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ORIGINAL ARTICLE

Koji Murata · Minoru Masuda

Microscopic observation of transverse swelling of latewood tracheid: effect of macroscopic/mesoscopic structure

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Abstract In order to study the transverse swelling/shrinkage of wood, the microscopic swelling behavior of latewood tracheid was observed using confocal laser scanning microscopy and the digital image correlation method. A microcrater structure was created on the surface of the specimen by using the ion sputter etching technique to obtain a pattern-rich digital image for image analysis. Douglas fir specimens were conditioned by two methods of absorption: rapid absorption of moisture from hot steam, and slow absorption of moisture from the water vapor of saturated solutions. Latewood tracheid near the surface of the specimen deformed only in the radial direction when the relative humidity of the surrounding air changed rapidly (rapid absorption of moisture from hot steam or absorption/desorption of moisture during the observation). In addition, the diameter of the lumen decreased upon rapid absorption of moisture, whereas it expanded upon slow absorption of moisture. These results indicate that the microscopic swelling behavior of latewood cells is strongly influenced by the macroscopic/mesoscopic structure, for instance, the cell arrangement or the alternation of latewood and earlywood.

Key words Microscopic swelling behavior · Latewood trachied · Confocal laser scanning microscopy · Digital image correlation

Introduction

Wood is a hygroscopic material, the moisture content of which depends on the surrounding water vapor pressure. The hygroscopic nature of wood is advantageous when wood is used in interiors because it can condition the humidity of the surrounding air. On the other hand, the same hygroscopicity causes undesirable movements, such as shrinkage or swelling, and the formation of weak points in the wood material resulting in twists or bows in the timber. Wood is an orthotropic material, and its swelling/shrinkage behavior depends on the material direction. Apart from this, the mechanism of swelling/shrinkage is complicated, particularly, in the transverse direction. The mechanism of transverse swelling/shrinkage with changing moisture content is summarized as follows:[1–3]

1. Interaction between two tissues that differ in their properties, such as the restricting effect of rays on a radial plane and the earlywood–latewood interaction.
2. Elastic anisotropy that depends on the geometric factor of cell shape and cell arrangement.
3. Ultrastructure and chemical distribution in the cell wall.

Many researchers have described the restricting effect of rays and the earlywood–latewood interaction. We[4] also succeeded in visualizing the interaction by using the digital image correlation method (DIC). Gibson and Ashby[5] discussed the elastic anisotropy of cellular structure on elasticity, and, recently, Watanabe et al.[6] discovered that the anisotropy of transverse shrinkage of earlywood is related to the deformation of the cell. Mikajiri et al.[7] observed deformations of the cell and lumen at low moisture contents by using low voltage scanning electron microscopy (LVSEM). The effect of ultrastructure or chemical distribution in the cell wall has been theoretically analyzed in relation to the small difference in microfibrillar angle[2] and the difference in the degree of lignification[8] between the radial and tangential cell wall. However, the microscopic behavior of the cell wall with changing moisture content has not been directly observed. We[9] also observed the deformation of the cell and lumen with increasing moisture content using DIC and confocal laser scanning microscopy (CLSM). However, it was difficult to observe the microscopic behavior of the cell wall because the images of cross sections of the cell wall show poor contrast and pattern for DIC analysis.

K. Murata (✉) · M. Masuda
Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan
Tel. +81-75-753-6235; Fax +81-75-753-6300
e-mail: murata@kais.kyoto-u.ac.jp

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In order to obtain a high contrast image with a rich pattern, we created a microcrater structure on the cross section of the cell wall using the ion sputter etching technique. CLSM allows observation of the surface of the semi-transparent material, i.e., the wood cell wall, and the microcrater structure could be visualized as a high contrast and pattern-rich image. When the microcrater structure was created on the surface of coniferous wood specimens, we[10] succeeded in directly observing the microscopic swelling behavior of latewood cell wall on absorption of moisture from hot steam. In this article, we discuss the differences in the cell wall deformation under conditions where the relative humidity changes rapidly and slowly.

