ORIGINAL ARTICLE

Yutaka Ishimaru · Ikuho Iida

Transverse swelling behavior of hinoki (*Chamaecyparis obtusa*) revealed by the replica method

Abstract Transverse swelling and its anisotropy in hinoki (*Chamaecyparis obtusa*) in several kinds of organic liquids and in water were investigated by means the replica method. There was more cross-sectional swelling of cell walls and cell wall thickness in earlywood than in latewood. Marked swelling toward cell lumens was observed in wood swollen in liquids that had higher swelling potentials than water. This suggests that the swelling of cell walls in these liquids is much greater than the external swelling. Feret's diameters of the cell lumens were reduced by swelling in all the observed cases except in the tangential direction of earlywood, suggesting that cell walls swell to a much less extent in width than in thickness. Deformation of cell shapes caused by the tensile force from the latewood were observed in the earlywood and in the transitional region from earlywood to latewood. When swollen in water, transverse swelling anisotropy caused only by the swelling in cell wall thickness were calculated to be 1.2 for the whole region over an annual ring and 1.4 for the earlywood. These values could not account for the external swelling anisotropy of 2.1. Considering obvious deformations of cell shapes in the earlywood and in the transitional region, we conclude that the interaction between earlywood and latewood is one of the prime factors contributing to the transverse swelling anisotropy of coniferous wood.

Introduction

Transverse swelling anisotropy in wood has been studied for many years, and various explanations of anisotropic swelling have been proposed. The factors considered to be of primary importance for anisotropic swelling are (1) interaction between earlywood and latewood,[1,2] (2) restriction of radial swelling by ray tissues,[3] (3) differences in microfibril orientation in the tangential and radial cell walls,[4] (4) differences in chemical composition (especially lignin content) in tangential and radial cell walls,[5-7] and (5) differences in the sum of cell wall thickness in the tangential and radial directions.[7,8] Factors (1) and (2) relate to the interaction between different tissues with different swelling potentials. Factors (3) and (4) are based on swelling anisotropy of the cell wall itself, and factor (5) relates to the shape of the cell cross section and the thickness of cell walls. Major investigators[7,9] do not currently considered (1) as a prime factor for anisotropic swelling, with only a few exceptions.[10] Thin cross sections of wood have usually been used to investigate transverse anisotropic shrinkage. However, minute cracks occurred during the sectioning, and the sections were necessarily fixed by some procedure to prevent their curling. Therefore, a thin section does not represent the actual shrinkage (swelling) behavior of the whole wood. On the other hand, Adachi et al. used the replica method, which duplicated wood surfaces on a plastic film softened by heating to investigate the transverse anisotropic swelling behavior of some hardwoods in several liquids and showed that this method was useful for tracing cell deformation of whole wood in the same position.[11]

In this study, the transverse swelling behavior of a coniferous wood in water and in some organic liquids was investigated using the replica method to obtain new information regarding the factors affecting the transverse anisotropic swelling of coniferous wood.

Y. Ishimaru (✉) · I. Iida
Laboratory of Wood Technology, Faculty of Agriculture, Kyoto Prefectural University, Shimogamo Nakaragi-cho, Kyoto 606-8522, Japan
Tel. +81-75-703-5638; Fax +81-75-703-5639
e-mail: ishimaru@kpu.ac.jp

Materials and methods

Wood samples

Wood samples to be used for external swelling measurements and for making replicas were obtained from the outer region of heartwood in a log of hinoki (Japanese cypress: *Chamaecyparis obtusa*) with 1.0mm average annual ring width. For external swelling measurements, more than 20 cross-sectional wood pieces of 40 (R) × 20 (T) × 4 (L) mm were successively cut from a wood stick with a cross section of 40 (R) × 20 (T) mm. Their dimensions were measured using a screw micrometer in the tangential (T) and radial (R) directions with a precision of 0.01mm after swelling in water (1 week at 30°C), vacuum drying (more than 50h at room temperature), and oven drying (15h at 105°C). The samples with abnormal shrinkage were discarded. These almost successive pieces were divided into four groups of five pieces each, which were used to determine swelling in different liquids.

