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Relation between growth stress and lignin concentration in the cell wall: Ultraviolet microscopic spectral analysis*

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Abstract Lignin content in the cell wall was investigated to examine its relation with growth stress, using an ultraviolet microscopic spectrum analyzer. Although a weak correlation existed between the growth stress and lignin concentration in the compound middle lamella, it was believed that the compound middle lamella did not contribute to compressive growth stress generation as there was no correlation between growth stress and lignin concentration in the cell corner part of the intercellular layer. In the secondary wall, larger compressive growth stress was associated with higher lignin concentration especially in the outer part. This finding confirms that lignin contributes positively to the generation of compressive longitudinal growth stresses in the compression wood and more substantially in the outer part of the secondary wall. This experimental result strongly supports our hypothesis of growth stress generation given by the model.

Key words Growth stress · Compression wood · Lignin · UV · Secondary wall

Introduction

We have discussed the generation mechanism of growth stress, using a cell wall model that takes into account the cell wall structure and the chemical components. We concluded that growth stress was generated as a complex stress comprising tensile stress induced in the microfibrils in the axial direction and isotropic compressive stress evolved in the matrix.[1–3] This result was based on the elucidation of relations between growth stress and cell wall structures and the amount of the chemical components.[4] For instance, a good correlation was recognized between growth stress and the lignin content, evaluated with Wise's method in compression wood.

However, the experimental result lacked accuracy without the qualitative analysis of the lignin concentration because Klason's lignin includes the lignin of both the cell wall and the intercellular layers. In the present study lignin content in the cell wall was investigated to examine its relation with growth stress using an ultraviolet (UV) microscopic spectrum analyzer.

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Method

Measurement of growth stress

A tilted 11-year-old sugi tree (*Cryptomeria japonica* D. Don), 8.6 m tall, 17 cm DBH, was used for the test. The stem leaned up to 1.3 m from the ground and formed compression wood on the lower side of the trunk. Released strain was measured on the standing tree in a conventional manner.[5] Eleven measuring stations were set continuously along the axial direction on the upper and lower sides of the inclined stem. Ten more stations were set around the periphery 0.6 m from the ground, where the stem was bent drastically. The measurement was carried out on June 8, 1994.

After measuring the released strains, test pieces were removed from the measuring stations for UV microscopic observation and determination of Young's moduli. The released strains were converted to the growth stresses using Young's moduli.

UV microscopic spectral analysis

A UV microscopic spectral analyzer, Carl Zeiss MPM800, was employed to evaluate the lignin concentration in the microscopic sections.

Specimen

Small sticks 4 mm square were cut from the wood blocks, which had been stored in 50% ethanol solution in a refrigerator after removing them from the measuring stations. Strips 4 mm × 200 μm × 4 mm (longitudinal, tangential, radial directions, respectively) were sliced using a sliding microtome. After dehydration in a series of ethanol-water mixtures, five or six sections were piled in strips and were embedded in epoxy resin (Epok 812 with methyl nadic anhydride and 2,4,6-triphenol), after substitution by propylene oxide. Sections 1 μm thick were cut from the embedded blocks using an ultramicrotome (Micron HM 350) and mounted on the quartz slide in a glycerol medium for UV absorption measurement.

The thickness of the thin sections were measured with an accuracy of 0.01 μm by a universal surface shape profiler (Kosaka, SE-3E) to confirm that the variation in the thickness within a section was less than the reading accuracy. If the thickness between sections was different, the UV absorption of each section was corrected with a linear relation between the UV absorption and the thickness of sections continuously sliced from a piled piece.

Estimation of lignin concentration

Absorption (A) can be obtained by

$$A = 2 - \log\left[(O - P)/(S - P) \times 100\right] \quad (1)$$

where O = intensity of UV light after passing through the objects; S = intensity of light through the cell lumen; and P = the intensity of background or stray UV light.