Material and methods

Specimens

All samples were cut from air-dried Douglas fir (*Pseudotsuga menziesii*). The interval between annual rings was 1.0–1.5 mm, and the thickness of latewood layers was 0.3–0.5 mm. The dimensions of the cubic specimens were approximately 7 (tangential) × 7 (radial) × 7 (length) mm. The end plane was softened by impregnating with water at room temperature for a few days, and, subsequently, it was planed using a sliding microtome. The planed specimens were dried for a few months with silica gel in a desiccator and were conditioned for 1 month with $CaCl_2$. Thus, the moisture content was reduced to 1%–2%.

The conditioned specimens were etched using the ion sputter system (JFC-1100E, Jeol). The current was 10 mA, the gas pressure in the chamber was 10–20 Pa, and the process time was 10 min. A total of 12 specimens were prepared.

Conditioning and observation of specimens

All specimens were conditioned for 1–3 weeks in a desiccator that contained saturated solutions of LiCl, $MgCl_2$, $Mg(CO_3)_2$, NaCl, or KCl. The specimens were conditioned at room temperature and they were observed using CLSM, ensuring only a small variation in the conditioning temperature. The weights of almost all the specimens did not change after 1 week of conditioning, indicating that the moisture contents of the specimens were in equilibrium with the water vapor pressure in the desiccator. The cross section (end plane) of the latewood cell wall of the dried etched specimens was observed using CLSM (1LM15H, Lasertec).

Digital images of CLSM were 640 × 480 pixels in size. One pixel is equivalent to 0.365 μm and 0.073 μm in the case of the 20× and 100× objective lens, respectively. After conditioning with the water vapor of a saturated solution, the same area was observed by the same procedure. The moisture contents of the dried specimens were gradually increased by sequential conditioning with saturated solutions of LiCl, $MgCl_2$, $Mg(CO_3)_2$, NaCl, and KCl. On the other hand, we also observed the swelling behavior of specimens that rapidly absorbed moisture from hot steam for 1 min. The temperature of hot steam discharged from a humidifier was 47°C. Obtaining a still image of a cell immediately after steaming was impossible because the cell was moving rapidly. However, after 30–60 sec, the cell movement was sufficiently slow so as to enable microscopic observation by CLSM. In this study, this cell state that could be observed was defined as the state of rapid absorption of moisture from hot steam. Eight conditioned specimens were used to study the slow absorption of water vapor of the saturated solution, and the remaining four specimens were used for studying the rapid absorption of hot steam.

Table 1. Average moisture contents of eight specimens conditioned with saturated solutions

Solution	MC	Weight gain[a]	Atmospheric temperature	Atmospheric humidity
LiCl	3.5%	0.4%	25.0°C	39% RH
$MgCl_2$	5.3%	0.2%	25.6°C	38% RH
$Mg(NO_3)_2$	7.8%	0.0%	25.3°C	59% RH
NaCl	11.8%	–0.2%	27.8°C	68% RH
KCl	13.3%	–0.2%	28.1°C	58% RH

MC, moisture content; RH, relative humidity
[a] Weight increment during observation

The day (or weather) selected for observation was such that it was optimal for preventing the absorption/desorption of moisture by the specimens during the observation period. In other words, the specimens with low moisture contenting were observed under low relative humidity conditions (i.e., on a clear day), whereas the specimens with high moisture content were observed under high relative humidity conditions (i.e., on a rainy day). The moisture contents of the specimens were measured at each stage of moisture conditioning using the oven-dry method (105°C, 2 days), after all the observations had been carried out.

Table 1 lists the moisture contents of the conditioned specimens. Variation in the moisture contents among the specimens was less than 0.5% at every stage, and a limited increment/decrement in weight was found after the CLSM observation. The changes in moisture content that occurred during the observation are also shown in Table 1. These changes in moisture contents were small (less than 0.5%). However, these were the average values of the whole specimen, and the changes in the moisture content were greater near the surface. The increase in nominal moisture content was approximately 3%, when specimens rapidly absorbed moisture from hot steam for 1 min. However, a large gradient in moisture content might be present around the surface. In this study, the basis of the measurement of the swelling strain was the specimen that was conditioned with the LiCl saturated solution. Thus, the swelling strain at a moisture content of approximately 3.5% was defined as zero. Furthermore, a "slowly absorbing specimen" refers to a specimen that absorbed moisture slowly until it was in equilibrium with the water vapor pressure of the saturated solution, and a "rapidly absorbing specimen" implies a specimen that rapidly absorbed moisture from hot steam.

