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STUDIES ON THE FORMATION AND STRUCTURE OF THE COMPRESSION WOOD CELLS INDUCED BY ARTIFICIAL INCLINATION IN YOUNG TREES OF *PICEA GLAUCA*

II. Transition from normal to compression wood revealed by a SEM-UVM combination method

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Introduction

Concerning to the perception of the stimulus responsible for the formation of compression wood cells, KENNEDY and FARRAR[25] first suggested a hypothesis that if a tree is tilted, differentiating cells on the lower side would be destined to show a characteristic feature or features of a compression wood cell (*i. e.*, rounded outline in cross section, thicker cell wall and heavy lignification), depending on their developmental stages at the time of tilting and that the "susceptible" stage of a characteristic feature would be situated immediately before the site where its formation begins.

To verify the hypothesis, we studied numerical and morphological changes of the cambial and differentiating cells of the lower and upper side following the artificial inclination, and reconstructed a theoretical standard course of the alteration from the formation of normal wood to that of compression wood[49]. Based on the standard course, the first cell with slightly thicker wall was found to have been situated in or near the earliest stage of the secondary wall formation at the beginning of the inclination, as expected by the hypothesis of KENNEDY and FARRAR. However, the cell showing the least excessive lignification and that having intercellular spaces were thought to have been situated in far earlier developmental stages, the fact contradicting the hypothesis. This contradiction comes mainly from the fact that the slightly thicker wall and the least excessive lignification

was found in the same cell. According to the hypothesis, the latter should appear faster than the former in the transition from normal to compression wood.

However, the observation on the transitional zone in the previous paper[49] was limited within only a light microscopic level, and therefore, a detailed observation on the transitional zone was required. On the transitional zone, almost no anatomical studies has been reported except for FUJITA *et al.*[9]. JUTTE and LEVY[24] simply stated that the shallow ridges and grooves of the transitional tracheids were not as steep as in the strongly developed compression wood and the shape of the bordered pit dome changed with the transition. Recently, FUJITA *et al.*[9] investigated the transition using SEM and a variety of light microscopy and stated that following changes were observed in succession from normal to compression wood; (1) disappearance of the S3 layer, (2) increases of the intensity of lignification and the wall thickness, (3) development of the spiral grooves and rounding-off of tracheids in cross section and (4) formation of intercellular spaces. However, since their observations by SEM and light microscopy were made on different cells, comparison of information given by these two methods can not necessarily be made.

In our laboratory, compression wood has been studied either by SEM[11,39] or by ultraviolet microscopy (UVM)[12], or both[40] for these several years. A SEM has proved its availability to observe the features of the inner surfaces of compression wood cells[5,24,36], and an UVM is a very usefull and convenient apparatus to show lignin distribution[12,35,48]. The microscope has also a higher resolving power than an ordinary light microscope. However, because these two apparatus have been used independently, relation between the structural features of the inner surface observed by SEM and those in a cross section especially lignin distribution by UVM of a particular cell have not been known. In the transitional zone from normal to compression wood, these two kinds of features change progressively, and whether these changes occur in parallel or independently could not been determined by the methods hitherto used. To disclose the problem, SEM and UVM observation should be made on the same cell.

In the present paper these two methods are intimately combined and structural features observed on the inner surface and those in the cross section of the same cells along a radial file are shown, and the transition from normal to compression wood is revealed in detail.

Materials and Methods

Young trees of *Picea glauca* ca. 1.7 m high grown in the nursery of

The Laboratory of Forest Tree Breeding in Nayoro, College Experiment Forests, Faculty of Agriculture, Hokkaido University, were bent at the level of ca. 30 cm above the ground to be inclined at 45° from the vertical and tied to woody stakes with strings. No compression or tension is expected to have been exerted on the stems to be examined. Experimental inclinations were made in June 1977, August 1977 (one sample tree resp.), September 1977 and July 1978 (two trees resp.). After appropriate periods (3 weeks or more) the 1st and 2nd internodes of the leaning stems were harvested.

For a general light microscopic observation, materials including both the lower and upper side of the tilted stem were severed from 3 portions, *i. e.*, distal, middle and proximal portion, of each internode, and fixed in either chromium — acetic acid or Zirkle's reduced chromium solution[52], the latter giving the most excellent results. After washing in running water, materials were dehydrated in n-butanol series and embedded in paraffin. After softening in water[2], sections of 15 μ were cut on a sliding microtome and fixed with Ullrich's adhesive[22] on a slide glass and stained with an iron haematoxylin — orange G combination[21].

Specimens for a SEM-UVM combination method were taken from the middle portions of both the internodes and fixed in FAA. Lower part of the severed stem including the transitional zone was cut into small pieces (ca. $2\times2\times2$ mm) and dehydrated in ethanol series and embedded in methacrylate resin mixture (butyl methacrylate : methyl methacrylate $=6:4$). A cross section of either 0.5 or 1 μ thick was cut on a ultramicrotome (LKB, Ultrotome III) with a diamond or a glass knife and fixed on a quartz slide and mounted in glycerin with a quartz cover slip. Photographs were taken under an UVM (Carl Zeiss, Type MPM-01) at a wavelength of 280 mμ using ordinary commercial films.

After sectioning, remainder of the embedded specimen, its transverse surface matching directly with the section, was cut out from the resin block with a razor blade and one of the radial surface of the specimen was attached to the top of a slender alminium stub (6 mm ϕ, 25 mm long, self-made from an alminium rod with a lathe) with Aron Alpha. Cautions should be taken not to damage or spoil the transverse surface.

