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Growth stresses in tension wood: role of microfibrils and lignification

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Summary — In order to clarify the role of microfibrils in the generation of growth stresses in trees, an experimental analysis was carried out on 7 Appalachian hardwood species which were with or without gelatinous fiber in the upper region of the leaning stem. In the species that had gelatinous fibers, large longitudinal tensile stresses appeared in the region where the cross-sectional area of gelatinous layers were large. In the species that had no gelatinous fibers the following relationships were observed: (a) the smaller the microfibril angle, the larger the longitudinal tensile stress; (b) the larger the tensile stress, the larger the α-cellulose content; (c) tensile stress becomes larger as crystallinity increases; and (d) tensile growth stress had no or a slightly negative correlation with lignin content. These results suggest that the high tensile longitudinal growth stress is mainly due to the tensile stresses of cellulose microfibrils as a bundle in their axial direction. Thus the microfibrils tension hypothesis can be applied to elucidate the growth stress generation in the region of normal and tension woods.

growth stress / tension wood / gelatinous fiber / microfibril / cellulose

Résumé — Les contraintes de croissance dans le bois de tension. Rôle des microfibrilles et de la lignification. *Afin de clarifier le rôle joué par les microfibrilles dans la genèse des contraintes de croissance dans l'arbre, une analyse expérimentale a été réalisée sur 7 essences feuillues des Appalaches produisant ou non des fibres gélatineuses dans la partie supérieure des tiges inclinées. Dans le cas des essences produisant des fibres gélatineuses, des contraintes élevées sont observées au niveau des zones à forte proportion surfacique de couches gélatineuses en section transverse. Pour les essences ne produisant pas de fibres gélatineuses, la contrainte longitudinale de tension est d'autant plus grande que l'angle des microfibrilles et petit ; elle est d'autant plus grande que le taux d'alpha-cellulose est élevé ; elle est d'autant plus grande que le taux de cristallinité est élevé ; elle n'est pas corrélée, sinon par une légère relation négative, avec le taux de lignine. Ces résultats suggèrent que les microfibrilles jouent un grand rôle dans la genèse des contraintes de croissance en traction dans la direction longitudinale. Celle-ci serait due principalement à la mise en tension axiale des microfibrilles. Ainsi l'hypothèse d'une tension des microfibrilles peut être admise pour expliquer la genèse des contraintes de croissance dans le bois normal et le bois de tension.*

contrainte de croissance / bois de tension / fibre gélatineuse / microfibrille / cellulose

INTRODUCTION

The mechanism of growth stress generation is usually discussed in terms of the lignin swelling hypothesis (Watanabe, 1965; Boyd, 1972; Kubler, 1987) and the cellulose tension hypothesis (Bamber, 1978, 1987; Kubler, 1987). We have recently proposed a new hypothesis that growth stresses are generated by the interrelation between the tensile stress of microfibrils generated positively in their axial direction and the compressive stress that is generated by the deposition of lignin into the gaps of the microfibrils (Okuyama *et al,* 1986). The tensile stress of microfibrils governs the longitudinal tensile stresses in normal and tension wood. The compressive stress from the deposition of lignin controls the level of the longitudinal compressive stress in compression wood and the tangential compressive stress of normal wood. This hypothesis has been corroborated by the experimental data and also by the analytical model of growth stress generation (Yamamoto *et al,* 1988). However, further data is required to substantiate the generation of tensile stress in microfibrils in their axial direction.

This report examines the contribution of microfibrils to the generation of tensile growth stresses based upon experimental data of some hardwood species from an Appalachian forest and the analytical model gives the detailed information on our hypothesis (Yamamoto *et al,* 1993). In addition, the cross-sectional area of gelatinous fibers, microfibril angle, degree of crystallinity and cellulose and lignin content are correlated with growth stresses. The generation mechanism of growth stress is discussed.

MATERIALS AND METHODS

Materials

Species of Appalachian hardwoods selected as the experimental trees are listed in table I. The first 2 species in the table do not have gelatinous fibers on the upper sides of leaning stems.