Estimation from Klason's lignin

The lignin concentration at the secondary wall was estimated using Klason's lignin, which was measured with wood powder prepared from around the position where the growth stresses were measured. Klason's lignin amount could be applied to each layer of the cell wall according to the ratios of the volume and the UV absorption of the secondary wall, the compound middle lamella, and the cell corner part of the middle layer, using the following relation.

$$W = aV_s + a \quad V_{cml}A_{cml}/A_s + a \quad V_{cc}A_{cc}/A_s \quad (2)$$

where W = Klason's lignin content obtained by wood powder (g/g); a = lignin concentration in the secondary wall (g/g); V = volumetric ratio of each layer of the cell wall; and A = UV absorption. The suffixes s, cml, and cc represent the secondary wall, the compound middle lamella, and the cell corner part of the intercellular layer, respectively.

The volume ratio of each cell wall layer was measured by means of cutting and weighing after taking the microscopic photograph at a wavelength of 280 nm. The measurement conditions of the UV absorption at the latewood region included an Ultrafluar (×100, N.A.1.20) as an objective lens and an Ultrafluar (N.A. 0.8) as a condenser lens. The scanning range of the wavelength was 250–310 nm, the step of the wavelength scanning was 1 nm, and the bandwidth was adjusted to 5 nm. The spot diameter was 0.5 μm for the measurements of A_{cml} and A_{cc} and 2.5 μm for A_s. Repetition of the measurement for one position was 30 times for the A_s and 45 times for A_{cml} and A_{cc} according to the results mentioned later. Measurements were taken at 15 positions and averaged to determine the UV absorpion. In the case of earlywood, because of the thinner layer the measurement was carried out using only 0.5 μm of the spot diameter and repeated 45 times for each position.

Estimation using Lambert-Beer's law

Almost all lignin of the softwood consists of the guaiacyl lignin, which has a UV absorption peak at 280 nm. Consequently, a linear relation between lignin concentration and UV absorption at 280 nm would be expected for sections of uniform thickness, so that:

$$A = \varepsilon \times C \times d \quad (3)$$

where A, ε, C, and d show UV absorption, an absorption coefficient ($cm^{-1}lg^{-1}$), the lignin concentration (g/l), and the thickness of the section, respectively. The lignin concentration (grams/gram) can be obtained after converting the unit grams per liter to grams per cubic centimeter and multiplying by the specific volume.[6] We used the value 1.07 cm^3/g for the specific volume of black spruce.[7]

The UV absorption is affected by the parallelism of the light when it passes through the section. After calculation according to Scott and Goring's method,[8] the UV absorption using nonparallel light gave values 1.06 times as large as those with parallel light. Therefore with this measurement the number divided by 1.06 was used as the UV absorption (A) in Eq. (3).

Literature values regarding ε (the UV absorption coefficient at 280 nm) range from 12.8[9] to 18.7.[10] In the present analysis we use 15.6 (that offered by Fergus et al.[6]) because the 280/305 nm UV absorption ratio was 3.44, which was similar to the 3.8 value obtained for spruce.[6]

Results and discussion

Determination of the measuring condition of UV absorption: repetition of the measurement

To measure the UV absorption at a measuring spot precisely, it would be better to measure the absorption on a spot as many times as possible to average the error caused by variations of the UV bandwidth and the electric source. On the other hand, it is necessary to shorten the time of UV exposure to avoid a reduction of UV absorption by lignin, as pointed out by Scott and Goring[8] and Takano et al.[11]

Reduced dispersion of UV absorption at 280 nm by repeating the measurement was investigated under wavelengths of 250–300 nm on one section. The maximum