The other exposed radial surface was finished on the ultratome with a glass knife to expose the inner surfaces, though still embedded, of cells of a radial file. Direction of the radial file was examined beforehand under a metallurgical microscope (Ollimpus, Neopak), and the specimen was attached to the ultratome and planed off roughly to near the radial file to be exposed. Then the specimen with the holder was detached from the microtome and

examined again under the metallurgical microscope whether the cut surface is parallel to the direction of the file. LKB Ultrotome III has a stable mechanism and a good reproductivity, therefore, by adjustment of the angle of the specimen little by little, following repeated inspections under the microscope, and enhanced renewal of glass knives, a radial surface can be good finished with an accuracy within a few microns along a radial file, if the file is not concavely curved to the surface.

After finishing, a small tangentially flat piece of the specimen with finished radial surface was cut from the specimen on the stub with a razor blade to give a new radial surface and the new surface was also finished in the same manner and cut off.

The resin was removed from the specimens in acetone or xylen for a week or more, with enough renewal of the solvent. Thus, usually 3 to 4 SEM specimens can be successfully obtained from an embedded sample.

After removal of the resin, specimens were dried in room conditions and mounted on specimen stubs with electron conductive paste or with pieces of double face adhesive tape and coated with gold in an ion coater (Eiko, IB : 3). Photographs were taken in a JSM-2 microscope at 15 kV.

To obtain matched SEM-UVM pictures in practice, serial SEM photographs were first taken at a low magnification (ca. ×250) along the edge of the specimen to show both the transverse and radial surface at the same time. Based on the transverse surface shown in the printed serial photographs, the radial file in quesition was sought on the matched section under the UVM. Generally some outstanding structures were used as markers, such as resin canals, cells of peculiar shapes, if such structures were not present, emergence and disappearance of rays and the number of the cells between rays were helpfull to seek the file.

Results and Discussion

I. The SEM-UVM combination method

The SEM-UVM combination method proved successfully its availability in the present study. Examples of matched SEM- and UVM-photographs are presented in Photos 1 a-1 b, 9 a-9 b, 10 a-10 b etc. Preparation of thin sections for UVM is not different from that in routine ones, therefore, problem may lie in that of SEM specimens.

Generally the surface of the specimen after removal of the resin was sufficiently neat and free from contaminations. The time required for the enough removal of the resin varied considerably. Genarally 3 to 4 days seem to give a good result, however, in some cases a week soaking was

not sufficient and occasionally polymerized methacrylate resin could not be dissolved in acetone, xylene and other organic solvents, even in a hot oven (ca. 60°C). Conformation of the polymerized resin is supposed to be altered by the existence of oxgen or water vapor in the atmosphere and/or alpha-cyanoacrylate in Aron Alpha.

The polymerized resin soaked in the solvent seems to swell before dissolution, therefore, the form of weak structures such as pit membrane near the surface might be altered to some extent.

SEM specimens prepared in the present method showed little shrinkage when dried in room conditions even in unlignified soft tissues such as cambium and phloem. The resin penetrated into cell walls and other structures is thought not dissolved after a prolonged soaking in the solvent. A treatment in a hot oven (60°C) resulted in also little shrinkage.

The specimen has a clear and excellent cut surface especially finished with a diamond knife. Usual specimens finished in unembedded states are liable to have remnants of the S3 layer on the cut surface in a bridge-like form arching from a tangential wall to another. The specimens prepared in the present method are completely free from such remnants, and therefore, provide more available surface area than usual materials of the same dimensions.

Considered from these facts, it is concluded that the present resin removal method gives excellent SEM specimens and has a general value for the study of wood anatomy, though it takes longer time than usual methods. To our knowledge, a combination of observations by a SEM and a UVM on the matched specimens has not been attempted. In the present investigation, and observation was made on a transverse and a radial surface by a SEM and on a transverse section by an UVM, while it is also possible in other combinations of surfaces and/or by other apparatus, *i. e.*, a SEM with a TEM or other optical microscopes. Application of the method can be made widely, ranging from a simple combination of an observation by a SEM on a surface with that by a light microscope on the matched section of the same surface, to a complicated combination in which a three-dimentional observation by a SEM is matched with examinations by a TEM, UVM or other microscopes on three sections. In our laboratory, a combination of an observation on a tangential surface by a SEM with that on a tangential section by an UVM has shown its ability in a study of trabeculae[50,51].

Methacrylate resin shrinks considerably when polymerized[13] and occasionally is not dissolved in organic solvents as mentioned above. Therefore,

chief troubles to be improved are thought to lie in the selection of embedding resin and the solvent. Use of other resin was not attempted.

II. Transition from normal to compression wood

A general view of the transition from normal to compression wood is shown in Photos 1 a and 1 b. The upper photograph (UVM) and the lower one (SEM) are matched exactly, the same number showing the same cell. The basic pattern of the transition was found similar among the seasons and sampling positions, therefore, description will be focussed mainly on the transitional zone formed in July.

