epidermal layer. The epidermal cells are often covered on the outside by a cutinized layer of cellulose and pectin with tangential lamellation. Younger culms show numerous unicellular hairs. Beneath the epidermis lies the hypodermis, consisting of several layers of thick-walled sclerenchymatous cells. To the inner side is the actual cortex, nearly without vascular bundles. Cortical parenchyma generally appears homogeneous. Cell size increase from the outer to the inner layers. The compact composition of the cortex and the wax coating prevent loss of water from the culm. It also hinders any lateral uptake of liquids. At the outside of a culm, a waxy layer appearing as irregular plates, rods or granules, often covers the surface as can be felt especially on young culms. This wax hinders the penetration of liquid. Particular hindering is observed for the penetration of chemicals during the pulping process and of preservatives during vacuum pressure impregnation. Bamboo consists for 0.8 % up to 9.7 % of inorganic components with a higher amount in the nodal region than in the internodes. Silica is a major constituent of the epidermis with values between 1.5 % and 6.4 %. It consists of small crystals of silicon dioxide and is mainly localized in the shorter epidermal cells. The culm tissue itself contains hardly any silica and the nodes contain only small amounts. The presence of silica affects the cutting and pulping properties of bamboo. Especially bamboo in sympodial taxa from tropical climates contains a siliceous deposit in the lacuna, called tabashir (used in traditional medicine, properties as a catalyst). Most silica appears situated in the cortex region, but more knowledge about its location would be useful for processing technologies. The selection of species with a lower amount of silica is significant for the manufacture of products as furniture, structural components and skewers (Hamdan & Abd. Latif 1992). Schmitt *et al.* (2002) localized silica polymers as extracellular deposits within the wall of epidermal cells of bamboo culms. Young internodes showed distinct silica deposition in epidermal wall regions underneath the cuticle. In the outer wall regions silica granular deposits were found in increasing amounts during maturation but were only present in inner wall regions in late development stages.

3. Experimental

3.1. Bamboo species

Bamboo species are endemic in all continents except in Europe. The utilization of bamboo in production processes comparable to the wood sector is predominantly present in Asia. Although many species are known, only a limited number is used on a large scale, e.g. the two natural-stand Malaysian bamboos are *Bambusa blumeana* and *Gigantochloa scortechinii* (Hamdan & Abd. Latif 1992). They are used alongside *Bambusa vulgaris*, a village bamboo cultivated in all parts of Peninsular Malaysia (Chew *et al.* 1992). In China *Phyllostachys pubescens* predominates in the bamboo resources with approximately 75 % (Shilin *et al.* 1994).

Since frost resistance is far more important to Europe the possibilities of species are mostly limited to certain *Phyllostachys* species. Within the frame of the EU-project 'Bamboo for Europe' materials used originated from a mixed bamboo plantation in Belgium based on *Phyllostachys aureosulcata* 'Spectabilis', *P. nigella, P. praecox* and *P. vivax.* The bamboo species used for large-scale industrial experiments were also included. They were collected from older stands of *Phyllostachys aurea* and *Phyllostachys nigra* in Tomino, Pontevedra, in the North West of Spain.

3.2. Material properties

The density was calculated for bamboo as the ratio between the oven-dry mass and the green volume. The volume of cylindrical elements of approximately 3 cm in length was measured by submerging the water-saturated blocks. Samples with regular shape were volume checked using precise measurements of all dimensions. The oven-dry mass was determined after exposure of the specimens to a drying scheme of 48h at 60 °C, 24h at 80 °C and 24 h at 103 °C.

From the second internode of each bamboo culm a cylinder of between 1 and 2 cm was cut for anatomical examination. A small block of 1 cm width was extracted and softened by soaking it for some days in a mixture of 30% glycerin and 70 % ethanol. Cross-sections of 20 to 30 µm thick were microtomed. These microtome sections were stained using safranine and astrablue. Safranine stains lignin red and astrablue gives a blue colour to the other components. Light microscopy enabled the analysis of the anatomical structure.

The microtome slices were analyzed by means of an image analyser giving a relative proportion of grey expressing the cell wall ratio. This ratio is an estimation based on pixel grey values varying from white (0) to black (256). The vessel or vascular bundle density was also counted on the cross-sections.