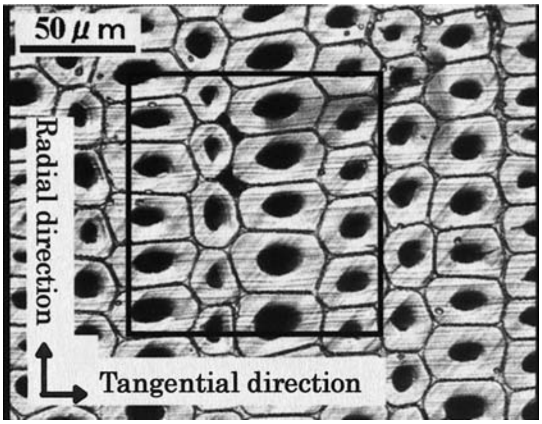

Fig. 1. Square area for the observation of latewood swelling

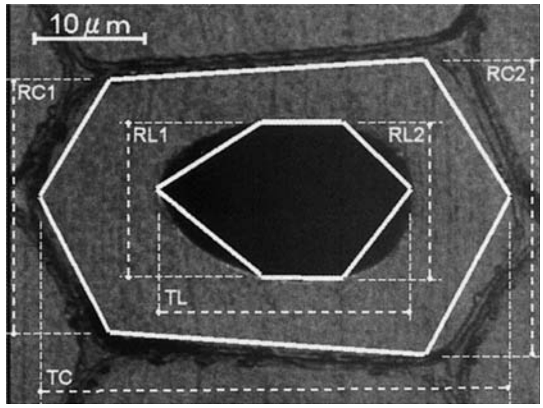

Fig. 2. Hexagons inscribed in cell and lumen

Analysis method

Anisotropy of swelling behavior of latewood

In the previous study[4], it was clarified that tangential swelling of latewood is hardly restricted by earlywood. Therefore, the swelling behavior of the latewood area in a whole specimen is almost equal to that of the isolated latewood. In order to understand the behavior of latewood, we analyzed the images of latewood that were obtained using a 20× objective lens with DIC. The square area of 300×300 pixels that was analyzed corresponded to approximately $110 \times 110\,\mu$m, and it included approximately four tracheids in the tangential direction and approximately five or six tracheids in the radial direction (refer to Fig. 1). The radial and tangential swelling strains were calculated from changes in the distance between the corners of the analyzed square. The size of the square must be synchronized with the periodicity of cell arrangement in order to obtain the true value of the tangential and radial swellings; however, this was found to be extremely difficult. In this study, the subset area for pattern matching of the corners was 31×31 pixels (approximately $11.3 \times 11.3\,\mu$m). Because the subset area was large enough to trace the latewood tracheid (larger than half of the tracheid) and the square included a number of tracheids, the effect of disagreement with the periodicity appears to be inconsequential.

Change in diameters of latewood tracheid with swelling

Based on the results of several studies[11] performed over the past decades, it has been suggested that the cell lumen remains essentially constant when wood shrinks or swells, because, for many wood species, the mean ratio of maximum percent shrinkage/swelling to specific gravity was almost equal to the fiber saturation point. However, there were wide variations in the ratio, which was believed to result from the variation in the fiber saturation point or slight shrinkage/swelling of the cell lumen. In addition, Quirk[12] found that the lumens of the earlywood cells of Douglas fir expanded to some extent during the shrinkage of the wood, while those of latewood contracted. For the purpose of analyzing such deformations of the cell and lumen, the deformation of the latewood tracheid, which was not adjacent to the ray tissues, was observed in detail. We observed a tracheid with a 100× objective lens and using DIC, we traced vertices of virtual hexagons inscribed in the cell or lumen, that is, the primary wall side or the lumen side of the cell wall (Fig. 2). Tangential and radial expansion of the tracheid or lumen were calculated from the change in the diameter of the hexagon. In Fig. 2, TC and TL refer to the tangential diameters of the tracheid and lumen, respectively, and (RC1 + RC2)/2 and (RL1 + RL2)/2 are the radial diameters of the tracheid and lumen, respectively. It is essential for the vertices to coincide with the corner in the middle lamella in order to precisely analyze the change in cell shape. However, because the aim of this analysis was to measure the radial and tangential swelling strains, pairs of vertices selected for the radial swelling measurement and for tangential swelling measurement were aligned along the vertical axis and horizontal axis, respectively. The virtual hexagon in the case of the lumen coincided with the maximum radial diameter and the maximum tangential diameter of the lumen. Furthermore, when a tracheid underwent excessive deformation on absorption of moisture, it was often impossible to trace the hexagon using DIC. The positions of the vertices were manually adjusted in order to trace the hexagon.