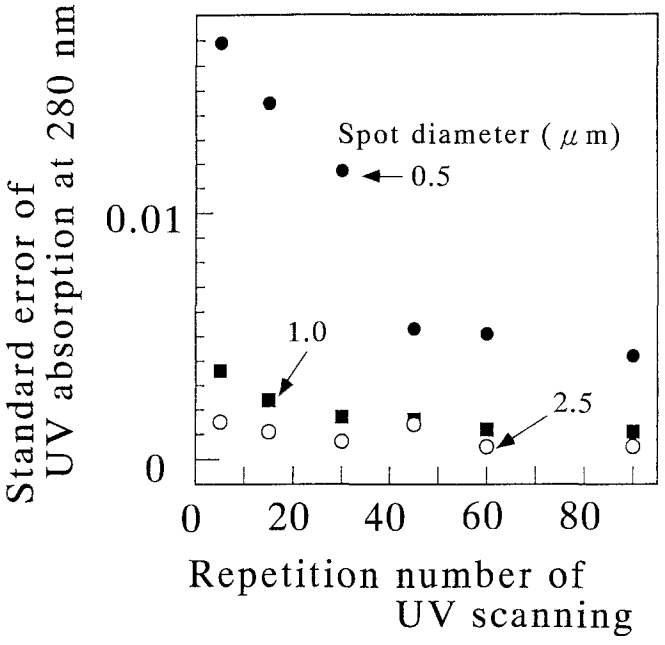

Fig. 1. Reductions in the standard error of Ultraviolet (UV) absorption with repeated scans

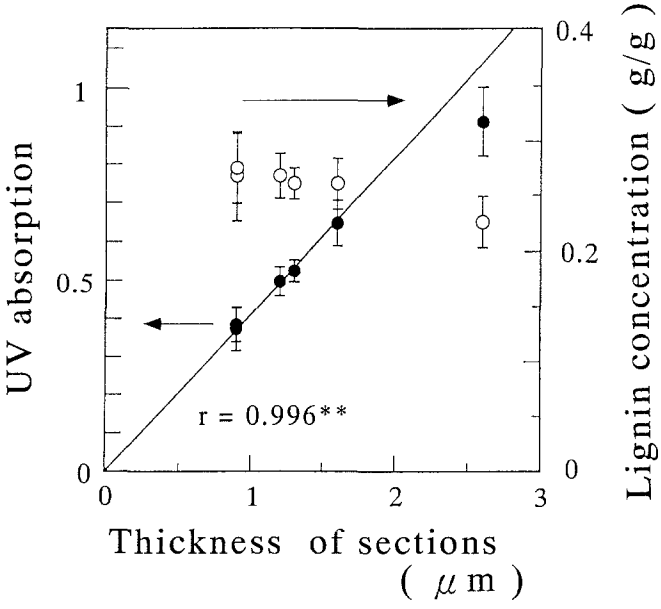

Fig. 2. Relations between thickness of sections and UV absorption and lignin concentration. *Error bars* show the standard deviation

repetition was 90 times for each spot diameter of 0.5, 1.0, and 2.5 μm.

Figure 1 shows the experimental relation between the standard error for UV absorption and the number of repetitions: the narrower the measuring area (spot diameter), the larger the standard error. This is attributable to the increase in error caused by aviations of the bandwidth and the electric source, comparing them with the light that permeates the section. The standard error is reduced as the number of measurements increases and approaches a certain value.

This result suggests that an appropriate number of repetitions is 45 for a 0.5-μm spot diameter and 30 for a 1.0-μm spot diameter.

Thickness of the section

When we measure the lignin concentration by means of Lambert-Beer's law, it is necessary to measure precisely the thickness of the section and choose a thickness that yields the lowest error for UV absorption. Six sections with various thicknesses (0.9–2.6 μm) were sliced consecutively from an embedded sample block and mounted on quartz glass; the thickness was then measured at least 15 times to obtain the average value. The overall accuracy of the thickness measurement was 0.01 μm. After measuring the thickness, UV absorption was measured 15 times for each section with various thicknesses and the results compared.

Figure 2 shows the relation between UV absorption and the thickness of the section. The sections with various thicknesses used in this measurement can be considered to have the same lignin concentration because they were cut continuously from the same block.

A straight line going through the origin of the coordinate axes fits the relation between UV absorption and section thickness. This line gave the highest correlation coefficient, with less than 1% level of risk when drawn by omitting the data from the thickest sections (of 2.6 μm). This relation supports the exactness of Lambert-Beer's law.