More than 24 samples for making replicas of 20 (R) × 10 (T) × 15 (L) mm were successively cut from a wood stick with a cross section of 20 (R) × 10 (T) mm. Four edges of a transverse surface of each sample were cut diagonally to obtain a transverse surface of about 10 (R) × 5 (R) mm. After the samples were soaked in distilled water under vacuum, the transverse surfaces were smoothed with a sliding microtome. These successive pieces were divided into four groups of six pieces each; they were then swollen in various organic liquids.

Liquids used as swelling agents

The liquids used as swelling agents are listed in Table 1. These liquids swell wood to different extents. Table 1 also shows the tangential, radial, and cross-sectional swelling obtained by measuring external swelling in the liquids relative to those in water. Organic liquids of the highest grade available and deionized and distilled water were used without further purification.

Swelling in organic liquids

Five groups of sample pieces were placed into separate sample bottles. Immediately after being dried at 105°C

Table 1. Swelling agents used and the relative swellings of wood in the agents

Swelling agent	Relative swelling (%)		
	Tangential	Radial	Cross-sectional
Dimethylsulfoxide (DMSO)	147.3	100.6	132.4
Formamide (FA)	122.4	99.7	115.2
Water	100.0	100.0	100.0
Ethanol (EtOH)	86.2	82.6	82.6
Ethyl acetate (EtAc)	47.8	48.2	48.2

for 15h in an oven to avoid adsorption of moisture for the samples to be swollen in organic liquids, and without drying the samples to be swollen in water, the organic liquids listed in Table 1 were poured in individual sample bottles under a vacuum. Each sample bottle was sealed using a screw cap with a Teflon liner packing. The samples were kept at a constant temperature of 30°C for the preliminary determined durations while attaining the swelling equilibriums (i.e., 120 days for ethyl acetate and 1 week for the other liquids).

After these periods, the tangential and radial dimensions of the samples were measured to determine the external swelling. The degree of swelling was evaluated from the swelling relative to that in water to eliminate variations among wood pieces.

Method for making replicas

A glass slide with a polyethylene film (0.01mm thickness) softened by heating to about 130°C was placed on a mount supported by three screws to adjust its slant. A smoothed surface of the sample attached to the chuck of a pressing apparatus was pushed onto the film to obtain a replica. Replicas of the same sample were obtained in water-swollen, oven-dried, and organic liquid-swollen states. When the replicas of the oven-dried sample were prepared, the apparatus for making replicas was covered with a box made with acrylic resin, in which dry nitrogen gas was introduced to avoid sorption of moisture. During the preparation of the replicas of samples swollen in a volatile liquid (ethyl acetate), the liquid was added to maintain the samples' swollen state. For more details regarding the method for making replicas, refer to a previous paper.[11]

Analysis of images obtained from the replicas

The replicas were observed by light microscopy, and the portions showing clear images over an annual ring in all states (water-swollen, dried, organic liquid-swollen) were entered into a personal computer using a CCD camera system. The images, shown in Fig. 1, were printed out from the computer. From the images thus obtained, figures of the middle lamella and the edge between the cell walls and the lumens in small portions that included three or four cells were separately traced onto tracing paper and were entered into a personal computer using an image scanner. The cross-sectional areas of the cell lumens and cell walls were then measured using software for image analysis (NIH Image). Cross-sectional swelling of cell walls, enlargements in the area of the cell lumens, and the ratio of the cell wall area/whole area of the small portion (hereafter referred to as "cell wall ratio") were obtained from the values measured by the method described above.