Swelling behavior of the cell wall

We analyzed the microscopic swelling behavior of the cell wall using the images that were obtained in the above analysis. As shown in Fig. 3, virtual nodes were set at every 20 pixels (approximately $1.5\,\mu$m) on the tangential wall (T-wall) and the radial wall (R-wall). Swelling strains at the node were analyzed using DIC. Four virtual nodes were set along the circumferential direction and were averaged to determine the swelling strain distribution along the direction of the thickness of the cell wall. In this study, the method of calculation of the swelling strain differed from that used in the above analysis. The subset pattern of the

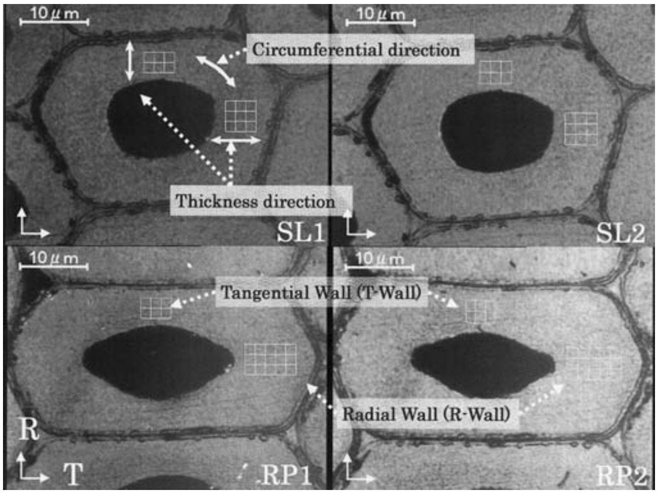

Fig. 3. Analyzed areas for the observation of cell wall. *Top*: *SL*, slowly absorbing specimen. *Bottom*: *RP*, rapidly absorbing specimen. *SL1* and *RP1*, the dried tracheid before absorbing moisture; *SL2*, in equilibrium with vapor of KCl solution; *RP2*, after hot steaming

node was matched with the six variables shown in Eq. 1, and the strains were calculated with Eq. 2. In this analysis, the strains refer to the value of the subset area (25 × 25 pixels, approximately 1.8 × 1.8 μm) at the virtual node of interest. The calculation time increased substantially with the pattern that matched six variables. We used the Newton-Raphson method[13] to accelerate the calculation with DIC.

$$S\left\{u, v, \frac{\partial u}{\partial x}, \frac{\partial u}{\partial y}, \frac{\partial v}{\partial x}, \frac{\partial v}{\partial y}\right\} = \frac{\sum\sum a \cdot b}{\sqrt{\sum\sum a^2} \cdot \sqrt{\sum\sum b^2}} \tag{1}$$

where x and y are the tangential and radial coordinates, respectively, u and v are the tangential and radial displacements, respectively, and a and b are undeform and deform images, respectively.

$$\varepsilon_x = \frac{\partial u}{\partial x} + \frac{1}{2}\left\{\left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial v}{\partial x}\right)^2\right\}, \quad \varepsilon_y = \frac{\partial v}{\partial y} + \frac{1}{2}\left\{\left(\frac{\partial u}{\partial y}\right)^2 + \left(\frac{\partial v}{\partial y}\right)^2\right\} \tag{2}$$

where ε_x and ε_y are the tangential and radial swelling strains, respectively.