The lignin concentration obtained by means of Eq. (3) is also shown in Fig. 2. The differences in lignin concentrations were not recognized between sections of less than 2 μm thickness, but the thickest sections showed lower lignin concentrations (at <1% level). The fact that the UV absorption of the thickest section is not on the straight line is attributed to the fact that part of the light went not only thought the cell wall but also through the lumen owing to the nonparallelism of UV in the section.

Fujii et al. noted that a section of >4.0 μm thickness is not suitable for UV spectrum analysis.[12] In the present study we found that sections of >2.0 μm thickness could not be used for measuring lignin concentration by the UV absorption method.

Growth stresses

The distribution of growth stress in the longitudinal direction of the tree is shown in Fig. 3 and the peripheral distribution in Fig. 4. In these figures, compressive growth stresses corresponding to compression wood had existed where the release strains were positive values. The release strains show continuous distributions, similar to previous results.[1]

As observed in past investigations.[1,4,13] the longitudinal release strain has good correlations with the Klason's lignin

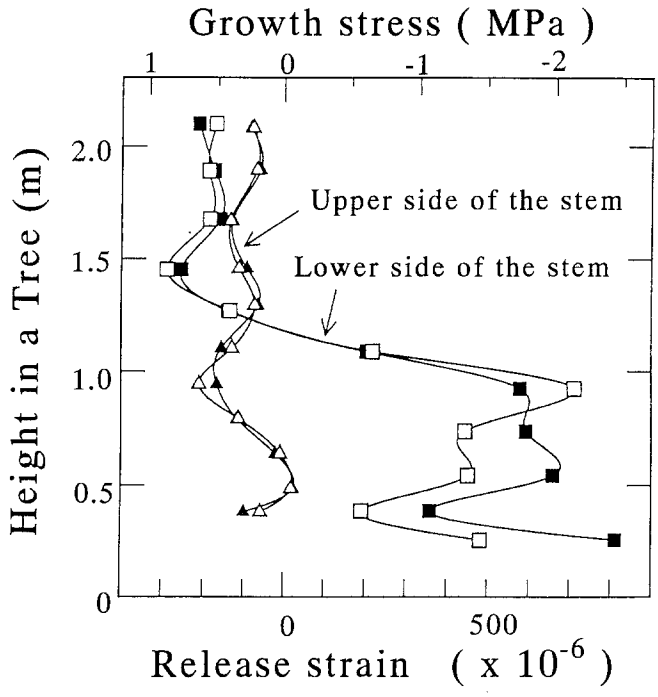

Fig. 3. Distribution of the growth stresses in the longitudinal direction along the height in a tree. A leaning stem of sugi (*Cryptomeria japonica* D. Don)

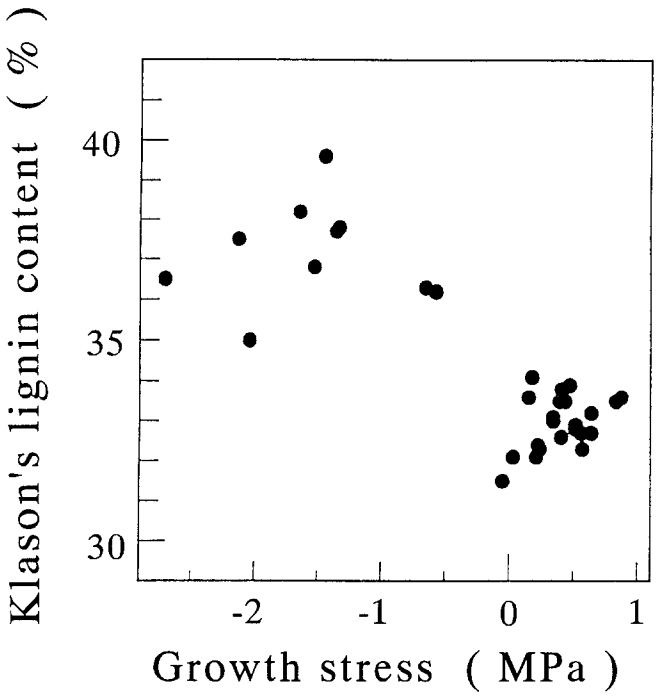

Fig. 5. Relation between Klason's lignin content and growth stress in the longitudinal direction