Feret's diameters of the cell lumens and thickness of cell walls in the tangential and radial directions were measured by caliper from the printed images. The values obtained by each measurement were averaged separately for small portions that included three or four cells.
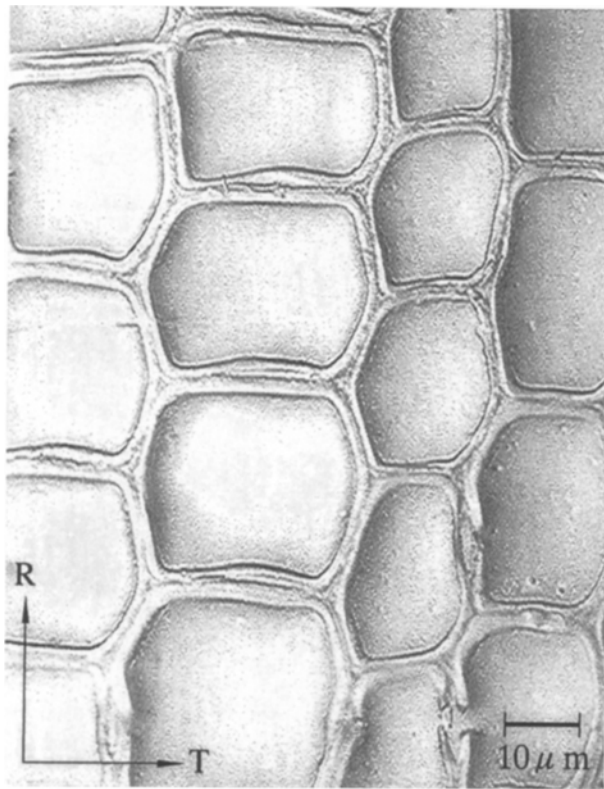
Fig. 1. Image obtained from the replica of a cross-sectional surface of oven-dried wood. *R*, radial; *T*, tangential

Fig. 2. Changes in cross-sectional swelling of cell walls relative to the cell wall ratio. *DMSO*, dimethylsulfoxide; *FA*, formamide; *EtOH*, ethanol; *EtAc*, ethyl acetate

Results and discussion

Cell wall swelling

Figure 2 shows the cross-sectional swelling of cell walls in the test liquids as a function of the cell wall ratio, measured in the dry state. It can be seen that the cross-sectional swelling was larger in the liquids that swelled wood to a larger extent (Table 1). Furthermore, it is notable that the cross-sectional swelling of cell walls in all the test liquids was not constant in relation to the cell wall ratio and decreased with an increase in the cell wall ratio; that is, there was more swelling in earlywood than in latewood. This tendency was significant above the 99% confidence level for all the liquids except ethyl acetate (EtAc) with a 98%–99% confidence level.