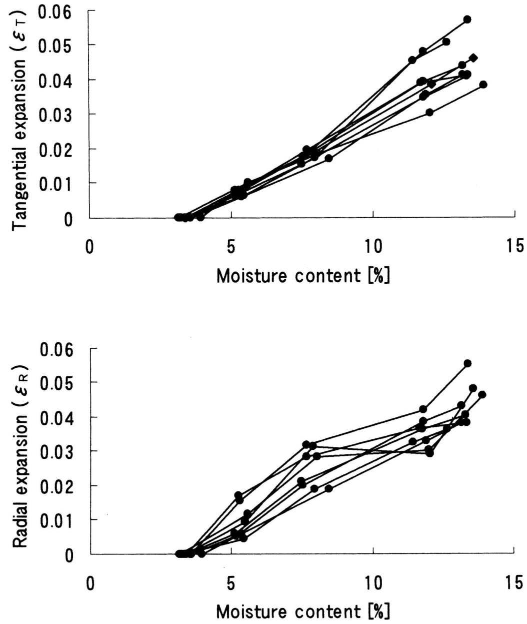

Fig. 4. Relationships between moisture content and swelling of latewood. *Top*, tangential expansion; *bottom*, radial expansion

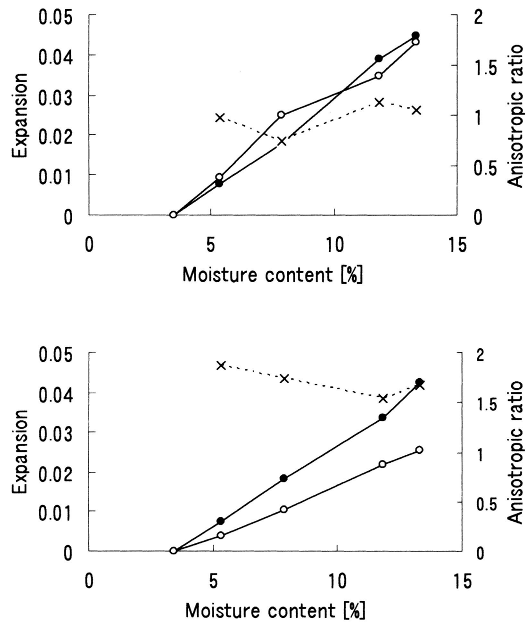

Fig. 5. Average tangential and radial expansion, and anisotropy of swelling expansion. *Top*, latewood only; *bottom*, whole specimens; *filled circles*, tangential expansion; *open circles*, radial expansion; *crosses*, anisotropic ratio

Results and discussion

Anisotropy of the swelling behavior of latewood

Figure 4 shows the relationship between the moisture content and the swelling strain of slowly absorbing specimens. In both the tangential and radial directions, the latewood area expanded linearly on absorption of moisture; however, in the radial direction the expansion was slightly irregular. Figure 5 (top) shows the changes in average expansion and the anisotropy of the swelling expansion, where the anisotropic ratio implies the ratio of tangential expansion (ε_T) to the radial expansion (ε_R). The anisotropic ratio of latewood has an almost constant value of approximately 1.0. For the purpose of comparison, Fig. 5 (bottom) shows the swelling behavior of the block specimens measured using a screw micrometer. The tangential swelling of the whole specimen was almost equal to that of the latewood area. However, the radial swelling of the whole specimen was smaller than that of the latewood area such that the anisotropic ratio ($\varepsilon_T/\varepsilon_R$)

of the specimens was 1.5–1.8. We presumed that the isolated earlywood, which has a low density, will move to a limited extent with an increase in the moisture content. However, in the tangential direction, the earlywood area in the whole specimen expanded to a great extent on account of the adjacent latewood layer, which has a large density. This interaction between earlywood and latewood will cause that the anisotropic ratio ($\varepsilon_T/\varepsilon_R$) of the whole specimen is such large.

In Table 2, the behavior of the rapidly absorbing specimen is compared with that of the slowly absorbing specimen that was conditioned with the vapor of the KCl solution. The latter specimen expanded equally in the tangential and radial directions. On the contrary, it was noted that the rapidly absorbing specimen did not expand in the tangential direction. On a different day, we also measured the change in dimensions of the other specimens upon rapid absorption of moisture ($n = 8$). The weight increased by approximately 1.6%, based on the conditioning with $CaCl_2$, and the whole specimen expanded by 0.2% in both the radial and tangential directions. Accordingly, only the surface of the specimen appeared to swell by rapid absorption of moisture.