The first appreciable feature of the transition from normal wood is disappearance of the S 3 layer (Photos 2 a, 2 b and 2 c). In the present paper, cells in a radial file are numbered; the first cell without the S 3 layer is numbered "1" and the number is increased to the cambial side and decreased to the pith side as "0", "−1", "−2" etc. The cells of lesser numbers than "1", namely, cells 0, −1 or so, give an impression that the S 3 layer becomes thinner towards cell 1 (Photo 2 c). Disappearance of the S 3 layer in the SEM-photograph (Photo 2 c) is in accord with that in the polarizing micrograph (Photo 2 a). Sometimes the disappearance was uncertain and could not be determined by SEM. In such cases, the cell apparently without the S 3 layer was numbered "1".

In some specimens, largely in the transition in latewood, the S 3 layer reappeared after its initial disappearance and redisappeared (the second disappearance) (Photo 3). In these cases, the cell showing the second disappearance was numbered "1". Such a "hesitative" disappearance might happen to occur by the preexistent stimulus for the compression wood formation before the experimental inclination, namely, wind action or change in physiological balance within the tree. However, compression wood induced by a short term stimulation gives essentially different features[25,50]. Therefore, the hesitative disappearance of the S 3 layer would not be caused by the existance of the stimulus shortly before the experimental inclination. This might be occur by the difference of the sensitivity of cells to the stimulus. However, an essentially different explanation is possible and the problem will be discussed later. In a case, a cell showing the transition layer between the S 2 and S 3[14,19,20] was found between those with and without apparent S 3 layer (Photo 4). This was only once observed in the transition in latewood. Whether the S 3 layer is formed or not seems to be determined consistently throughout the long axis of a cell and no cells which showed partial disappearance of the S 3 layer was found.

Next to the disappearance of the S 3 layer, the change in the distribu-

tion pattern of UV-absorption in the S 2 layer occurs. Slightly higher absorption first appears in the inner region of the S 2 layer, then spreads outwards through the layer and finally the outer region of the S 2 layer, *i. e.*, S 2 (L)[7], becomes to show the most strong absorption (Photos 1, 5 a, 6, 11 and 12). The first higher UV-absorption in the inner region of the S 2 layer has not been reported as a pattern of the distribution of UV-absorption in compression wood cell walls[12,48], and therefore, thought to be unusual. On the other hand, the strong absorption in the outer region appeared later is one of the typical features of compression wood cells. This change of the pattern begins at cell 3 to 6 in the 1st internode and at cell 4 to 8 in the 2nd and accomplished within a few number of cells along a file. FUJITA *et al.*[9] reported that the first excessive lignification was observed in the outer region of the S 2 layer at cell corners, based on the observation on thin sections stained with safranine. The first unusual UV-absorption in the inner region of the S 2 layer revealed by UVM is considerably faint, and therefore, thought not able to detect in the stained sections. The unusual UV-absorption in the inner region is clearly shown in the transition in latewood (Photo 6).

Cells situated between the disappearance of the S 3 layer and the appearance of the unusual UV-absorption show some anomalous features. The most usual one is a pebble-like deposit (Photos 2 c and 7). This deposit is obviously not an artifact brought about by insufficient removal of the embedding resin, because Photo 7 was taken from a specimen finished in water saturated state on a freeze microtome and dried in room conditions. Chemical nature of the deposit was not studied. The deposit assumes sometimes a wart-like appearance, though the relationship between them is uncertain. Photo 8 shows an extreme case of anomalous modification of the S 2 layer itself. This modification might be formed under an influence of longitudinal compression. Although, the stem examined had been kept straight with woody stakes and strings, as already mentioned, therefore, no compression is expected to have been exerted on the lower side of the stem. These anomalous features can be found slightly beyond the zone between the disappearance of the S 3 layer and the appearance of the unusual UV-absorption, and the anomalous modification (Photo 8) is not necessarily restricted in the transitional zone. It was observed also in normal wood. The abnormalities, if present, are distributed over a fairly long distance along the cell axis, while in the neibouring cells sometimes no abnormalities can be found. In latewood the formation of these features seems to be reduced to some extent.

Appearance of the unusual UV-absorption is followed by the increases in the thickness of the wall, the strength of UV-absorption and the roundness, and the formation of spiral grooves in the S 2 layer. The first cell giving the strong UV-absorption in S 2 (L) is, in most cases, identical with that showing the first appreciable roundness and situated at the foot of the increase of cell wall thickness (Photos 1, 6 and 9 a). The first cell with spiral grooves has already strong UV-absorption in S 2 (L) and considerable roundness and wall thickness (Photos 1 a, 1 b, 9 a, 9 b, 10 a and 10 b). These two types of cells are sometimes the same or adjoining, or one or two cells are between them. Generally changes are rather abrupt. From SEM observation on the radial surfaces, change in the degree of the development of spiral grooves seems sometimes abrupt, in other cases gradual. In the case of the former (Photos 9 a and 9 b), cell 9 has no spiral grooves and weak UV-absorption in S 2 (L), on the other hand, cell 11 has thicker wall with well-developed spiral grooves and strong UV-absorption, therefore, in these respects, is thought to be almost a typical compression wood cell, though being without intercellular spaces. The spaces appeared around cell 20 in this radial file. In the case of the latter (Photos 10 a and 10 b), cell 5 and 7 show almost the same degree of the development of the grooves, though slight difference in the strength of UV-absorption is found, and cell 9 has not yet the typical grooves. Well-developed grooves appear eventually at cell 11, which is the first cell with an intercellular space. Thus, morphological changes up to the appearance of intercellular spaces are rather variable. This variability is apparently not caused by the difference of the width of the cambial and differentiating zones, because the abrupt change in Photo 9 was found in the 2nd internode and the gradual one in Photo 10 was in the 1st, the width of these zones in the latter being narrower than in the former[49].