The increases in cell wall thickness in the test liquids are shown in Fig. 3. These values also changed with the cell wall ratio as seen in the cross-sectional swelling of cell walls. These results are quite reasonable, as latewood (with thick cell walls) must deform more extensively than earlywood (with thin cell walls) if latewood swells to the same extent as earlywood. In other words, this result suggests that some factor restricts swelling toward the outside of the cells. A similar conclusion was obtained from the study on the external swelling of coniferous wood in various liquids, where restrictions by the parent wall or the primary wall (or both) were considered candidates for the restricting factors.[12]
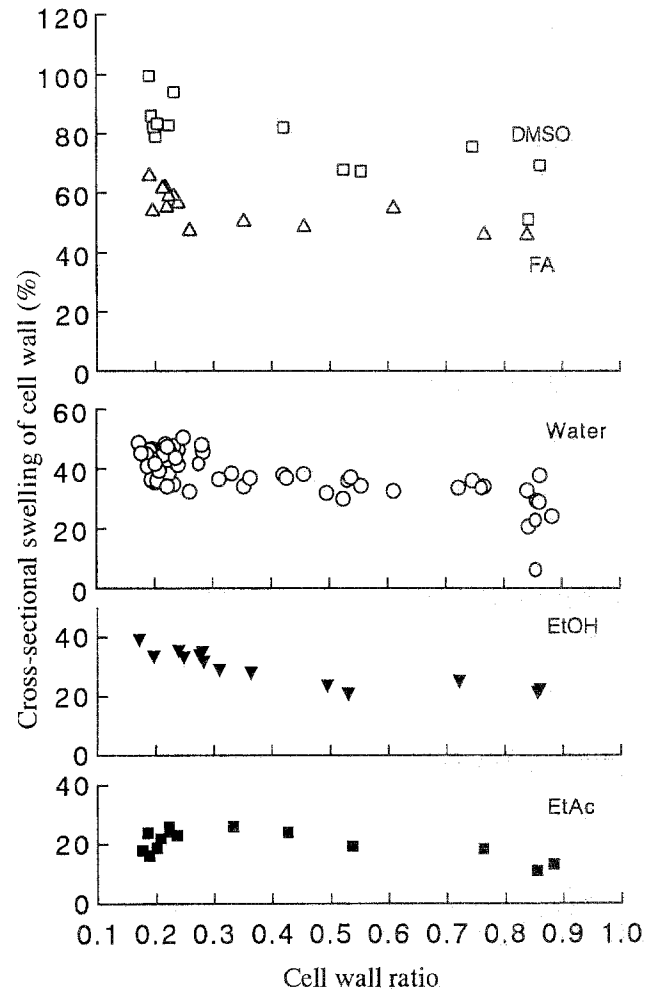
Figure 3 also shows that the increases in thickness of the tangential wall were larger than those of the radial walls for the wood swollen in dimethylsulfoxide (DMSO) and formamide (FA) except in the portion where the cell wall ratio is extremely small. This suggests that the number or size of the fine voids formed in cell walls by swelling differ for the radial and tangential walls, or that the restriction mentioned above acts more strongly for the increased thickness of the radial walls than the tangential walls in highly swollen states. However, in the cases of swelling in water, ethanol (EtOH), and EtAc, there was little difference between the increased cell wall thickness in the radial and tangential directions.

Deformation of cell lumen

Figure 4 shows the enlargements in the area of cell lumens as a function of the cell wall ratio. A tendency toward increasing reduction in the area with increases in the cell wall ratio was observed. This tendency was significant above a 99% confidence level for each of the liquids.
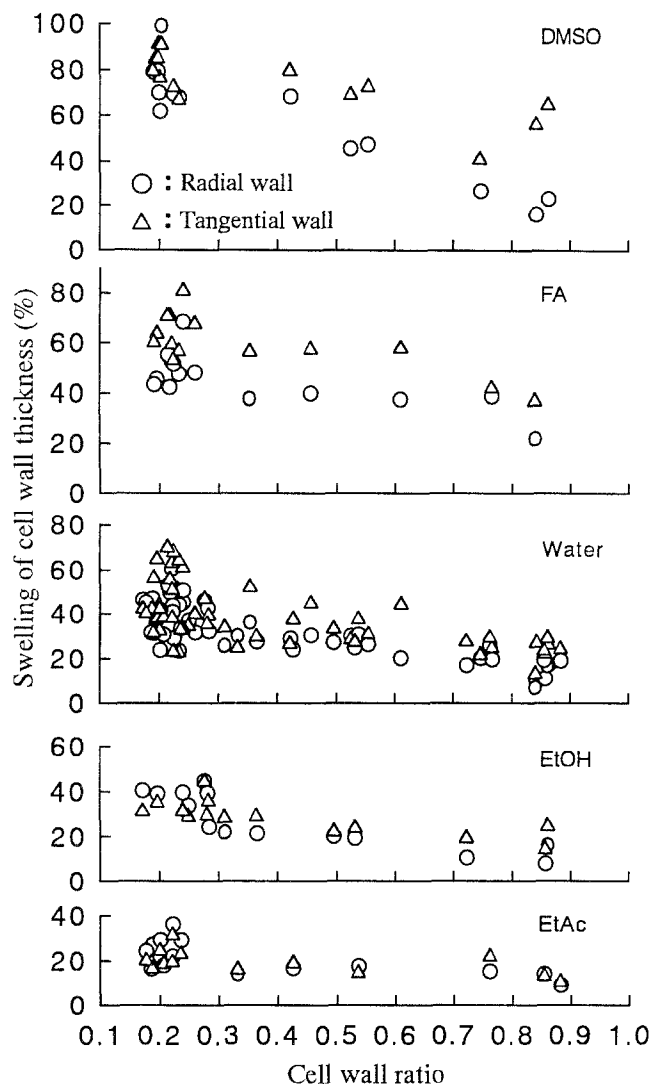
Fig. 3. Changes in the swelling of cell wall thickness relative to the cell wall ratio