For fibre length measurements, the bamboo material was macerated according to the method of Jeffrey. Tangential slices of approximately 100 to 200 μm were cut from the bamboo specimens by means of a microtome. These blocks were softened using a glycerin-ethanol mixture and then transferred to a solution of 30 % nitric acid and 30 % sulfochromic acid. After soaking in a hot water bath (60°C) for 24 hours, the fibres were regularly mixed, filtered and washed with distilled water. For analysis some fibres were transferred onto a micro slide, covered with 1 cm² glass and closed on the sides with nail varnish to avoid water evaporation. Using an image analyzer connected to the microscope the length of 100 fibres was measured on each microslide.

A further material property, the silica content was looked at because of the importance when dealing with wood processing systems. Bamboo contains 0.5 to 4 % silica mainly localized in the epidermis (Liese 1987). In order to get insight in the distribution of silica an Environmental Scanning Electron Microscope (ESEM) was used. For the ESEM-analysis microtomed bamboo cubes had 0.5 cm sides. The ESEM was equipped with an EDXA-unit (Energy Dispersive X-ray Analysis) enabling qualitative detection of silicon. More quantitative data were obtained by chemical analysis. Preparation of samples and the spectrophotometric determination of silica as silicon dioxide was performed according to the methods described by Sulthoni (1989). The chemical analysis of silica was performed on two selected species from the ESEM work. From each culm 4 samples were extracted: three from the lower part of the culm (one covering the entire culm cross section; one of the epidermis zone and one from the inner part, excluding the epidermis) and one from the upper part of the culm (entire cross section).

4. Results and discussion

Basic data of the culms from the older *Phyllostachys aurea* and *P. nigra* stands that were selected for detailed investigations are given in Table 1.

Table 1. Age, number of internodes, total length and total green mass of the different bamboo culms tested.

Species	Culm	Age (year)	#Internodes	Total length (cm)	Total mass (g)
P. aurea	11	1	37	391	424
	12	2 – 3	39	448	495
	13	4 – 5	36	453	951
	21	1	33	326	254
	22	2 – 3	30	361	417
	23	4 – 5	37	502	820
P. nigra	11	1	22	418	310
	12	2 – 3	19	349	194
	13	4 – 5	17	304	211
	21	1	20	439	385
	22	2 – 3	27	452	497
	23	4 – 5	26	469	464

All *Phyllostachys* species showed quite similar density values ranging mostly from 600 up to 750 kg/m³ (Tables 2 and 3). The overall variation and the density level indicated that *Phyllostachys* bamboo can be used for most industrial applications as an alternative to wood without further species differentiation. Only some culms show a lower density, especially *Phyllostachys vivax* (Table 3). Both *Phyllostachys aurea* and *Phyllostachys nigra* show similar density patterns (Table 2). Density increases distinctly with height in the culm. The impact pattern of age on the density could not clearly be derived.

Table 2. Density of different internodes of *Phyllostachys aurea* and *P. nigra*.

Density (kg/m³)								
Species	Internode	Culm 11 1 year	Culm 12 2-3 year	Culm 13 4-5 year	Culm 21 1 year	Culm 22 2-3 year	Culm 23 4-5 year	Mean
P. aurea	2			545	616	628		596
	6	491	453	559	672	788	644	601
	10	575	484	595	799	689	648	632
	14	632	534	592	790	734	640	654
	18	665	587	591	799	732	660	672
	22	707	647	631	831	835	694	724
	26	676	647	720	831	835	752	744
	30	637	701	705			820	716
	34		740	740			861	780
	Mean	626	599	631	763	749	715	680
P. nigra	2	592	626	594	600	653	531	599
	6	640	668	618	698	699	663	664
	10	656	699	645	741	747	656	691
	14	653	728	698	724	766	715	714
	18	756				781	743	760
	Mean	659	680	639	691	729	662	686

Table 3. Mean relative grey value (cell wall ratio), the vascular bundle density and the culm density of different *Phyllostachys* spp.

Species	Mean grey value	Vascular bundle density (number/cm²)	Density (kg/m³)
P. aurea	161 (1)	489 (1)	701
P. aureosulcata	201 (3)	981 (3)	747
P. nigella	184 (2)	801 (2)	713
P. nigra	178 (2)	425 (1)	649
P. praecox	195 (3)	1088 (4)	713
P. vivax	182 (2)	921 (3)	595

(*) Significant differences at 95 % level

All *Phyllostachys* species show a very similar anatomical tissue composition mainly because of identical Type I vascular bundles. On cross section the vascular bundle density is significantly higher near to the epidermis. The compact epidermis is identified as being obviously different from the inner side which may cause difficulties when used in traditional wood processing systems.

