CELL WALL MORPHOGENESIS AND STRUCTURE IN TROPICAL TENSION WOOD

by

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Summary
Differentiating tension wood was observed in order to analyse the changes occurring during cell wall morphogenesis. Specimens were taken from trees in Guyana. Wall texture was analysed by means of ultrastructural cytochemistry. Modifications were encountered in fibre and vessel walls of tension wood when compared to typical wood. The changes were twofold: variation in the layering of polyamellate walls, and the deposition of a gelatinous layer in the fibre cell walls. Results are discussed in terms of variations in the rhythmic nature of cell wall deposition. Data confirm that the morphogenesis of the wall is a modular process allowing the cells to adapt to growth constraints.

Key words: Cell wall cytochemistry, helicoidal pattern, tension wood, flexible structure, Macrosamanea, Eperua.

Introduction
The recent development of new cytochemical techniques has allowed a fine visualisation of cell wall ultrastructure (Parameswaran & Liese, 1981; Roland, 1981). It has been suggested that the variability encountered in cell wall textures can be traced back to a common morphogenetic process. The mechanism is characterised by a continuous rotation of the microfibril planes. Variations in the rate of microfibril deposition could explain the different cell wall organisations observed (Roland & Mosinia, 1983; Roland et al., 1984). Tension wood appears a good material to study this hypothesis thoroughly. It allows one to analyse spontaneous changes occurring in cell wall morphogenesis. Tension wood has been studied in many trees and exhibits various cell wall organisations (Détiennne, 1976; Wicker, 1979). In most cases it has been characterised by the occurrence of a gelatinous layer, the G layer, in fibre walls (Wardrop, 1964; Côté et al., 1969; Scuffield, 1973; Trenard & Gueneau, 1975; Mariaux & Vitalis-Brun, 1983).

Cytochemical techniques were applied to tension wood in order to analyse cell wall differentiation from cambial cells. A methylamine extraction was associated to the PATAg test, i.e. a test for polysaccharides at the ultrastructural level. Methylamine is known as a lignin extrac tant. The PATAg staining reveals the vic-glycol groups carried on polysaccharides. In most types of walls, a mild extraction with methyl amine generally re-establishes both a fibrillar texture and a PATAg reactivity in places where they would not be seen without extraction (Czaninski & Monties, 1982; Roland & Mosinia, 1983; Harche & Catesson, 1985). Therefore, variations in extractability and reactivity in lignified walls should provide indications on chemical variations existing in these walls. The present paper suggests that the changes occurring in cell wall structure are not only the result of a variation in the rate of rotation of microfibril planes, but also the result of a variation in the chemical composition of the cell wall.

Material and Methods
The observations were made on two straight trees from Guyana, Assao (Macrosamanea pedicellaris) and Wapa (Eperua rubiginosa) (see Lindeman et al., 1963, and Détiennne et al., 1982, for wood anatomical descriptions). The trees were chosen because of their strong longitudinal growth stresses noticed on their circumference. The preliminary biophysiological studies of the trees were made by the Centre Technique Forestier Tropical (CTFT). The longitudinal strains were first estimated by the 'drilling hole method', at the cambium level, on the standing tree (see Mariaux & Vitalis-Brun, 1983). Then the trees were barked on a little area. A small triangular stick was cut of 10 cm length (1) and 3 mm² in cross section. The longitudinal strain was then calculated from the linear shrinkage (ΔL) of the stick harvested. The tension stresses are expressed in $\Delta L = \frac{1}{1} \times 10^{-6}$ microdeformations (see Chardin & Sales, 1983, for details). The specimens observed in this paper were taken from sticks in which the measured tensions corre-
sponded to 1000–2700 microdeformations (Satiat-Jeunemaître, 1984a). These values correspond to very tense woods, capable of exp
dloding during the cutting down of the trees.

For the light microscopic observations, clas
tical stainings were used in order to visualise the aspect, the distribution and the compo
osition of the fibres (cf. Johansen, 1940). Some sections were mounted in 1% NH₃OH and ob
served under UV light (λ = 470 nm) in order to see the fluorescence of lignin.