Fig. 4. Changes in enlargement of the areas of cell lumens relative to the cell wall ratio

Moreover, in the case of DMSO and FA, in which wood swelled more than in water (Table 1), the cell lamina area reduced not only in latewood but also in earlywood. For wood swollen in the other liquids, the area was reduced in latewood, though little enlargement was found in the lower regions of the cell wall ratio.

The elongation in Feret's diameter of the cell lumens is shown in Fig. 5 as a function of the cell wall ratio. The reduction in diameter of the cell lumens of wood swollen in each of the test liquids was found in the radial direction over the whole range of cell wall ratios, and such reductions increased extensively with increases in the cell wall ratio, especially with liquids that have high swelling potentials. This result suggests that the swelling toward the outside of cells is restricted, and that the operative restrictions strongly affected swelling in the radial direction in latewood, which had become swollen in liquids such as DMSO, FA, and water (which have high swelling potentials). Accordingly, it can be concluded that these restrictions contribute to the transverse swelling anisotropy of conifer-
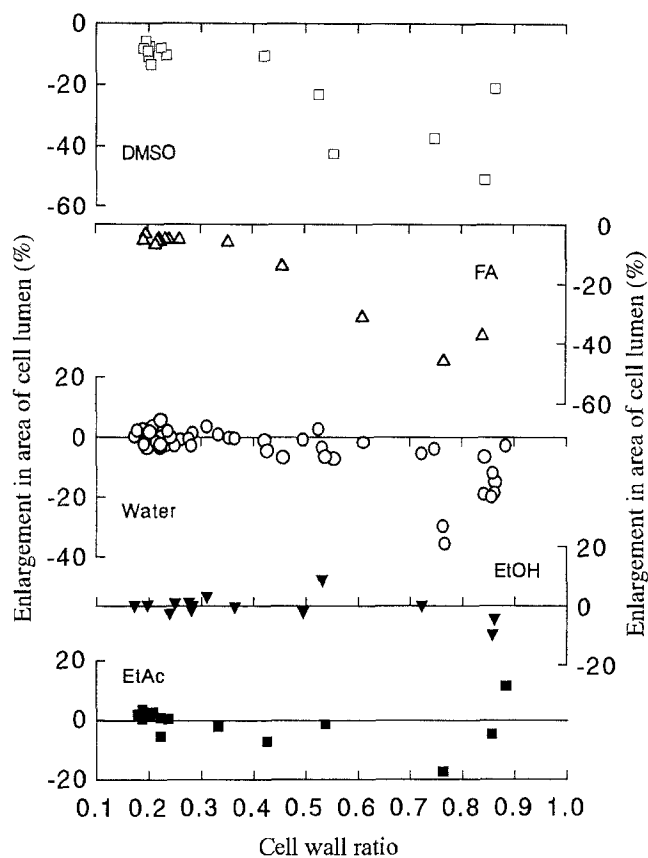
ous wood, as they reduce radial swelling in latewood, which has a higher swelling potential. Small reductions in Feret's diameter were also found in the tangential direction at higher cell wall ratios for all liquids, though elongations in the diameters were observed at lower cell wall ratios (i.e., in earlywood). These elongations in the diameter of the cell lumens in the tangential direction in earlywood can easily be explained by the stretch of the earlywood based on a tensile force from latewood. Therefore, we conclude that the increased width of the cell walls is much less than the increased thickness of the cell walls.

Changes in shapes of cells

Figure 6 shows a comparison between a transitional region from earlywood to latewood of dried wood and of the wood swollen in DMSO, demonstrating changes in cell shapes caused by swelling. Changes in the angles of hinges of cell walls were observed. To examine these changes more clearly we used traced images, shown in Fig. 7.