No appreciable changes in the fibril angle of the S 2 layer was observed through the transition from normal to compression wood. FUJITA *et al.*[9] reported a drastic change in the angle in 20-year-old *Pinus densiflora*. Since compression wood cells generally have a greater fibril angle of the S 2 layer than normal wood cells[4,6], possible changes in the angle may exist. However, materials examined in the present study are juvenile wood and the fibril angle of the S 2 layer of this type of wood is known greater than that of mature wood[8], therefore, it can be thought that no appreciable changes in the angle was observed in the present investigation.

The observation stated above was made on the wood free from other structures in xylem and was restricted in axial tracheids. However, structures

other than axial tracheids exist in the xylem. The occurrences of a ray (Photos 1 a, 6 and 12) and a horizontal resin canal (Photo 11) do not seem to affect the course of the transition appreciably. On the other hand, the occurrence of a vertical resin canal in the early stage of the transition apparently shifts the transition inwards to some extent (Photo 12). Cells A to D in Photo 12 show strong UV-absorption in S 2 (L) and some of them have apparent spiral grooves, on the other hand, cells a to c situated in similar radial positions do not. Since the maturation of cells around a vertical resin canal is known to be delayed, these cells would remain sensitive to the geotropic stimulus for a longer period than usual. In a specimen the epitherial cells of a horizontal resin canal change from flat and relatively thin-walled to expanded thick-walled ones accompanying the transition. VERRALL[42] reported epitherial cells had thicker walls in compression wood than in normal wood, however, TIMELL[41] noted they were similar in these two types of wood. SATO[34] observed vertical resin canals in normal and compression wood of *Larix leptolepis* by SEM and concluded that there was no differences of epitherial cells between them.

In the course of the present investigation, ray tracheids with spiral grooves were observed (Photo 13). They have thicker wall and resemble to axial compression wood tracheids. However, not all the ray tracheids in compression wood are so and there are found thin-walled seemingly normal ray tracheids. TAKAOKA[40] made a similar observation in a young tree of *Larix leptolepis* using SEM. Compression ray tracheids would only be formed in young trees. Ray tracheids in compression wood had been believed normal[30,41]. Structural changes of ray tracheids accompanying the transition could not be observed, because a ray tracheid has a smaller tangential dimension, and therefore, it is difficult to expose their inner surfaces over a long enough distance along the file. Structural valiability of compression ray tracheids also disturbed the confirmation.

III. Increases in the degree of the development of compression wood cells accompanying the transition

In the previous paper[49] an increase in the degree of the development of compression wood cells in the transitional zone towards the typical compression wood was suggested from a light microscopic observation. In the study of the severity of compression wood cells, it is an important matter to choose an adequate characteristic among many as a marker of the severity. Although attention has increasingly been called to the severity of compression wood recently[16,37,38,47], little is known about the fine structure of intermediates between normal and typical compression wood cells (confer with references[11],

[12,16,48]). For the present investigation several characteristic features of compression wood cells were scanned to seek the most suitable marker ; roundness and the value of UV-absorbance can not be simply determined quantitatively ; fibril angle of the S 2 layer did not change appreciably in the materials used in the present study ; there remains still some questions in the relation between the degree of the development of spiral grooves (*i. e.*, depth of the grooves and the height and width of the ridges) and that of a compression wood cell ; patterns of the lignin distribution found in UV-micrographs are available only for the cells of lesser degrees[12]. On the other hand, cell wall thickness can be easily measured and could be an available marker.

Although no reports has been published concerning to the relation between cell wall thickness and the degree of compression wood cells, there are good reasons to believe the positive relation. Specific gravity is thought determined mainly by density of cell wall materials and wall percentage of

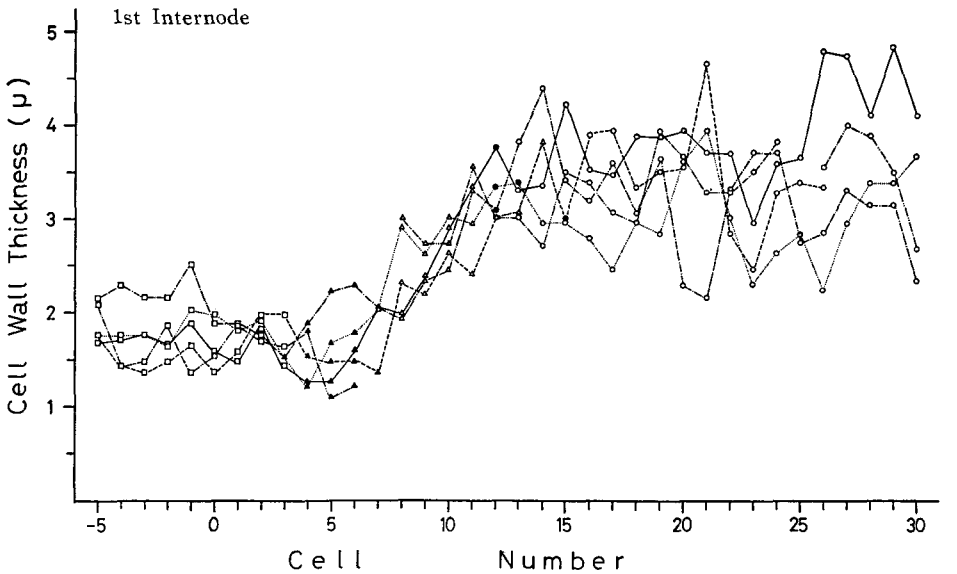

Fig. 1. Change in the thickness of radial single wall from cell −5 to 30 of five radial files in the 1st internode of a sample tree inclined in July. Files were all observed by the SEM-UVM combination method and are shown in different patterns of lines. Simbols; □: normal and transitional cells without S 3 layer, UV-absorption of these cells remaining normal; ▲: transitional cells with unusual UV-absorption in the inner region of the S 2 layer or in the S 2 (L) but without spiral grooves; △: transitional cells with spiral grooves but no intercellular spaces; ●: compression wood cells with an intercellular space between the cell and the next; ○: typical compression wood cells.