Change in diameters of latewood tracheid with swelling

Figure 6 and Table 3 show changes in the diameter of latewood tracheids and their lumens. With regard to the slowly absorbing specimen, the relationship between the moisture content and the diameter of the tracheid was similar to the result described in the last section, and the diameter of the lumen also expanded with an increase in the moisture content. Only one tracheid lumen expanded to a large extent in the radial direction (Fig. 6; right bottom). This might be attributed to the shape of the cell that appeared as a pentagon. However, on rapid absorption of moisture, the diameter of the lumen contracted to a large extent. Moreover, the contraction in the radial direction was greater than that in the tangential direction.

Swelling behavior of the cell wall

Examples of typical swelling behavior are shown in the swelling distributions in Fig. 7. In the slowly absorbing specimen (Fig. 3; top), the swelling strains tended to be unrelated to the distance from the edge (see filled symbols in Fig. 7). The swelling distribution curves were found to be noteworthy in some of the other specimens; however, the distribution curve depended on the individual specimen and was probably influenced by the adjacent tracheids.

Table 2. Swelling expansion of latewood with increasing moisture content

Adsorption	Number of specimens	MC	Tangential swelling	Radial swelling
Hot steaming	4	4.6%	–0.004	0.032
Vapor of KCl solution	8	13.3%	0.045	0.043

Table 3. Swelling expansion of cell shape and lumen at latewood tracheid

Adsorption	Number of cells	MC	Cell		Lumen	
			Tangential	Radial	Tangential	Radial
Hot steaming	2	4.3%	0.007	0.041	–0.048	–0.072
Vapor of KCl solution	8	13.3%	0.044	0.043	0.041	0.046

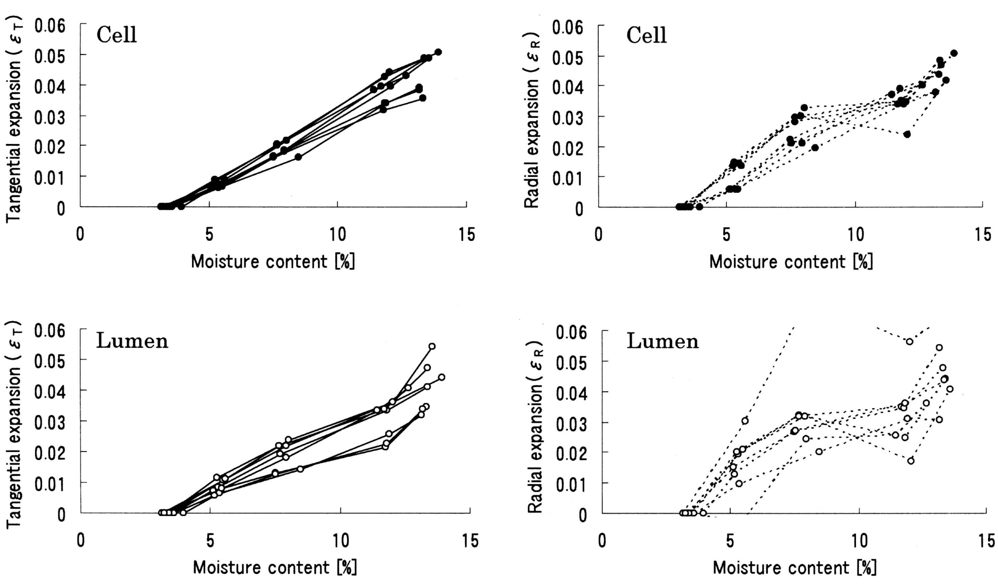

Fig. 6. Relationships between moisture content and swelling of latewood tracheid. *Top* (*filled circles*), diameter of cell; *bottom* (*open circles*), diameter of lumen; *left* (*solid lines*), tangential expansion; *right* (*dotted lines*), radial expansion