Figure 7 also shows the direction in which the tensile forces acted on the wood swollen in DMSO in comparison to that in dried wood (indicated by arrows). A similar result was obtained by Watanabe at al.[10] In any event, the cell was deformed by the tensile force caused by the more extensive swelling in the tangential direction of latewood than that of earlywood, and the hinge angles of cell walls changed not
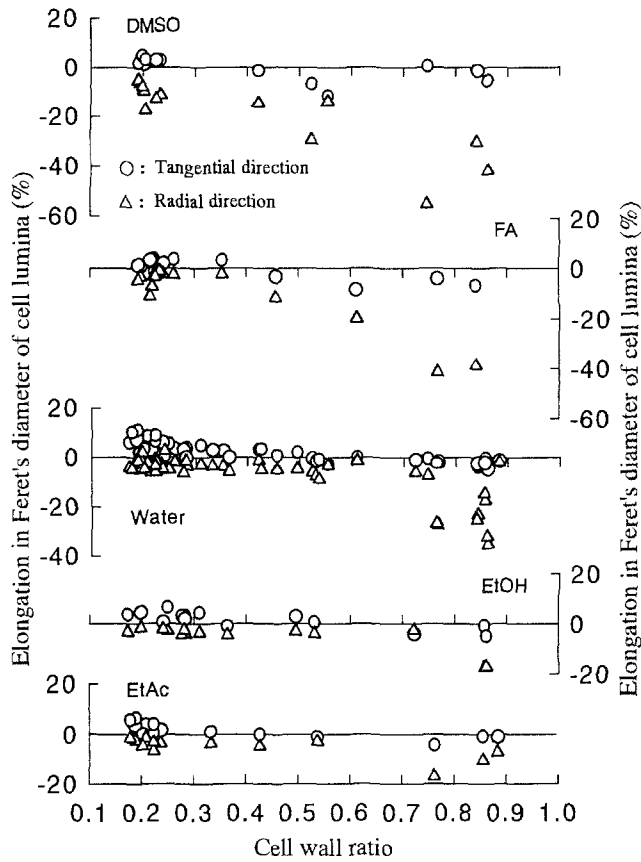
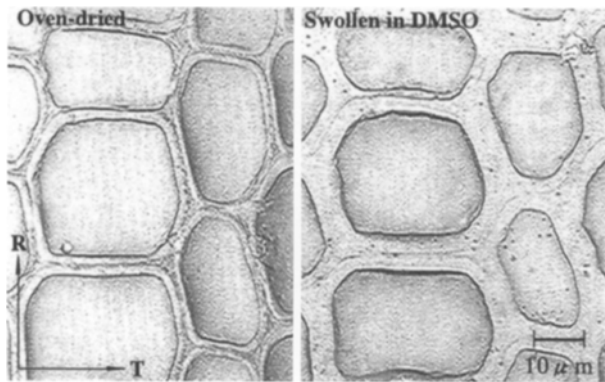**Fig. 5.** Changes in the elongation of Feret's diameters of the cell lumens relative to the cell wall ratio

Fig. 6. Image obtained from replicas of a cross-sectional surface of oven-dried wood and wood swollen in dimethylsulfoxide

Fig. 7. Deformation of cell shape caused by swelling. *Arrows* indicate tensile forces

Fig. 8. Relative swelling of whole wood (*filled circles*); earlywood (*open circles*); and latewood (*diamonds*) in the radial direction. *Error bars* indicate the 95% confidence range