Experimental method

The released strains were measured by a strain-gage method. Several measuring stations were fixed at various heights in a standing tree stem and 10–15 measuring positions were made around the periphery of each station. Two strain gages of 8 mm in length were glued perpendicularly on the measuring position in longitudinal and tangential directions. The measuring position was arranged selectively on the upper side of a leaning stem so as to determine the released strain in tension wood. The strain was measured

Table I. Species used in this experiment.

Species	*Age of tree (yr)*	*BHD (cm)*	*Mean height (m)*	*No of trees*
Cucumber tree (*Magnolia acuminata* L)	51	25–27	12	2
Yellow poplar (*Liriodendron tulipifera* L)	51	28–30	14	3
Black cherry (*Prunus serotina* Ehrh)	45	20	17	1
Black locust (*Robinia pseudoacacia* L)	23	15	7	1
Red maple (*Acer rubrum* L)	53	15	9	1
Red oak (*Quercus rubra* L)	55	22	11	1
Sassafras (*Sassafras albidum* (Nutt) Nees)	28	15	12	2

with a strain meter with a multi-scanner of 40 strain bridges, each bridge had one active gage connected with 3 wires. Soon after taking the initial reading the 2 dimensional growth strains were released by making grooves of 10–15 mm in depth around the strain gages. Two-dimensional released strains can be detected by means of the above procedure and converted into two-dimensional growth stresses using elastic moduli.

After the measurement of released strain, a wood block surrounded by grooves was removed from each measuring position for the specimens of elastic moduli, microfibril angle and anatomical analysis of gelatinous fiber. The specimen for analysis of chemical composition was matched longitudinally with the strain-measured position.

Elastic moduli were determined by a tensile test using small test specimens of 10 x 20 x 1 mm in a green condition. Young's moduli in the longitudinal and tangential directions and Poisson's ratios were measured to convert the released strains into growth stresses.

Mean microfibril angle was measured by X-ray diffraction using flat-sawn air-dried sections, 0.2 mm thick (Meylan, 1967) only for the species with no gelatinous fibers. The X-ray diffraction meter was also used to determine the cellulose crystallinity of wood powder prepared from the wood block.

The fraction of cross-sectional area of gelatinous layer was determined on microscope sections of 8–10 μm thickness. After being stained with fast green and safranin and mounted on a glass slide with a water-soluble glycerine-gelatin compound, the specimen was photographed at 50 and 250 magnifications. The photographs were processed with an image analyzer, IBAS-II, which discriminated the cross-sectional image of gelatinous layer from that of the other layers and the lumen, and converted it into digital images of 512 x 512 pixels, and the cross-sectional area of the gelatinous layers was measured.

The chemical composition was analyzed on wood powder of 42–60 mesh prepared from the wood blocks taken from positions matching each measuring position of released strain. The lignin content was determined by the Klason method. The α-cellulose content was obtained by extraction of the holocellulose with 17.5% NaOH aqueous solution and then determined by the chlorite method.

RESULTS AND DISCUSSION

Contribution of gelatinous fibers to generation of growth stress in longitudinal direction

The results are shown in figures 1–6. In figures 1, 2 and 4–6 the uppermost measuring station of the leaning stems corresponds with the zero degree and the lowest with

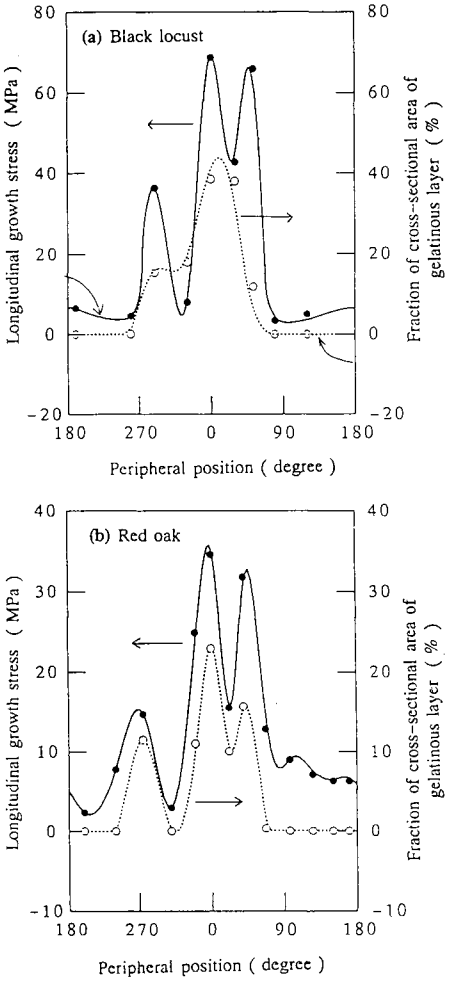

Fig 1. Peripheral distribution of longitudinal growth stress and the relationship to the cross-sectional area of the gelatinous layer.