The mean grey value obtained by image analysis and reflecting the cell wall ratio was not an adequate predictor of density and seemed to be highly influenced by the vascular bundle density (Table 3). High and low vascular bundle densities linked very well to the mean grey value. However none of both parameters was able to predict the lower density of *Phyllostachys vivax*. Variation in vascular bundle density as indicated in table 4 showed important differences both between inner and outer part of the cross sections and between different heights in the culm. Outer and inner parts were differentiated based on the transition of individual sclerenchyma sheats into an amalgamated ellipsoid of xylem sheats.

Table 4. Vascular bundle density of *Phyllostachys aurea* and *P. nigra*.

Vascular bundle density (number/cm²)				
Species	Internode	Inner part	Outer part	Overall
P. aurea	2	194	533	356 (1)
	6	217	420	308 (1)
	10	206	512	357 (1)
	14	253	675	568 (2)
	18	362	654	489 (2)
	22	468	883	743 (3)
	26	752	989	865 (4)
	Mean	350	667	530
P. nigra	2	180	301	251 (1)
	6	175	350	283 (1)(2)
	10	213	380	281 (1)(2)
	14	233	396	295 (1)(2)
	18	257	392	312 (2)
	22	393	653	464 (3)
	Mean	242	412	329

(*) Significant differences at 95 % level

The mean fibre length for all *Phyllostachys* species studied varies between 1700 and 2000 µm (Table 5), except for *Phyllostachys vivax*. Mean fibre length values from literature already indicated major variation depending on the origin of the bamboo culms (Liese & Grosser 1972). Figures for mean fibre length reported are 980 µm (Japan) and 1610 µm (USA) for *Phyllostachys aurea* and 2100 µm (Brazil), 1860 µm (Philippines) and 1040 µm (Japan) for *Phyllostachys nigra*. Such density and fibre length values enable the use of European grown *Phyllostachys* bamboo

as an alternative for wood in e.g. MDF production. The lower value for *Phyllostachys vivax* added an additional argument not to include this species in future bamboo plantations in Europe.

Table 5. Mean fibre length of different *Phyllostachys* spp.

Species	Mean fibre length (µm)
P. aurea	1756 (2)
P. aureosulcata	1701 (2)
P. nigra	1918 (3)
P. nigra 1 year	1962 (3)
P. nigra 2-3 year	1888 (3)
P. nigra 4-5 year	1753 (2)
P. praecox	1750 (2)
P. vivax	1514 (1)

(*) Significant differences at 95 % level

Middle and top section of each internode showed higher fibre length than the bottom part (Table 6). Variation coefficients were approximately 30 %. Variation in fibre length was important while no clear impact of the internode level could be observed. The feeble relation between fibre length and age could not be substantiated and is clearly not essential to the overall variation.

Table 6. Fibre length variation in an internode of *Phyllostachys aurea* and *P. nigra*.

Fibre length (µm)								
Species	Internode 6				Internode 18			
	Bottom	Middle	Top	Mean	Bottom	Middle	Top	Mean
P. aurea	1591 (1)	1754 (2)	1861 (3)	1735	1688 (4)	1832 (4)	1808 (4)	1776
P. nigra	1748 (1)	2109 (2)	2130 (2)	1996	1651 (3)	1967 (4)	1902 (4)	1840

(*) Significant differences at 95 % level

The ESEM study showed clearly that the presence of silicon is linked to the epidermis (Fig. 3). The findings were confirmed by the chemical analysis of two species with clearly different silicon response when using ESEM-EDX analysis (Table 7). The silica content of the epidermis of *Phyllostachys nigra* was merely approximating the lowest figure (0.4 %) reported in literature, while the silica content of *Phyllostachys praecox* was substantially lower and even meaningless for industrial processing of bamboo.