only in earlywood but also in the transition region from earlywood to latewood. The contribution of the interaction between earlywood and latewood to the transverse anisotropic swelling of wood depends strongly on the difference in Young's modulus between them when calculating this contribution using the formula derived by Pentoney.[2] Nakato calculated this contribution on the assumption that the Young's modulus was proportional to the density of each portion of wood and obtained a small contribution (33%) in Japanese larch (*Larix leptolepis*).[13]
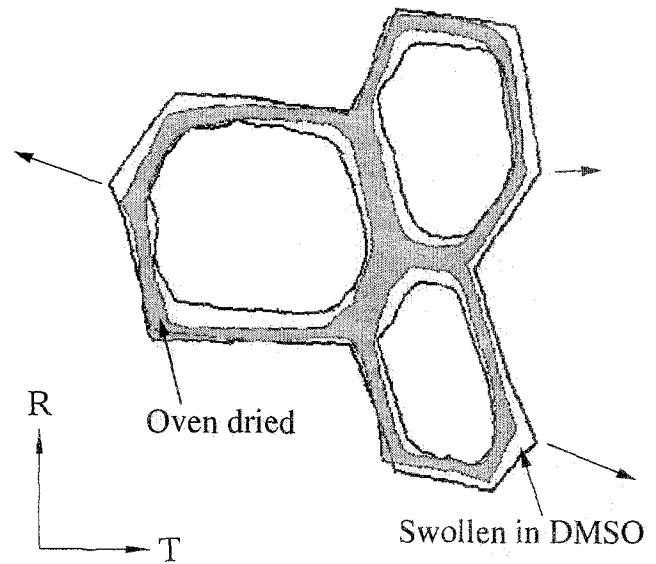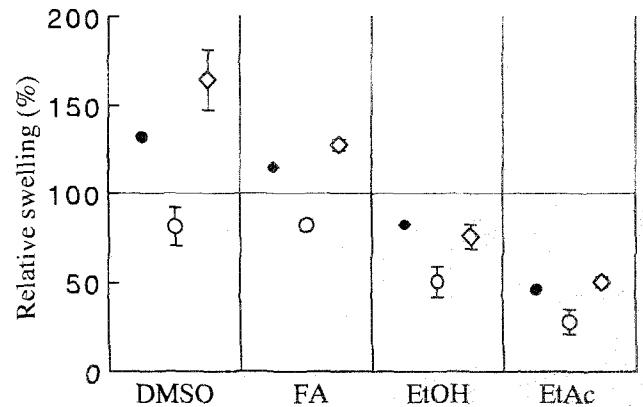
However, according to the results obtained here, bending force originating from the tensile force on the tangential direction must act in the radial cell wall in earlywood and in the transition region from earlywood to latewood. In this situation, the latewood/earlywood ratio of Young's modulus must be much larger than the ratio of their densities because the flexure by bending varies in inverse proportion to the third power of cell wall thickness. Indeed, a similar result was obtained by Watanabe et al. in a study using cell models of coniferous wood.[14] Consequently, a much larger contribution should be calculated in such a case.

Swelling in the radial direction of earlywood and latewood

Figure 8 shows the relative swelling in the radial direction in the whole wood and in the earlywood (<25% of the cell wall

ratio) and latewood (>30% of the cell wall ratio). Latewood shows more swelling than both earlywood and whole wood in all the organic liquids. It is notable that the swelling of earlywood in DMSO and FA, in which the wood swelled externally beyond the water-swollen state, was less than that in water, though the swelling of latewood was more than that in water. Because the cross-sectional swelling of cell walls in earlywood and latewood in both of the organic liquids was more than that in water (Fig. 2), this result is thought to be due to Poisson's effect, resulting from the tension in the tangential direction of the earlywood generated from the increased swelling of latewood in the same direction. This result suggests that the effect of the interaction between earlywood and latewood on swelling exceeds the effect of the increased swelling of the cell wall in both liquids. In other words, the result demonstrates that the interaction between earlywood and latewood is a significant factor in the transverse anisotropic swelling of wood.