the wood, and the latter would be related primarily to the wall thickness. Since specific gravity was reported to increase as the severity of compression wood increases[16,31,32,37,38], cell wall thickness would show a similar relation to the severity, though density of wall materials is known lower in highly lignified tissues such as compression wood[30]. Difference in cell wall thickness between earlywood and latewood in compression wood seems to change in parallel with the severity of compression wood cells[12,48]. In the present study cell wall thickness was determined to use as a marker of the severity.

Radial single wall (center of the middle lamella to the inner surface of the secondary wall) was measured on UV-micrographs (ca. ×1000) under a binocular. Radial cell diameter was also measured. All measurements were made on the trees inclined in July. Figs. 1 and 2 show changes in wall thickness from cell −5 to 30 in the 1st and 2nd internode respectively. The thickness decreases after the disappearance of the S 3 layer to the minimum at cell 3 to 7 in the 1st internode and at cell 7 to 10 in the 2nd, then increases steadily towards the typical compression wood containing

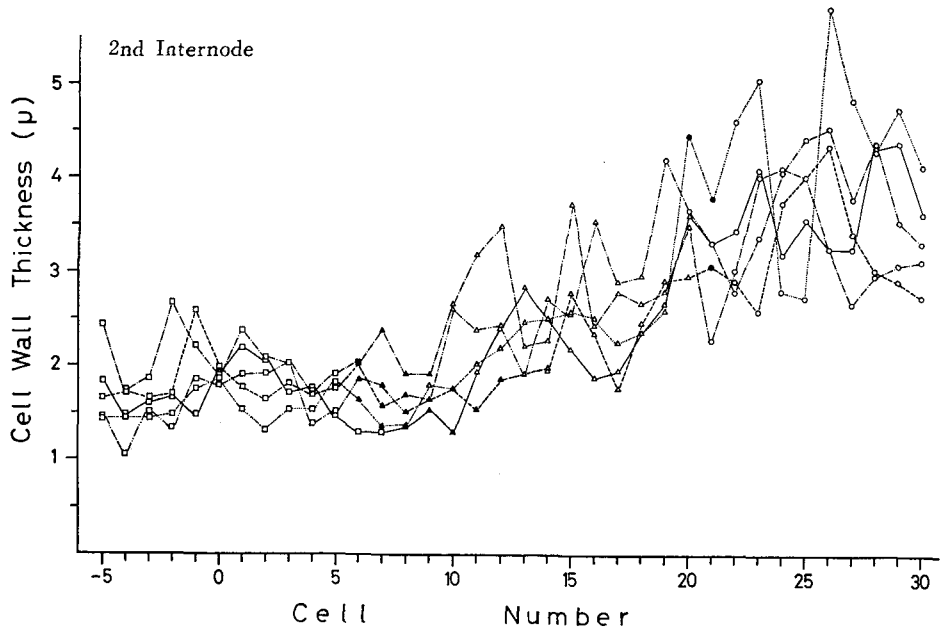

Fig. 2. Change in the thickness of radial single wall from cell −5 to 30 of five radial files in the 2nd internode of a sample tree inclined in July. Files were all observed by the SEM-UVM combination method and are shown in different patterns of lines. Symbols; see the explanation of Fig. 1.

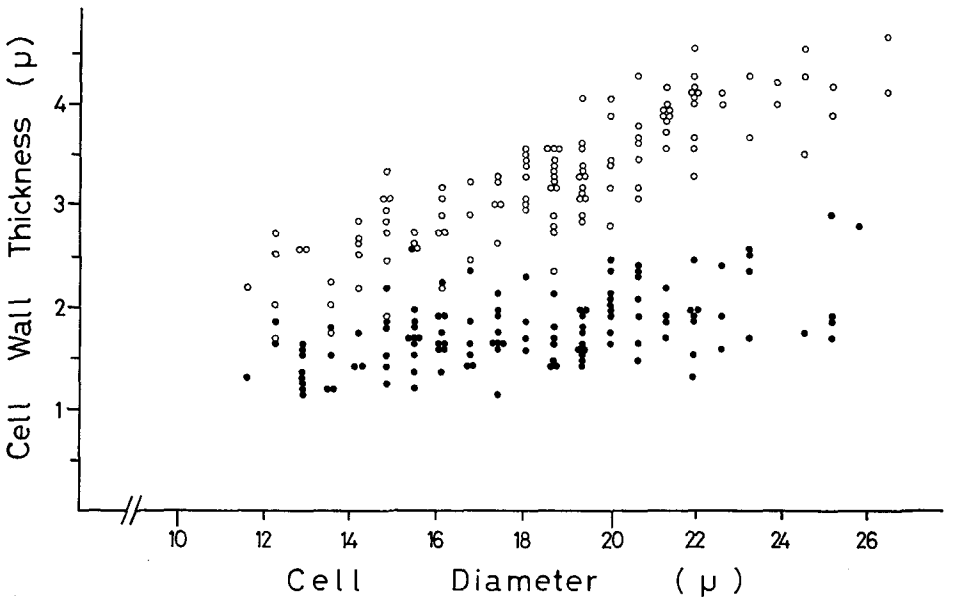

Fig. 3. Relation between radial single wall thickness and radial cell diameter of normal and compression wood cells in the 1st internode; ○ : compression wood cell; ● : normal wood cell.