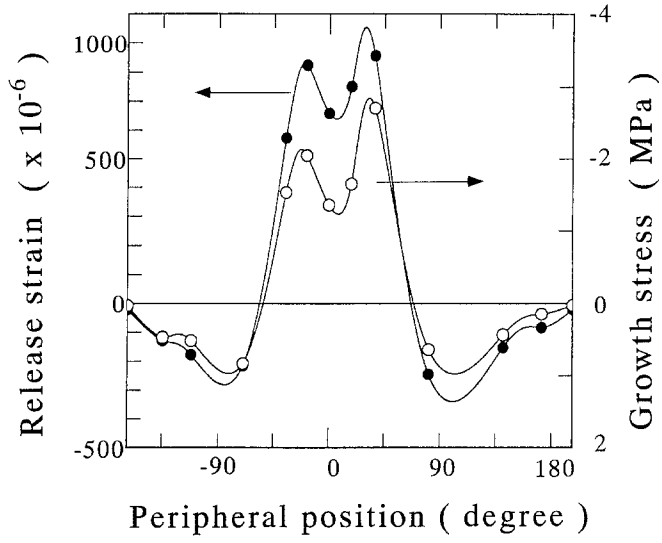

Fig. 4. Peripheral distribution of release strain and growth stress in the longitudinal direction 0.6 m above the ground

content and the cellulose microfibril angle (i.e., the larger the compressive growth stress, the larger are Klason's lignin content and the microfibril angle). These results and the mechanical analysis elucidated that the longitudinal compressive growth stress in the compression wood was generated by elongation of tracheids induced by the isotropic compressive stress, which was evolved by an irreversible swelling due to the lignin deposition between microfibrils.[2,3]

Growth stress and Klason's lignin content

Figure 5 shows a relation between longitudinal growth stress and Klason's lignin that is similar to past results. The normal wood region, where growth stress is positive, contains 32%–34% of Klason's lignin, whereas the compression wood region, where it is negative, contains much more Klason's lignin. However, a linear relation was not confirmed within the region of compression wood. Klason's lignin content usually shows a dispersion with the stress value because the wood powder cannot be prepared from exactly the same position where growth stress was measured. However, such as result is enough to estimate that compressive growth stress has been generated in a region of high lignin content. Consequently, the lignin swelling hypothesis[13,14] proposed by Boyd is supported by many researchers and by the theoretical analysis using a cell wall model[13].

Difference between the results using equations (2) and (3)

Table 1 compares the lignin concentrations obtained by Eqs. (2) and (3). High significance, within a 0.1% critical level, was recognized in the difference between the lignin concentrations at the positions that showed positive and negative stress. As shown in Table 1, the lignin concentration obtained by Eq. (2), based on Klason's lignin, was higher than that obtained from Eq. (3), that of Lambert-Beers's law. We cannot determine immediately which method is more reliable, but Klason's lignin has some prob-

Table 1. Lignin concentrations in secondary wall in normal wood and compression wood regions calculated from Eqs. (2) and (3)

Growth stresses (MPa)	Lignin concentration (g/g), mean ± SD		By Eq.
	Latewood	Earlywood	
0.58	0.288 ± 0.003	0.277 ± 0.004	(2)
	0.231 ± 0.015	0.239 ± 0.032	(3)
−2.76	0.332 ± 0.003	0.315 ± 0.006	(2)
	0.288 ± 0.021	0.289 ± 0.023	(3)

Table 2. ANOVA of difference of lignin concentration between growth stresses (factor S) in earlywood and latewood (factor P) in the secondary wall

Condition	s.s.	ϕ	V	F0	F (0.01)
S	0.213	6	0.0354	30.6	2.80
P	0.0235	1	0.0235	20.3	6.63
S × P	0.0201	6	0.0033	2.9	2.80
E	0.22	196	0.0012		

Table 3. ANOVA of difference of lignin concentration between growth stresses (factor S) in earlywood and latewood (factor P) in compound middle lamella