Cross-sectional swelling of cell walls

Table 2 shows the cross-sectional swelling of cell walls measured and calculated over an annual ring with the cross-sectional relative swelling measured externally. The calculated values were based on the assumption that swelling does not change the volume of the cell lumens, that cell walls swell volumetrically in terms of the volume of liquids sorbed into the cell walls without longitudinal swelling, and that the fiber saturation point of the wood swollen in water is 28%. The calculated value for water was a close approximation to the measured value, so the reliability of these measured values is considered to be quite high. In the cases of FA and especially DMSO, the measured values were much higher than the calculated values. This result obviously shows the swelling of cell walls toward the cell lumens in these liquids, as demonstrated in Fig. 4. Moreover, it is obvious that the swelling of the cell walls in these liquids was much more than the swelling measured externally, which also suggests the existence of some restrictions acting on the swelling toward the outside of the cells as described above.

Transverse swelling anisotropy based on swelling of cell wall thickness

Swelling anisotropy (the tangential swelling/radial swelling ratio) in an entire annual ring and in earlywood based only

on the increased cell wall thickness is shown in Table 3 along with the anisotropy of external swelling. The values calculated using the data of the measured thickness and swelling of each cell wall throughout an annual ring and the number of cell walls are in unit lengths in the tangential and radial directions. The most reliable value was obtained for water because of the most measurements being 1.2. This value was much smaller than that of the external swelling of 2.09. Accordingly, the swelling anisotropy of the coniferous wood used in this study could not be attributed only to the increased cell wall thickness. Nevertheless, a swelling anisotropy of about 1.4 in the water-swollen state of earlywood, where the cell wall ratio was less than 0.25, was due only to the increased cell wall thickness. This result corresponds to the previous result obtained by Nakato, who found that earlywood isolated from whole wood showed significant anisotropy.[15] Nakato also found that shrinkage in the width of the tangential tracheid cell wall was lager than that of the radial wall in isolated earlywood.[16] Based on these results, Nakato concluded that the interaction between earlywood and latewood was not a prime factor in the transverse swelling anisotropy, and that the causes for anisotropy should be sought in the cell wall structure.[13] The fact that isolated earlywood showed significant anisotropy does not constitute proof that the earlywood–latewood interaction is an unimportant factor in anisotropy: The results obtained in the present study (obvious deformation of cell shape in earlywood and the transitional region, and the fact that swelling in the radial direction of earlywood in liquids that have higher potentials than water were less than that in water) demonstrate the presence of an interaction between earlywood and latewood.

Conclusions

The coefficient of the swelling of cross sections of cell walls and of cell wall thicknesses was larger in earlywood than in latewood. In the case of liquids such as DMSO and FA, which have high swelling potentials, marked swelling toward the cell lumens were observed. This finding suggests that these liquids induce more swelling of cell walls than external swelling. Feret's diameter of the cell lumens was reduced by swelling in all the observed cases except in the tangential direction of earlywood, where small elongation of the diameters was observed. This means that the increase

Table 2. Cross-sectional swelling of cell wall: measured and calculated

Liquid	Cross-sectional relative swelling (%)	Cross-sectional swelling of cell wall	
		Measured	Calculated
DMSO	132.4	82.1	55.3
FA	115.2	55.6	48.2
Water	100.0	40.0	41.8
EtOH	82.6	30.5	34.5
EtAc	48.2	21.3	19.3

Table 3. Cross-sectional swelling anisotropy based on swelling of the cell wall thickness

Liquid	Anisotropy based on the swelling of cell wall thickness		Anisotropy of external swelling
	Whole annual ring	Early wood	
DMSO	1.38	1.29	3.04
FA	1.30	1.52	2.26
Water	1.17	1.37	2.09
EtOH	1.10	1.39	2.34
EtAc	1.47	1.83	2.23

in the width of the cell walls is much less than the increase in cell wall thickness. Deformations of cell shapes caused by tensile forces from latewood were observed in earlywood and in the transition region from earlywood to latewood. In the case of swelling in water, swelling anisotropy based only on the increase in cell wall thicknesses were calculated as 1.2 for the whole of an annual ring and 1.4 for earlywood. These values were much smaller than the external swelling anisotropy of 2.1. According to the results obtained in the present study, we conclude that the interaction between earlywood and latewood is one of the prime factors for transverse swelling anisotropy of coniferous wood.

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