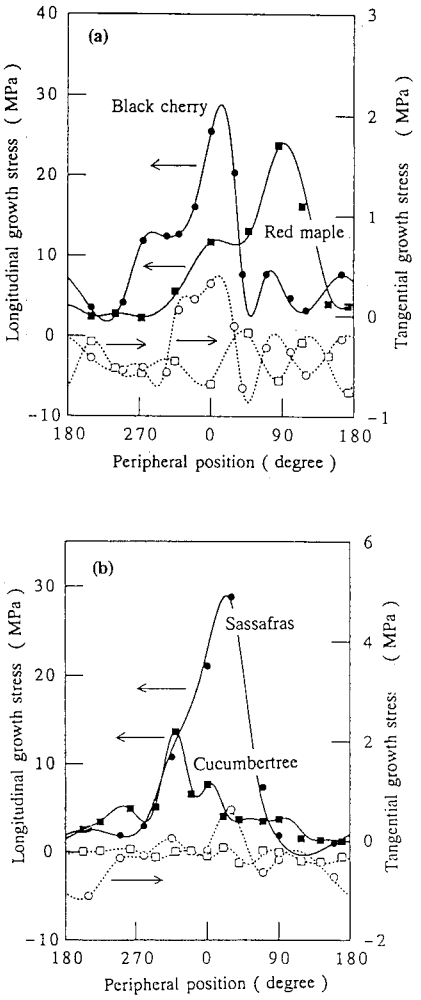

Fig 2. Examples of peripheral distribution of growth stresses in longitudinal and tangential directions.

the 180 degree position. In figure 1 the relationship of gelatinous fibers to longitudinal tensile growth stress is shown. It can be seen that black locust has very large stress, approximately 70 MPa. This stress is roughly equal to half of the longitudinal tensile strength of green wood. Their asymmetrical distribution of the stress with respect to the stem axis produces a large recovery moment. Normal cell walls cannot support such large stresses for a long time without stress relaxation. A highly reinforced fiber, *ie* gelatinous fiber, would support a larger stress. As we previously reported (Okuyama *et al,* 1990) the gelatinous layer (G-layer) has a large Young's modulus and in maple (*Acer mono* Maxim) this was estimated to be approximately 3 times as large as that in the normal cell wall, and to have a large released strain.

As clearly shown in figure 1, in the cases of the 2 species above, the fraction of cross-sectional area of G-layer is large corresponding to the presence of large growth stress. Large Young's modulus and released strain are attributable to the G-layer, *ie* cellulose microfibrils. A G-layer has a highly crystallized, pure cellulose (Norberg *et al,* 1966) with a low microfibril angle. Therefore we are led to the conclusion that the gelatinous fibers develop a large longitudinal tensile stress during cell maturation to support the large stress in the wood.

Important phenomena were also observed in the form of the growth stress distribution. As shown in figures 1–5, the growth stresses in the normal wood region on the periphery containing tension wood become smaller than that of the other normal wood region, *ie* the straight part in the upper position of the leaning trees. In the case of a leaning stem of yellow poplar (figures 4a and 5a), growth stresses on the periphery became almost zero in the lateral to lower part of the stem despite the presence of a large amount of tension stress on the upper side. Figure 2 shows the peripheral distributions of growth stresses of other species. Their largest stresses appeared around zero degree of peripheral position and the growth stresses in the lateral or lower position on the periphery containing tension wood were also smaller than that of the upright part in a tree as in figure 4a and figure 5a. However, no anatomical dif-