Condition	s.s.	ϕ	V	F0	F (0.01)
S	0.117	6	0.0194	3.48	2.80
P	0.095	1	0.0095	17.0	6.63
S × P	0.147	6	0.0244	4.37	2.80
E	1.096	196	0.0056		

lems regarding the relation with growth stress: (1) We are obliged sometimes to prepare the wood powder from regions other than where the growth stress was measured. (2) The ray tissue fraction is not taken account when preparing the wood powder. (3) As pointed out by Fukazawa[15] the fluctuation in lignin concentration is large in the compound middle lamella and the outer part of the secondary wall in the compression wood cell. Hence although Eq. (3) contains some uncertain factors, such as the value of the absorption coefficient, the lignin concentration obtained by Lambert-Beer's law is considered more reliable.

Relation between growth stress and lignin concentration in the cell wall

Figures 6–8 show the relations between growth stress and lignin concentrations in the middle layer of the secondary wall, the compound middle lamella, and the cell corner region of the middle lamella. The regression lines (a) and (b) in Fig. 6 show that an inverse relation with high negative correlation exists within the secondary wall in earlywood and latewood (i.e., the larger the compressive growth stress, the higher the lignin concentration). As shown in an ANOVA (Table 2), the lignin concentration in earlywood is higher than in latewood, which agrees with the results of Fukazawa and Imagawa[16] and Takano et al.[11] Because the interaction between earlywood and latewood is also significant, the difference of lignin concentration between earlywood and the latewood is reduced as the compressive growth stress increases. The reason the plots of the largest compressive stress (−2.76 MPa) are off the straight line are discussed in the next section.

Figure 7 shows the lignin concentration in the compound middle lamella (CML). The ANOVA results are given in Table 3. The earlywood has a higher lignin concentration than latewood in the CML. Although it appears that there is no relation between growth stress and the lignin concentration, a weak correlation exists between them within the compressive stress region. It is attributable to the fact that the UV absorption of the CML contains partly the absorption of the secondary wall due to the nonparallelism of the UV light in the section. A weak correlation could be influenced by the strong correlation in the secondary wall. Thus we can say that the lignin in the CML does not contribute to compressive growth stress generation.

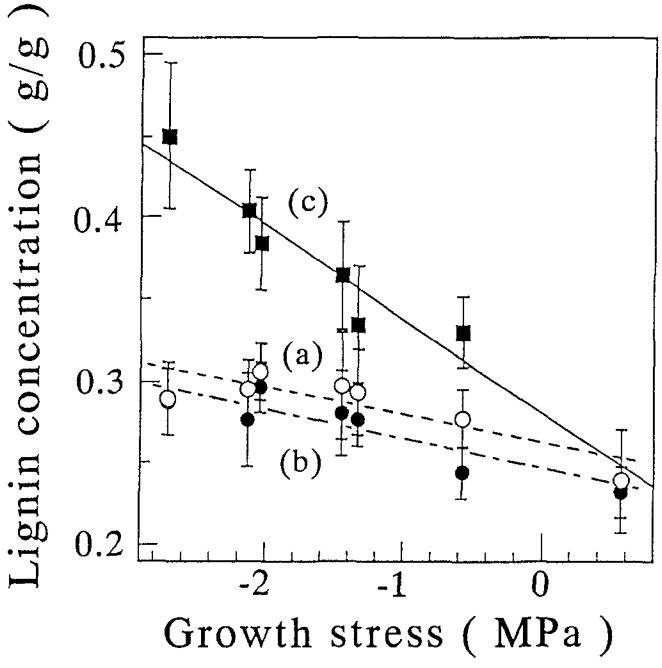

Fig. 6. Relations between growth stress and lignin concentration in the secondary (S) wall. *Filled squares* (*c*), outer part of S wall in latewood ($r = -0.941$**); *open circles* (*a*), S wall in earlywood ($r = -0.847$**); *filled circles* (*b*), S wall in latewood ($r = -0.912$**)