intercellular spaces, *i. e.*, cell 12 to 15 and cell 19 to 21 in the 1st and 2nd internode respectively. A general correlation between radial wall thickness and radial cell diameter is found especially in compression wood cells (Fig. 3), and therefore, the value of wall thickness per cell diameter would give more regulated figures. The trend is thus more clearly illustrated (Figs. 4 and 5). Radial cell diameter did not show any definite patterns.

FUJITA *et al.*[9] reported a similar decrease of the thickness in the early stage of the transition and attributed this to the lack of the S 3 layer. However, it is apparent that the decrease occurs after the disappearance of the S 3 layer and cells with thinnest wall are those with unusual UV-absorption in the inner region of the secondary wall. Therefore, the decrease of the thickness is apparently not related to the lack of the S 3 layer, but the S 2 layer itself decreases, though the change in the thickness of the S 1 layer was not studied. The incipient decrease would imply the interruption of the normal secondary wall formation. This is also suggested by the occurrence of the deposits found on the inner surface of these cells (Photo 7). The increase thereafter would mean an increase of the severity of compression wood cells. In the present study changes in the UV-absorbance and the roundness were not measured, however, roundness seems increases

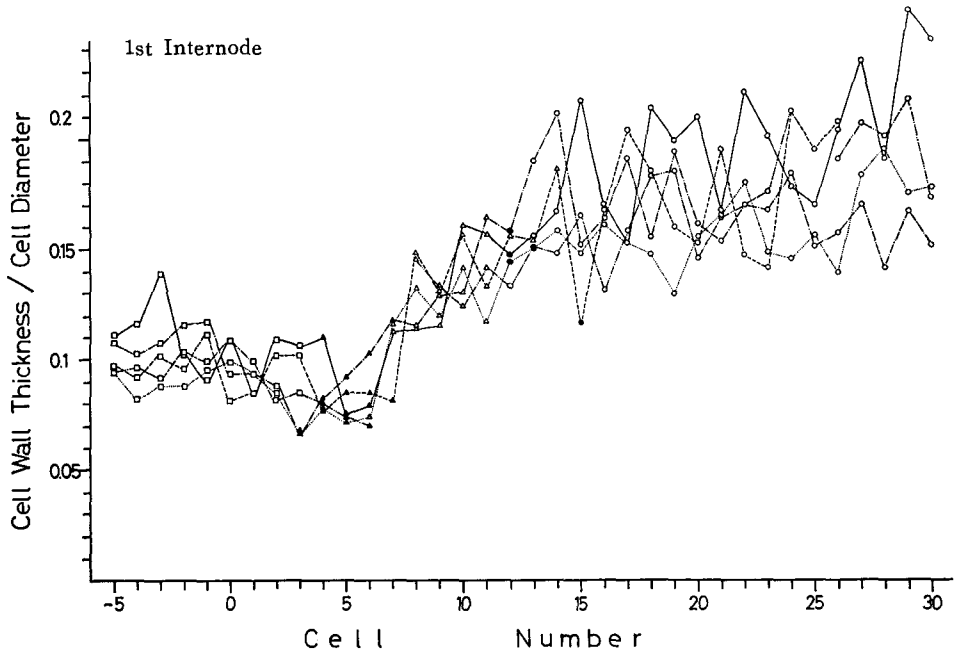

Fig. 4. Change in the values of radial single wall thickness/radial cell diameter from cell −5 to 30 of five radial files in the 1st internode. The same pattern of the line with that used in Fig. 1 represents the same file. Symbols; see the explanation of Fig. 1.