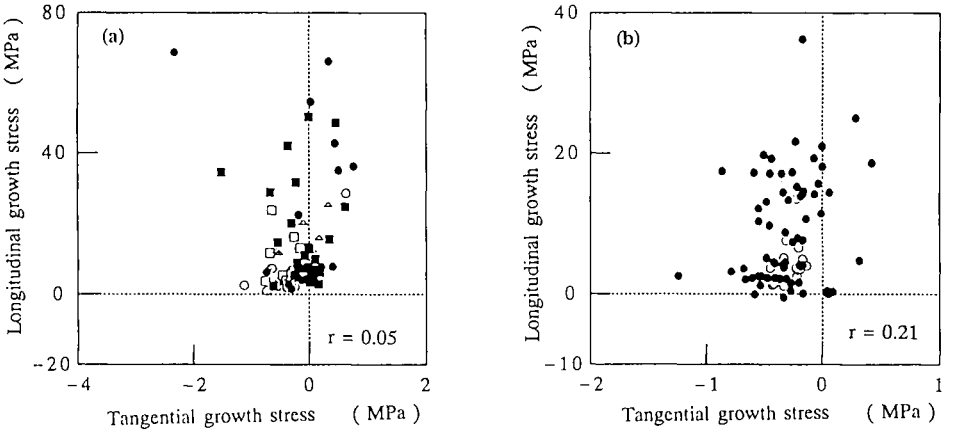

Fig 3. The relationships of growth stresses in longitudinal and tangential directions. (a) ● black locust; ■ red oak; □ red maple; O sassafras; Δ black cherry; (b) ● yellow poplar; O cucumber tree.

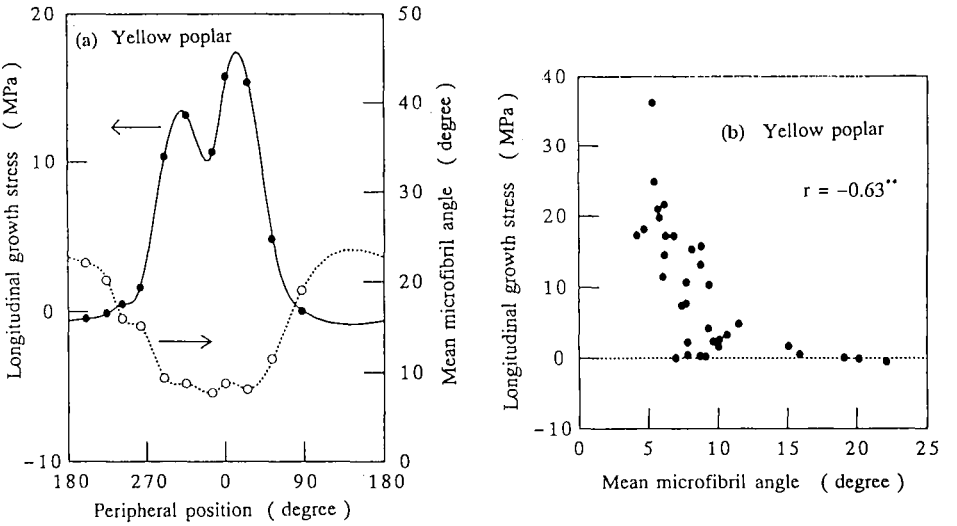

Fig 4. (a) Peripheral distribution of longitudinal growth stress and mean microfibril angle in a leaning stem of yellow poplar. (b) Relationships of longitudinal growth stress and mean microfibril angle in 3 leaning yellow poplar stems.

ferences were observed between the lateral or lower part of the periphery of leaning trunks and that of the upright part of the tree. It is possible that another factor may exist controling the level of longitudinal growth stresses serving to straighten their leaning stem.

Figure 3 shows the relationship between growth stresses in the longitudinal and tangential directions. No correlation can be