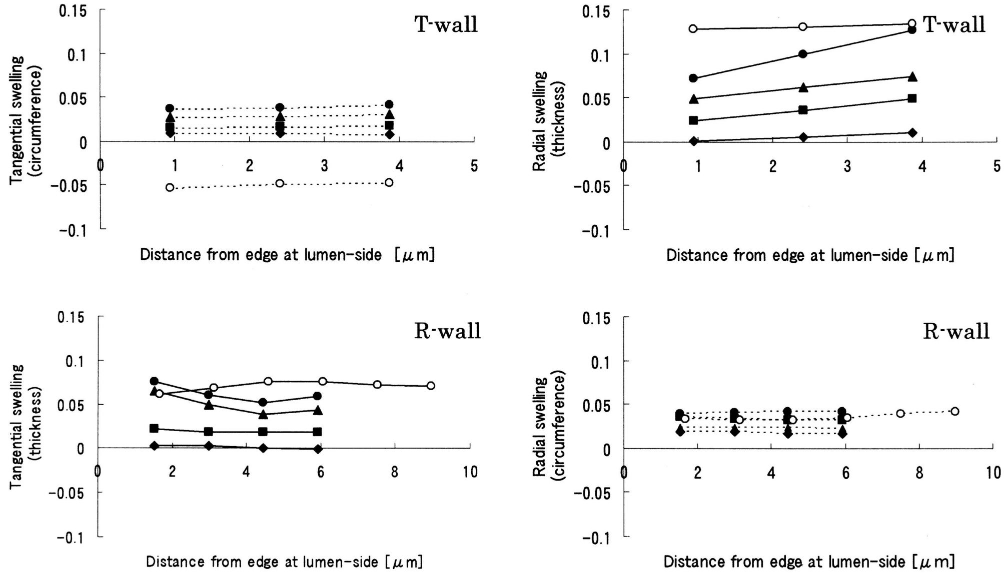

Fig. 7. Microscopic behavior of tangential wall (*T-wall*) (*top*) and radial wall (*R-wall*) (*bottom*). *Left*, tangential swelling; *right*, radial swelling. *Solid line* and *dotted line* indicate thickness swelling and circumferential swelling, respectively. *Diamonds*, moisture content 5.5% ($MgCl_2$); *squares*, 7.9% [$Mg(NO_3)_2$]; *triangles*, 12.1% (NaCl); *filled circles*, 13.6% (KCl); *open circles*, after hot steaming

Both T-wall and R-wall tended to expand more in the direction of thickness than in the circumferential direction. Meanwhile, in the rapidly absorbing specimen (Fig. 3; bottom), both types of cell walls, T-wall and R-wall, expanded to a greater extent in the direction of thickness (see open symbols in Fig. 7). However, only the T-wall did not expand in the circumferential direction; instead, it appeared to contract slightly.

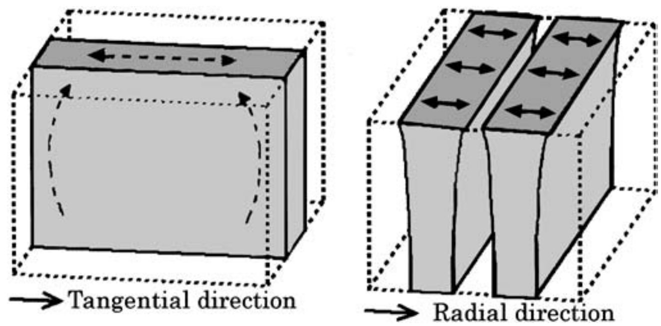

Fig. 8. Swelling models for rapidly increasing moisture in the surface region. *Solid lines* and *dotted lines* mean latewood and earlywood, respectively. *Left* model shows restriction of the inner latewood in the tangential direction, and *right* model shows swelling of the surface latewood in the radial direction

The difference in swelling behavior depends on the swelling rate

Based on the above mensioned observation, when a specimen rapidly absorbed moisture from hot steam or when it rapidly adsorbed/desorbed moisture during the observation period, the moisture content would change only on the surface resulting in a large moisture gradient in the specimen. Consequently, the latewood did not swell in the tangential direction but swelled rapidly in the radial direction, while the diameter contracted mainly in the radial direction.