For the observations with the electron micro
to-scpe, sticks were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.4 for 3 to 8
days. Sticks were cut in 3–5 mm segments and washed in buffer during 24 hrs. A mild extracti
on of cell wall components was performed by immersion of the samples in a 40% methy
amine solution (Reis, 1981; Czaninski & Monties, 1982; Roland & Mosniak, 1983) for 6 days. The samples were washed, dehydrated and embedded in an epon-araldite mixture (Roland, 1978). Ultra
thin transverse sections were stained by the PATAg test (Thiery, 1967). Ultrastructural ob
servations were made with a Philips 300 electron microscope.

Results

First the anatomical characteristics of ten
sion wood will be briefly described from ob
servations with the light microscope. Then the ultrastructural and cytochemical characteristics of cell walls during the differentiation process and in fully differentiated cells will be analysed.

Characteristics of tension wood

One of the most characteristic features ap
pearing at the light microscope level is the oc
currence in all the samples studied of a gelati
nous G layer. This layer appears rich in cellu
lose and hemicellulose, and little or not lignifi
ced, as deduced from the histological tests per
formed: blue staining with carmine-iodine green, red staining with congo red, faint staining with hydrochloric phloroglucinol and the Mäule reaction, lack of autofluorescence. This particular layer is often not firmly bound to the rest of the wall.

Cell wall texture during the differentiation process

Figure 1 shows cambial cells from which fi
bres, vessels and parenchyma cells are derived. Their walls are poorly extractable so that their texture is hardly visible, except in radial walls which appear fibrillar. Tangential walls are thinner and less extractable than radial walls. A diversity in fibrillar texture is seen in cambial derivative cells (Fig. 2). Some walls have an ordered texture, others present a loose fibrillar network. A general view of differentiating cells (Fig. 3) shows that a decrease in extraction properties in the wall is correlated with lignin deposi
tion: in lignifying cells the walls are less extractable and more reactive than in cambial cells. In fibres (Figs. 4–7), the typical S₁ and S₂ layers are recognisable in the wall. The S₁ layer is generally more resistant to extraction than the S₂ layer. The angle of microfibril rotation between the S₁ and S₂ layers changes ac

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Fig. 1–3. Cell wall texture at the beginning of differentiation. – 1: Cambial cells. Strong reactivity of the walls. The radial walls are extracted and show a fibrillar network. Note that radial middle lamellae are not connected with tangential middle lamellae. Extraction time: 58 hrs; x 5,800. – 2: First cambial derivative. Heterogeneity in wall texture of contiguous cell. Extraction time: 58 hrs; x 12,000. – 3: Cambial derivative (left) and lignified cells (right). The deposition of lignin is correlated with an increase in reactivity and a decrease in extractability of secondary walls. Note the strong extraction in the primary walls. Extraction time: 100 hrs; x 1,000.
Fig. 4–6. Fibre wall texture during differentiation. Deposition of $S_1$ and $S_2$ layers. Primary walls and $S_1$ layers are less extracted and more reactive than $S_2$ layers. Note that the orientation of fibrils in the $S_2$ layers changes according to the fibres observed. Extraction time: 58 hrs in Figs. 4 & 5; 100 hrs in Fig. 6; $\times$ 48,000.
Fig. 7–8. Fibre wall texture during differentiation. Occurrence of a gelatinous layer.

--- 7: Deposition of a thin G layer giving rise to a discontinuity in the inner part of S2. Extraction time: 58 hrs; x 40,000.