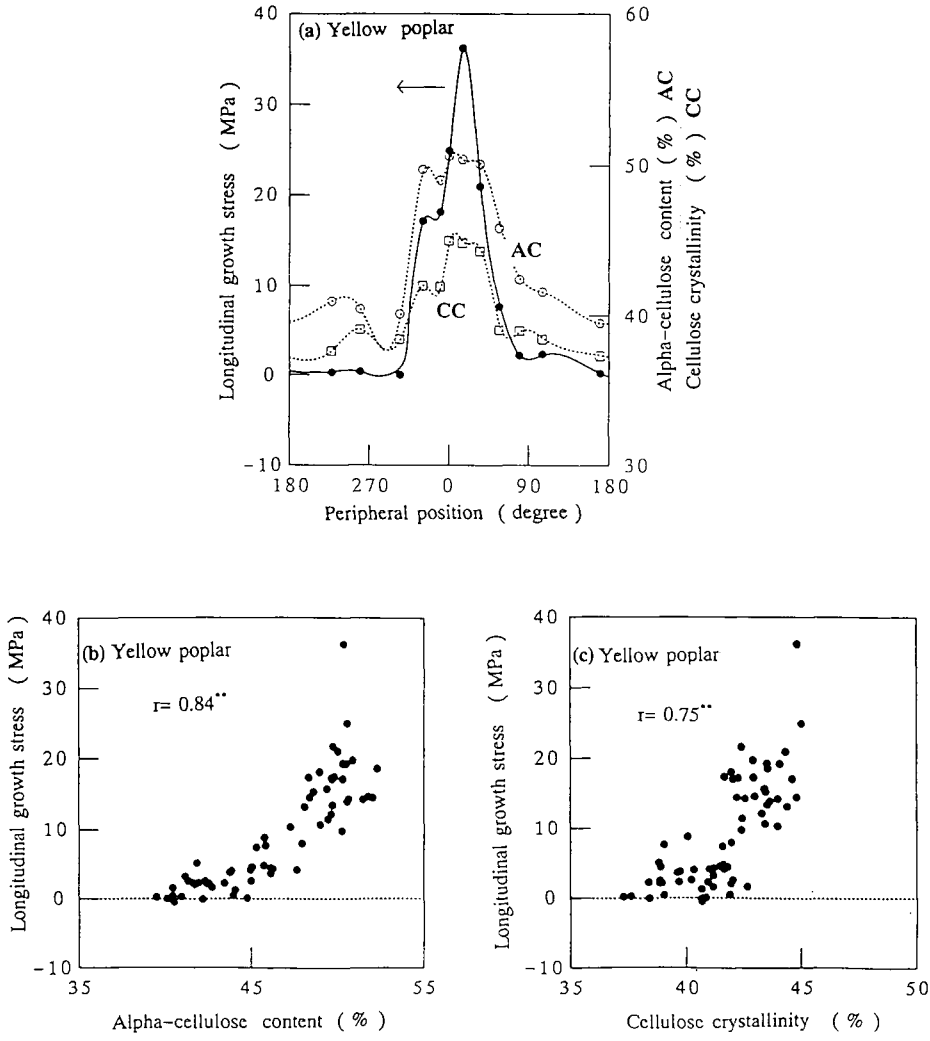

Fig 5. (a) Peripheral distribution of longitudinal growth stress, α-cellulose and cellulose crystallinity in one leaning stem of yellow poplar. (b) and (c) Relationships between longitudinal growth stress and alpha-cellulose content and cellulose crystallinity in 3 leaning stems of yellow poplar.

seen between them, in species with or without gelatinous fibers. This suggests that the large growth stresses in the longitudinal direction are generated mainly by the active longitudinal contraction of fibers and not only the transverse swelling of the cell wall as had been previously suggested (Okuyama *et al*, 1986).

The evidence presented here suggests that the G-layer generates a large tensile stress in its axial direction. This proposition is also supported by a growth stress gen-