The swelling behavior can be explained to occur by the following mechanism. As the relative humidity of the surrounding atmosphere changes rapidly, the surface of a specimen will absorb/desorb moisture; however, the moisture content of the inner regions of the specimen does not increase/decrease instantly. Accordingly, the swelling/shrinkage can occur only on the surface. A coniferous wood specimen comprises alternating layers of earlywood and latewood in the radial plane (Fig. 8; right). Thus, there are two types of planes in the tangential direction, that is, a plane of only earlywood cells and a plane of only latewood cells (Fig. 8; left). Young's modulus of latewood is large and that of earlywood is very small, so that the earlywood layer cannot affect the adjacent latewood layer.[4] If latewood cells on the surface try to expand or contract with increasing moisture levels, the inner latewood, which does not absorb/desorb moisture, will restrict the surface latewood cells in the tangential direction. On the contrary, the radial restriction caused by the inner latewood will be small because the radial plane is alternatively laminated with earlywood and latewood, and the rigid latewood layer is thin. As a result, the soft earlywood layer between the latewood layers will not disturb the expansion of the surface; hence, the rapid change in relative humidity affects only the radial swelling/shrinkage on the surface of the specimen.

A similar restriction mechanism can explain the observation that the lumen diameter is also easily affected by the rapid change in the relative humidity. The lumen diameter of the rapidly absorbing specimen contracted to a large extent, while that of the slowly absorbing specimen expanded irregularly with an increase in the moisture content. In both types of specimens, the lumen diameter changed to a greater extent in the radial direction than that in the tangential direction. This could probably be attributed to the microscopic composition of the cell wall or the shape of the latewood cell. The microfibrils in the outermost (S1) and inner (S3) layers are largely aligned along the transverse direction of the cell. The S1 layer is thin,[14] and the concentration of lignin in the compound middle lamella is high.[15] With the rapid change in moisture content, these layers prevented the cell wall from swelling in the outward direction; this effect is known as the "Crossband Effect."[16] On account of this, the cell wall (mainly S2 layer) expands only in the inward direction, i.e., the luminal side. In order to demonstrate that the S1 and S3 layers resist swelling, Nakano[17] thermodynamically analyzed the isotherm curve of wood block and powder. In the present study, the results of the rapidly absorbing specimen also suggested the restricting effect. Furthermore, almost all latewood tracheids are rectangular and have long edges along the tangential plane, and the curvature of the R-wall is larger than that of the T-wall. Hence, the R-wall with the large curvature hardly expanded along the direction of the thickness, whereas the T-wall with the small curvature could swell easily such that the lumen might contract to a great extent in the radial direction. In addition, a specimen is able to absorb moisture not only from the cross section of the cell wall, but also from the inner surface of the lumen. Hence, the contraction of the lumen diameter was possibly caused by the moisture gradient between the luminal side and the primary wall side of the cell wall.

Upon rapid absorption of moisture, the T-wall did not expand in the tangential direction; instead, it appeared to contract slightly (Fig. 7). This behavior was also observed in the previous study.[9,10] As stated above, the surface of the latewood that rapidly absorbed moisture appeared to be restricted by the inner latewood, which absorbed no moisture. Assuming that the cell wall of a multilayered structure will swell mainly in the direction of thickness, the swelling stress of the R-wall would develop largely in the tangential direction whereas that of the T-wall would develop to a small extent in the same direction. Because the inner latewood is restricted in the tangential direction, the expansion of the R-wall would result in a slight contraction of the T-wall until equilibrium of the swelling stress.

Conclusions

The swelling behavior of coniferous latewood was observed using confocal laser scanning microscopy and the digital image correlation method. The results are as follows:

In a specimen that rapidly absorbed moisture from hot steam, the latewood specimen mainly expanded in the radial direction.

When the specimen slowly absorbed moisture until it was in equilibrium with the water vapor pressure of the saturated solutions, the expansion of the latewood in both the radial and tangential directions showed a linear relationship with the increase in moisture content. However, the radial expansion was slightly irregular.

In a specimen that had rapidly absorbed moisture from hot steam, the diameter of the specimen tracheid lumen contracted. However, the tracheid lumen irregularly expanded when the specimen absorbed moisture slowly.

In summary, it appears that a latewood tracheid can deform only in the radial direction after rapid change in the relative humidity of the surrounding air. Furthermore, the deformation of the lumen is probably much more sensitive to the rapid change in the relative humidity.

These results indicate that the microscopic swelling behavior of latewood cells is strongly influenced by the macroscopic/mesoscopic structure, for instance, the cell arrangement or the alternation of latewood and earlywood.

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