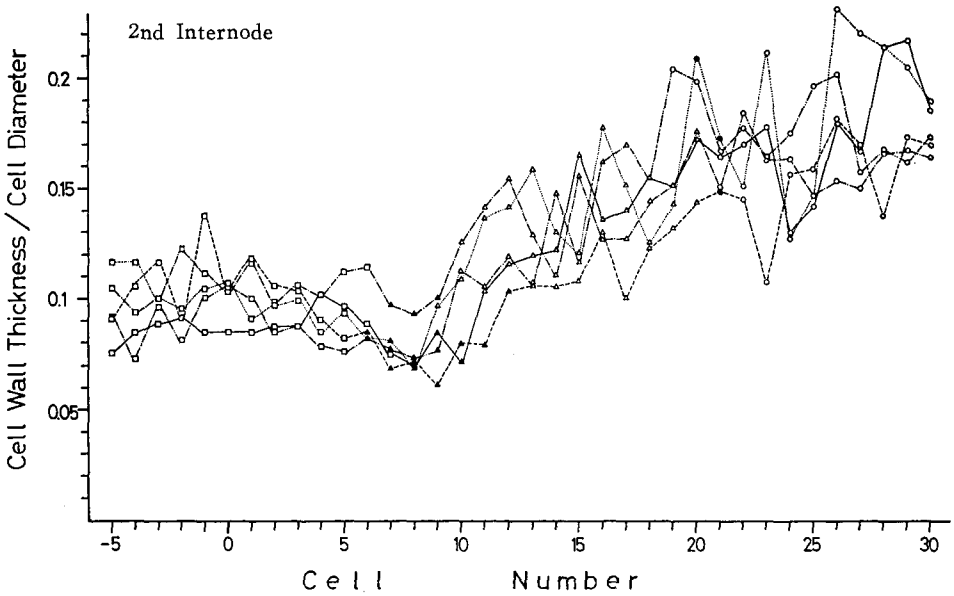

Fig. 5. Change in the values of radial single wall thickness/radial cell diameter from cell −5 to 30 of five radial files in the 2nd internode. The same pattern of the line with that used in Fig. 2 represents the same file. Symbols; see the explanation of Fig. 1.

towards the typical compression wood (Photos 1 a, 9 a, 10 a, 11 and 12). Although the strength of UV-absorption is difficult to estimate from printed photographs, several cells after the appearance of the strong UV-absorption in S 2 (L) show an apparent increase of the absorption especially in the inner region of the S 2 layer. These facts suggest also the increase of the severity of compression wood cells. However, it should be noted that this is not the increase of the severity as a whole cell but that of each characteristic feature. The increase in the wall thickness seems not to occur in parallel with that of UV-absorption, though the increases begin from almost the same cell. This might imply essential differences of the perceptional mechanism or those of the stimulus itself between these two features. On the other hand, the wall thickness and the roundness seem to change nearly in parallel, though the degree of the roundness is difficult to estimate exactly. Since the rounded shape of compression wood cells can be established through the formation of the secondary wall, even if the cell was square before the wall formation begins[49], the parallelism between the roundness and the thickness would be brought about by an essential cause.

IV. Perception of the geotropic stimulus

In a previous paper[49], the cambial and differentiating zones were divided into three developmental phases for convenience, and the position of the first cells which show characteristic features of a compression wood cell, at the beginning of the inclination, was infered from the observation on 15 μ thick sections. However, more detailed observation on the differentiating zone was needed for the present work. Cross and oblique sections[9,26] of ca. 1 μ were cut from the specimens which were severed from the vertical controls used in the previous study, and were examined under a polarizing microscope. The number of the cells in the S 1 and S 2 deposition was estimated 2 and 6 for the 1st internode and 3 and 10 for the 2nd respectively. The S 3 thickening begins at the 9th and 14th cell from the beginning of the secondary wall formation in the 1st and 2nd internode respectively. Since the sample trees inclined in July examined in the present study had similar height and diameter to the vertical controls used in the previous one and were harvested in similar season, it can be permitted to suppose that they had similar numbers of the cambial and differentiating cells.

In the previous paper, the first cell showing the least excessive lignification, which seemed also the first cell having slightly thicker wall was supposed to have been situated near the earliest stage of the secondary wall formation at the beginning of the inclination. From the present observation

this cell would correspond to cell 10 in Photo 9 and cell 5 in Photo 10, and cell 6 in Fig. 4 and cell 9 in Fig. 5. These cells seem to have essentially the nature of compression wood cells including the thicker S 1 layer, and FUJITA *et al.*[9] reported that the fibril angle of the transitional tracheids changed markedly at the foot of the increase in cell wall thickness. Therefore, the secondary wall of these cells should have been formed after the stimulus responsible for the compression wood formation had been percepted. The time required for the perception, *i. e.*, presentation time must be less than 2 days for the tree used in the present study, since 2-day inclination at 45° was sufficient to induce the formation of slight compression wood cells with somewhat thicker wall and mild UV-absorption in S 2 (L) in an equivalent tree[50]. In the previous paper the rate of cell division in the cambial zone was estimated 2 and 2.5 cell per day in the 1st and 2nd internode respectively. Assuming that the presentation time for the formation of thicker wall of compression wood cells is ca. 24 hours and taking the fact into consideration that cells in the 2nd internode are not so sensitive as those in the 1st[49], the cells in question (cell C) must be situated, at least, at XII and at XVII in the 1st and 2nd internode respectively (Figs. 6 a and 6 b), or more to the cambial side than those, if account of latent period is taken. However, the latent period for the formation of the thicker wall

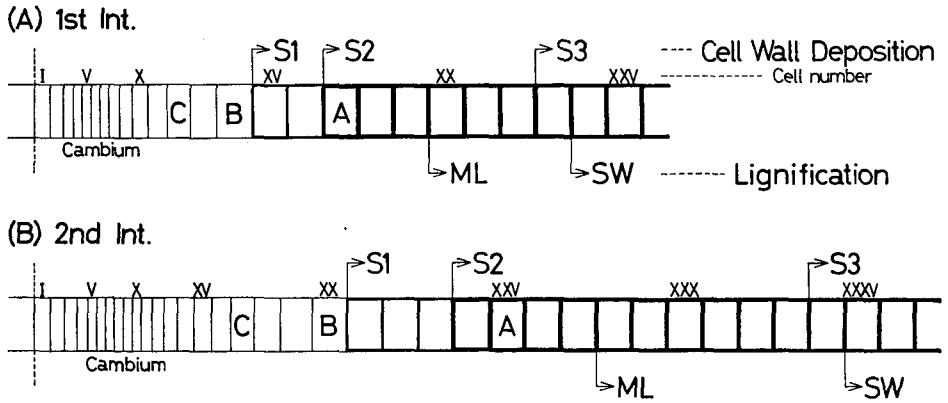

Fig. 6. Supposed sites of cells A, B and C at the begining of the inclination in the 1st internode (A) and in the 2nd (B). Cell A: the first cell without S 3 layer; cell B: the first cell with unusual UV-absorption in the inner region of the S 2 layer; cell C: the first cell with thicker cell wall and strong UV-absorption in S 2 (L); ML: middle lamella; SW: secondary walls. Arrows mean the beginning of cell wall deposition or lignification in the indicated parts of cell walls. The initiation of lignification was judged by the staining with gentian violet[49] and more sensitive method would reveal earlier initiation.