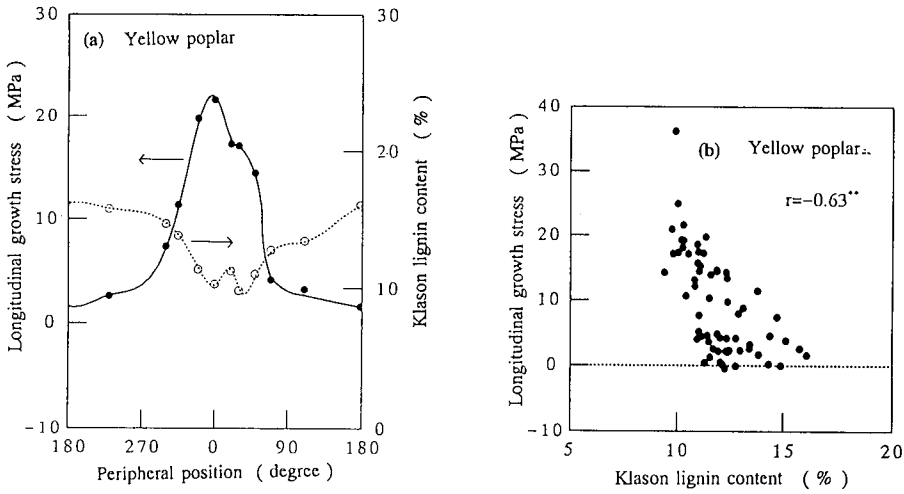

Fig 6. (a) Peripheral distribution of longitudinal growth stress and Klason lignin content in a leaning stems of yellow poplar. (b) Relationships of longitudinal growth stress and Klason lignin content in 3 leaning yellow poplar stems.

eration model of the cell wall proposed by Yamamoto *et al* (1993).

Contribution of microfibrils to generation of longitudinal growth stress

As shown above, large tensile growth stresses are also observed in regions where the anatomical properties, for example, the shape of the cross-section of fibers, are not different from normal wood.

What is the generation mechanism of tensile growth stress of species that have no gelatinous fibers on the upper regions of a tilted stem? As can be seen in figures 4a and 5a, yellow poplar, a species that does not have gelatinous fibers, generates high tensile growth stress. This indicates that high growth stress can be developed in the absence of gelatinous fibers. It should be noted that yellow poplar is in the family *Magnoliaceae*, which, together with the families *Tiliaceae*, *Sterculiaceae*, and *Rhinanthaceae*, was reported not to produce gelatinous fibers in tension wood (Onaka, 1949).

The peripheral distribution of mean microfibril angle (MFA) of yellow poplar shows a clear relationship with longitudinal growth stress (fig 4a), the MFA being small where the growth stress is large. The total data on 3 trees of yellow poplar are shown in figure 4b. The larger the tensile stresses are, the smaller the MFAs. A similar relation has also been given for hoonoki (*Magnolia obovata* Thumb) (Okuyama *et al,* 1990).

Figure 5a shows the peripheral distributions of the longitudinal growth stress, α-cellulose content, and cellulose crystallinity; figure 5b shows the relation between growth stress and α-cellulose content on 3 yellow poplars; and figure 5c shows their crystallinity. The longitudinal tensile growth stress shows a positive relation with both α-cellulose and its crystallinity. The magnitude of the stress is related to the amount of α-cellulose and its crystallinity. This relationship of tensile growth stress and α-cel-

lulose has also been shown in the normal wood region of softwood species sugi and hinoki (Sugiyama *et al,* 1993). These results suggest that α-cellulose has a strong influence on the generation of the high growth stresses.

Figure 6 shows the relationship between growth stress and lignin content. The larger the tensile growth stress, the smaller the Klason lignin content. This indicates that transverse swelling during lignin deposition is unlikely to be the origin of longitudinal tensile stress in tension wood and thus supports the hypothesis put forward by Bamber (1978, 1987). The high longitudinal growth stresses of species that have no G-fibers are generated in cell walls. The latter tend to be similar to the G-layer in that they have low MFA, high cellulose content and crystallinity, and low lignin content.

From the above discussions, it is obvious that cellulose microfibrils play an important role in the generation of growth stress of wood. During cell maturation the microfibrils not only resist the isotropic swelling of matrix substance but also have positive tensile stress in the axial direction: the larger the stress, the larger the amount of α-cellulose. The small MFA directly transfers the stress to the actual growth strain in the longitudinal direction as shown by numerical models (Okuyama *et al,* 1986; Archer, 1987; Yamamoto *et al,* 1988; Fournier *et al,* 1990).

Possibility of generation of tensile stress in cellulose microfibrils

The above experimental results predict that the high tensile longitudinal growth stress is mainly due to the tensile stresses of cellulose microfibrils (CMFs) in their axial direction. Thus, the microfibrils tension hypothesis can be applied to elucidate the growth stress generation in the regions of normal and tension woods. What is the generation mechanism of tensile stresses in the CMFs?