--- 8: Deposition of a thick G layer. Lightly stained by the PATAg test. No fibrillar network is distinguishable. Extraction time: 100 hrs; x 50,000.
Fig. 9–10. Fibre cell wall texture in differentiated tissues. – 9: The gelatinous layer, lightly stained, is closely bound to the S₂ layer. Extraction time: 120 hrs; x 22,000. – 10: Other aspect showing a slit in the G layer. G and S₂ layers remain closely bound. A very faint reactivity is seen in the G layer even after 120 hrs of extraction time. Extraction time: 120 hrs, x 8,000.
Fig. 11–13. Wall texture of other cell types in differentiated tissues. – 11: Parenchyma cells. The walls are typically three-layered. Extraction time: 120 hrs; x 120,000. – 12: Vessel-associated parenchyma cell. The wall is multilayered, the layering being irregular because of an unequal width of layers. Extraction time: 120 hrs; x 100,000. – 13. Vessels. Multilayered walls. The layering of walls varies according to the cell considered. Regular layering. Extraction time: 120 hrs; x 100,000.
Fig. 14–15. Wall texture of other cell types in differentiated tissues. Vessels. Multilayered walls. The layering of walls varies according to the cell considered. – 14: Disturbed stratification. Extraction time 120 hrs; x 110,000. – 15: Islets of longitudinal microfibrils (arrows) in a mainly transverse network. Extraction time: 120 hrs, x 110,000.
In other cell types, the fibrillar texture of the walls is clearly seen after the methylamine treatment (Fig. 11–15). In parenchyma cells, the walls are typically three-layered (Fig. 11). In vessels and vessel-associated cells, the walls are multilayered. In vessel-associated cells, the width of the layers often varies giving rise to an irregular layering (Fig. 12). In some vessel walls, the lamellation is regular (Fig. 13) and up to 10 layers can be counted within the wall. In some cases, the layering is not easily recognisable: transverse or longitudinal microfibril planes are still clearly seen, but the pattern is strongly disturbed (Fig. 14). In extreme cases, the longitudinal microfibrils are represented by little islets in a network of transversal microfibrils (Fig. 15).

**Discussion**

The structural and cytochemical variations encountered in tension wood are discussed in terms of adaptation of cell wall morphogenesis.

**Changes in the basic twisted model**

Several textures are usually described in the walls of wood: polylamellate texture, three-lamellate texture, layered texture, each texture being considered as a consolidated and fixed structure. Despite these different organisations, it has been suggested that such walls can be interpreted according to a common mechanism of helicoidal assembly (Roland & Mosiniak, 1983).

One of the characteristics of tension wood is the variability which occurs in the stratification of polylamellate walls. It is particularly clear in the walls of fibres in which the angle between the microfibril orientations of the S₁ and S₂ layers varies from fibre to fibre. This point has already been emphasised by Boyd in 1980. In vessels, the variability in wall texture is also expressed by an unequal width of the layers. Such differences may be traced back to the helicoidal pattern in which variations in the rate of rotation of microfibrils occur as described in the paper by Roland and Mosiniak (1983). To some extent, even a perplexing wall organisation as seen in Figure 15 can be explained in terms of the proposed model. It may correspond to a stop in the rotation of microfibril planes giving rise to mainly transverse depositions, the islets of longitudinal microfibrils being due to the sectioning through local deformations as already described (Satiat-Jeunemaitre et al., 1985).

**Cytochemical properties of the gelatinous layer**

A characteristic of tension wood is the occurrence of a particular layer, usually called the gelatinous layer or G layer. A mild extraction with methylamine re-establishes both a fibrillar texture and a strong PATAg reactivity in S₁ and S₂ layers but not in this G layer. Several chemical modifications occurring in this layer have been suggested (Mia, 1968; Ruel & Barnoud, 1978; Wicker, 1979; Lapière & Monties, 1982). The differences in staining after methylamine extraction confirm such chemical modifications. The absence of reactivity of the G layer to the PATAg test strengthens the hypothesis that the G layer is made of highly crystalline cellulose. Indeed, in tension wood an increase in crystallinity could prevent the action of methylamine and explain the inaccessibility of the reactive sites. Moreover, the fragility of the system and the frequency of breaks and slits at the border between G and the rest of the wall (in particular S₂) could be relevant of such differences in physical properties.

**Flexibility of the wood cell wall morphogenesis**

It has already been suggested that the flexibility in wall morphogenesis allows the cell to adapt to various growth constraints (see Roland et al., 1984). Our results strengthen this hypothesis. One can emphasise the importance of two adaptable systems: 1) an adaptation occurring at the level of the assembly of wall subunits, i.e., a variation in the rate of rotation of microfibril planes as described for elongating walls (Vian et al., 1983; Satiat-Jeunemaitre, 1984b); 2) an adaptation occurring once the assembly is done and consolidated, i.e., the construction of a highly crystalline and un lignified G layer. The high degree of crystallinity could help in the cohesion and the rigidity of the system (and thus play the role of lignin) and at the same time allow a greater elasticity to the fibre.

**References**