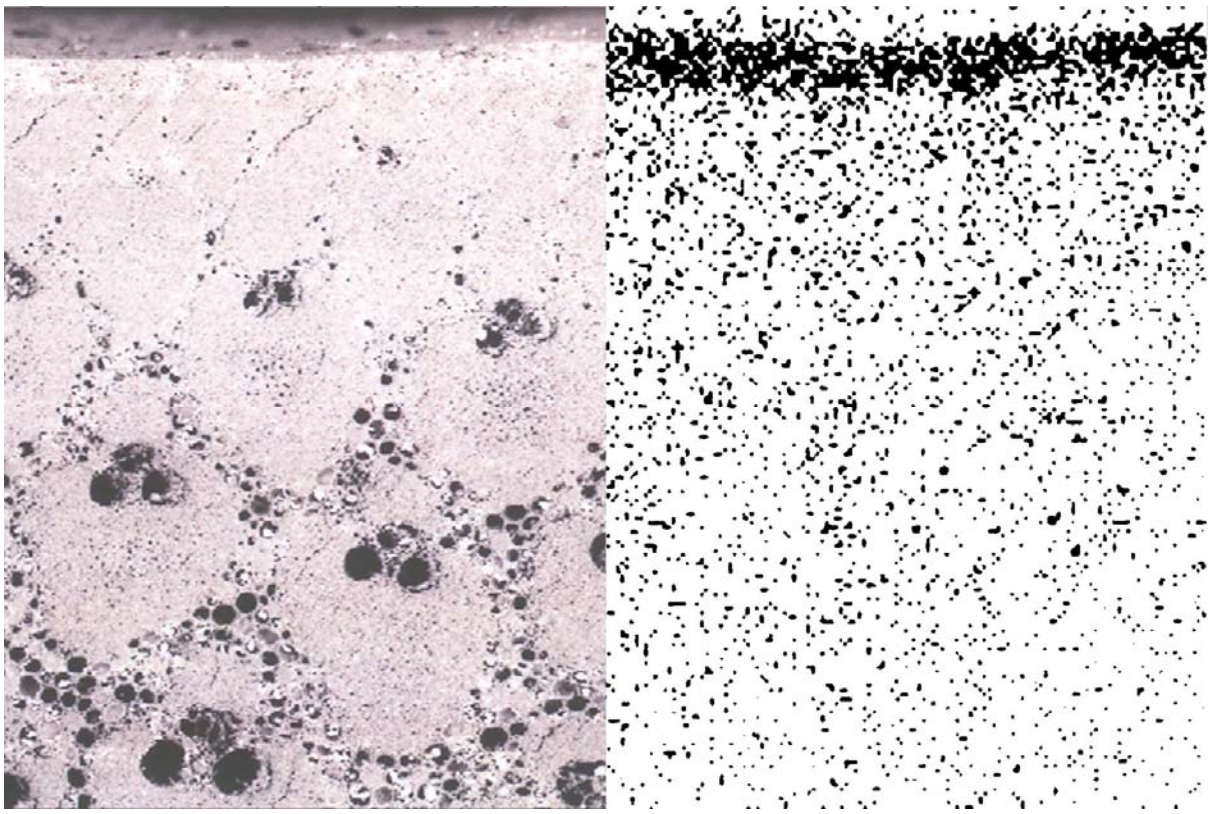

Figure 3. ESEM analysis of a transversial section of a *P. nigra* culm (internode 6). Left part presents the ESEM picture and the right part the corresponding silicium EDX mapping. Si is present in the outer zone.

Table 7. Silica content of Phyllostachys praecox and P. nigra

Si-content (%)				
Species	Lower part			Upper part
	Epidermis	Inner zone	Overall	Overall
P. preacox	0,056	0,018	0,066	0,053
P. nigra	0,268	0,012	0,120	0,355

5. Conclusions

Bamboo density is very similar to values recorded for many wood species, which allows for direct substitution. Variability in density and anatomical structures is important and the results obtained confirm literature data. The *Phyllostachys* species studied appear to be quite similar; hence it seems feasible to establish bamboo plantations using several species and enable production of relative uniform raw material.

Contrary to wood bamboo has no radial components but the tissue pattern with vascular bundles containing fibres embedded in ground parenchyma shows more similarity with fibre reinforced composites than wood does. In general, the fibre length in bamboo is situated in between softwood and hardwood fibres. The variation in fibre length is relatively low. The silica content can vary considerably between species but remains mainly located in the epidermis zone. The silica detected in the European grown *Phyllostachys* species was lower than generally reported in literature.

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