According to biochemical research, the process of cell-wall deposition is as follows: the cell wall is formed by successive and irreversible deposition of polymers, pectin, hemicellulose (HC), cellulose and lignin. The first step is the formation of the cell plate, composed of pectic substances, in the cambial zone during cell division. In the second step, the golgi apparatus supplies the terminal complexes (TCs), which generate CMFs, and the golgi vesicles then generate HC and lignin precursor and deposit their contents outside the plasma membrane. The TCs deposit CMFs in the sequence of primary wall, and outer, middle and inner layers of secondary wall.

The CMFs are oriented randomly in the primary wall but are highly oriented in the secondary wall, being fixed by the HC gels to form the rigid, twisted, honeycomb structure that forms secondary wall. Lignification occurs after this process (Fujita *et al,* 1978).

At first, lignification occurs at the cell corners and the compound middle lamella, then it extends to the secondary wall. The lignin and HC compounds fix the CMFs together as a honeycomb structure in the secondary wall during cell maturation (Terashima, 1990; Terashima *et al,* 1993).

During the above process, it is difficult to see how changes in the CMFs can generate such a large tensile stresses in its axial direction. It is understood that water molecules and calcium are removed from HC gels during lignin deposition, and an anisotropic shrinkage occurs in the direction perpendicular to CMFs (Terashima *et al,* 1993). Similar processes occur between the ends of adjoining CMFs and then a tensile stress might be generated in the axial direction of CMFs as a bundle. Such a phenomenon might be similar to the effects of longitudinal shrinkage during drying. These considerations are supported by the experi-

mental result that longitudinal shrinkage has a good correlation with the longitudinal released strain (Yamamoto *et al,* 1992). This is not contradictory to the generation of perpendicular compressive growth stress because the cell-wall thickening takes place according to the repetitive depositions of CMFs and matrix substance during cell-wall maturation.

Another physical factor could affect the stress generation during the cell maturation is the diurnal change of a turgor pressure as suggested by Bamber (1978, 1987). It is considered that turgor pressure cannot directly become growth stress as discussed by Boyd (1950) but affects cell-wall maturation.

The diurnal change of turgor pressure would induce an irreversible elongation of cells, for example, tracheids and fibers increase their lengths 10–140% of the initial during cell maturation (Bailey, 1920). The newly produced cell wall with CMFs would be stretched or loosened by turgor pressure change and lignin precursor would easy to penetrate and lignin deposition occurs between gaps of the CMFs. Tensile stress generated in the stretched CMFs under high turgor pressure cannot return entirely to the original state as a consequence of obstructions by adhesive force of adjoining cells and lignin-HC deposition between CMFs. The repetition of this process accumulates residual tensile stress in the axial direction of CMFs and compressive stress in the lateral direction of CMFs.

This factor should be investigated experimentally in order to further elucidate the generation process of the longitudinal tensile stress of CMFs.

CONCLUSION

The following conclusions can be drawn from the results. As regards longitudinal growth stresses of the species that have gelatinous fibers on the upper side of a leaning stem, large tensile stresses appear in the region where the cross-sectional area of gelatinous layers is large. Black locust develops an extremely large stress, above 70 MPa at the position where the gelatinous fibers are observed. This result suggests that the gelatinous fibers are responsible for the large tensile stress in the longitudinal direction.

In respect of longitudinal growth stresses in species that have no gelatinous fibers in the upper side of a leaning stem the following conclusions can be drawn: (a) the smaller the microfibril angle, the larger the tensile stress, a tendency which is similar to the situation in normal wood including softwood; (b) the larger the tensile stress, the larger the α-cellulose content; (c) tensile stress is larger when the crystallinity is higher; and (d) tensile growth stress has no or a slightly negative correlation with lignin content. These results suggest that CMFs produce tensile stress in the longitudinal direction. A low compressive stress was always found in the tangential direction and has no correlation with the longitudinal stress.

These results suggest a positive contribution of tensile stress by microfibrils to the generation of tensile growth stress in the longitudinal direction.

The existence of the molecular attraction in amorphous HC that is located in the gaps between the ends of adjoining cellulose microfibrils could take part in the generation of the tensile stress in the axial direction of CMFs. The diurnal change of turgor pressure would indirectly affect the tensile stress generation in CMFs.

It is suggested that the cellulose microfibrils as a bundle produce the tensile stress in the axial direction. This is a natural explanation that allows interpretation of stress phenomena without any contradiction.

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