Figure 8 shows the lignin concentration in the cell corner region (CC) of the intracellular layer. The lignin concentration in the CC is higher than in the secondary layer and the intercellular layer. Earlywood has a higher lignin concentra-

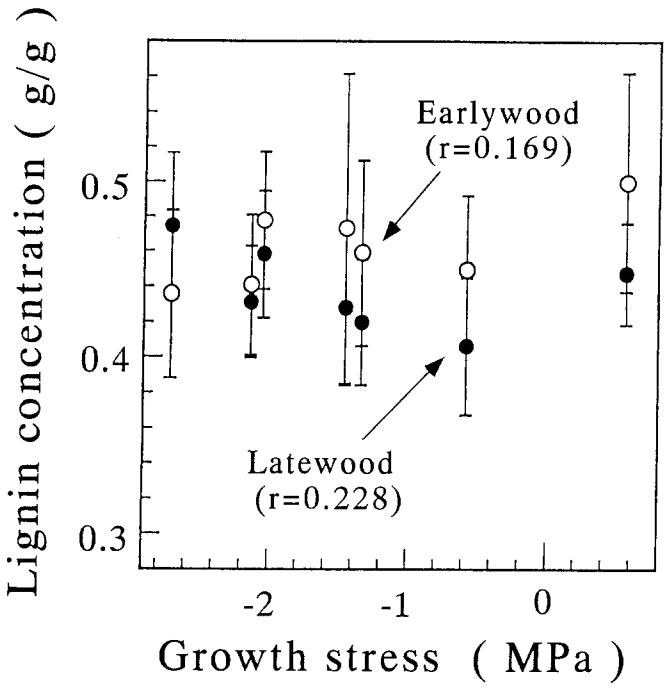

Fig. 7. Relations between growth stress and lignin concentration in the compound middle lamella

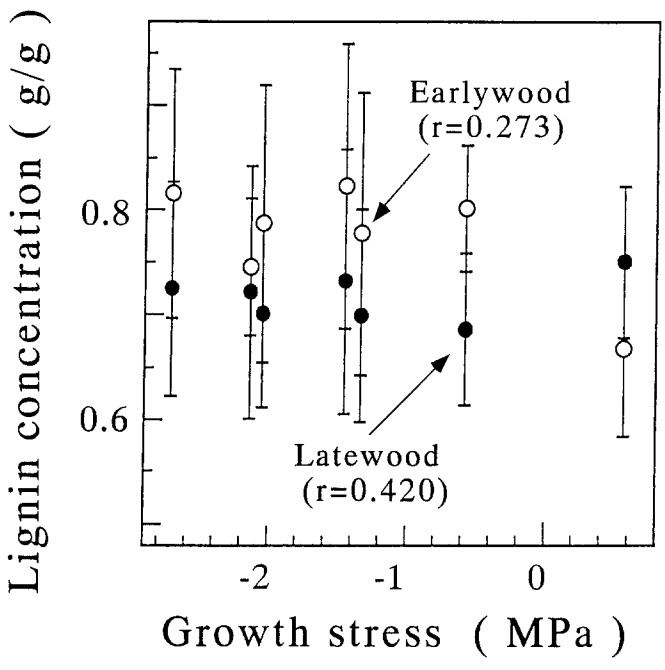

Fig. 8. Relations between growth stress and lignin concentration in the cell corner region of the intercellular layer

tion than latewood, but growth stress has no statistical relation with the lignin concentration in the CC. Thus the lignin in the CC has no positive effect on growth stress generation.

As mentioned above, it was made clear that the compressive growth stress in the compression wood increases as the lignin concentration in the secondary wall increases. Lignin in the CML and the CC does not affect growth stress generation.

Growth stress and distribution of lignin in the secondary wall

Figure 6 revealed a negative correlation between growth stress in the longitudinal direction and the lignin concentration in the secondary wall. Hereafter the distribution of lignin in the secondary wall is considered using the neighboring tracheids of latewood sections.

The UV absorption was continuously measured in the radial direction–first in the lumen and than in the secondary wall, the CML, the secondary wall, and the lumen—using UV at 280nm and a 0.5-μm spot diameter. The measurement was repeated 15 times for each section. Because each section is of varying thickness, the measured absorptions (A) were corrected to that of 1μm thickness; that is, the absorption of the section with T μm was calculated as A/T.

Figure 9 shows UV absorption patterns in double tracheids of earlywood and Fig. 10 that of latewood. The three patterns are shown for double cells corresponding to 0.58, −1.48, and −2.76MPa compressive stress, respectively.

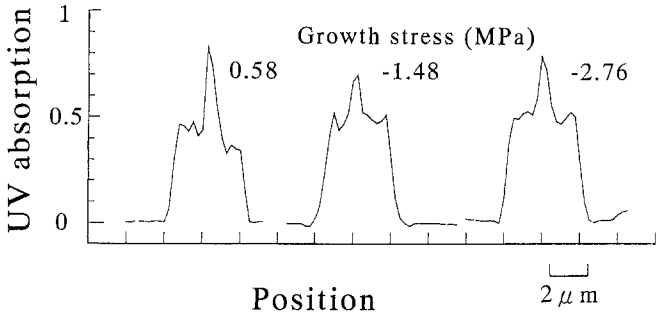

Fig. 9. Examples of the distribution of UV absorption across the earlywood double cell wall in the regions of various growth stresses

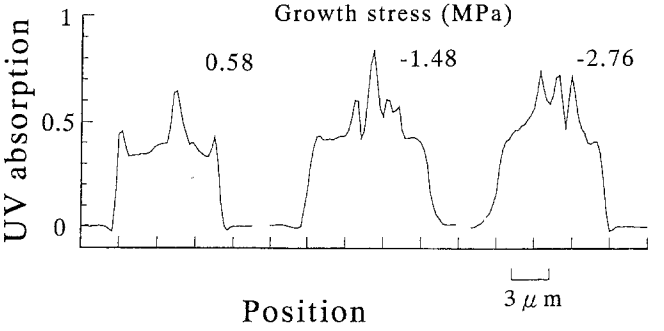

Fig. 10. Examples of the distribution of UV absorption across the latewood double cell wall in the regions of various growth stresses

In the earlywood tracheids (Fig. 9) the distribution pattern of UV absorption was almost the same within the secondary wall despite the different growth stress, but the level of the absorption increased as the compressive stress increased. In the latewood tracheids, which had large compressive stress (Fig. 10), the UV absorption peaks were recognized in the outer part of the secondary wall. The taller the peak, the larger was the compressive stress. As reported by some researches,[15,17,18] in some softwoods the outer part of the secondary wall has almost the same UV absorption as that of the CML in the region of high compressive stress.

The regression line (c) in Fig. 6 shows the relation between growth stress and the lignin concentration in the outer part of the secondary wall, which was converted from the UV absorption pattern. The relation has a high correlation coefficient (0.941), which is higher than that of lines (a) and (b) in Fig. 6. The lignin concentration obtained from the part where the largest compressive stress existed, which was off lines (a) and (b), is included in the regression equation (c).

Compressive growth stress is strongly affected by the lignin concentration in the cell wall, especially in the outer part of the secondary wall. This result strongly supports our hypothesis of growth stress generation as shown in the model. That is, the growth stress in compression wood is generated in the matrix between cellulose microfibrils as isotropic compressive stress.[1–3]

Conclusions

It had been thought that growth stress in compression wood was generated by isotropic compressive stress evolved by irreversible deposition of lignin between microfibrils. The tracheid, which has a large microfibril angle, is expanded in the axial direction by compressive stress. Consequently, compressive growth stress is generated in the longitudinal direction of compression wood. This idea was dependent on a good experimental correlation between growth stress and Klason's lignin content.

The findings of the present study confirm this correlation and provide a positive basis for the contribution of lignin to the generation of compressive growth stress in compression wood as speculated by theoretical analysis. Furthermore, it has become clear that the lignin concentration in the part of the secondary wall contributes substantially to the generation of compressive growth stress in the longitudinal direction.

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