would be negligible, because the site of cell C estimated by the observation on the cell wall structure in the present study agrees well with that estimated from the standard course in the previous one. If this is the case, the first cells lacking in the S 3 layer (cell A) and the cells of the first unusual UV-absorption in the inner region of the S 2 layer (cell B) should be situated as in Figs. 6 a and 6 b. Perception occurs from this site inwards for the period of presentation time. It should be noted, however, that the site estimated here is not the sole site of the perception. As will be reported elsewhere, the stimuli are percepted not in a site but in a considerably wide zone. The site infered from the present study is only the inner margin of the perceptional zone.

It is of interst that cell A is found in far earlier stage than the S 3 layer formation begins and that the similar is true in the case of cell B. Since the fibril angle of the transitional tracheids changed markedly at the foot of the increase of cell wall thickness[9], the secondary wall of the cells of lesser number than those would be formed normally. As stated above, they have thinner wall than usual and pebble-like deposits on the inner surfaces of these cells were observed. These facts would indicate that the normal wall formation was interrupted after the inclination. If so, it is not strange that the first cell lacking in the S 3 layer is found in far earlier stage at the commencement of the inclination, and there would be a good posibility the transition layer between the S 2 and S 3[14,19,20] to be observed adjoining to cell 1. Such a layer was actually observed (Photo 4), though only once as a convincing case through the scrutiny of ca. 60 files. The hesitative disappearance of the S 3 layer (Photo 3) can be thought to be also caused by the interruption of the normal wall formation, if the deposition of the S 3 layer would occur not regularly in succession in a radial file, or alternatively, if there would be the difference in the sensitivity of the cells to the interruption.

Another posibility is that whether the S 3 layer is formed or not is determined independently of the formation of the S 2 layer of compression wood cells. Since cell A is older than cell C in the developmental sequence, sensitivity of the cell A to the stimulus might be lower than that of cell C, and therefore, presentation time for the lack of the S 3 layer might longer than that for the formation of the characteristic secondary wall of compression wood cells. It also can be thought that longer latent period is needed for the lack of the S 3 layer. However, between the formation of the S 2 and S 3 layer, there would not be so essential differences as those found between the cellulose wall formation and lignification.

The fact that the first cell which shows heavy lignification in S 2 (L) is, in most cases, also the first cell with thicker wall was also reported by FUJITA *et al.*[9], though contradicting the hypothesis of KENNEDY and FARRAR[25]. According to the hypothesis, heavy lignification should be found in cells of more pith side. FUJITA *et al.*[9] attributed this to the fact that lignin precursors are already synthesized and stored in the cytoplasm of the cells under the S 2 formation. This seems highly reasonable. However, if the heavy lignification of compression wood cells is caused simply by the large quantity of the precursors, why lignin is not distributed evenly but characteristically? The reason must lie in the cell wall. Although the manner of lignification in the cellulose wall, which was already formed before lignification occurs, remains still a moot question, there might be left spaces to be occupied with lignin when the microfibrils are deposited[43]. Another possibility could be sought in the distribution of the lignin polymerizing enzymes in cell walls[15,17,18]. If the enzymes are incorporated in a definite pattern with the advance of the cellulose deposition, and if the activation of the enzymes occurs from the outer side of the cell wall, polymerized lignin would be distributed in accordance with that of the enzymes. In either case, distribution of lignin is determined at the time of cellulose deposition, and thus, longer latent period is required for the characteristic distribution of lignin in the secondary wall of compression wood cells.

The first unusual lignification in the inner region of the S 2 layer is difficult to explain. The secondary wall of these cells is thought to be formed normally as mentioned above. Since polymerization of the lignin precursors was reported to occur considerably after their synthesis[10], excessive quantity of the precursors incorporated into the normal secondary wall would simply result in excessive lignification distributed normally. However, if excessive quantity of the precursors is provided after the lignification of the secondary wall began, only the inner region of the secondary wall which remains non-lignified will be excessively lignified, because the lignified wall is thought to disturb the penetration of the precursors. Alternatively, even though the fibril angle appeared normal[9], the inner region of the secondary wall of these cells might be formed as that of compression wood cells, namely, in the course of the secondary wall formation, the nature of the wall might be altered from normal to that of compression wood cells after the perception of the gravitational stimulus.

In the present study, an increase of the severity of compression wood cells towards the typical ones was confirmed. Since the formation of compression wood cells is thought to be a geotropic manifestation[33,45,46], the severity

would be determined by the intensity of the stimulus, the duration of the stimulation and the sensitivity to the stimulus as in herbaceous plants[1,23]. In the study of geotropism in herbaceous plants, the actual intensity of the stimulus is thought not to increase gradually after the stimulation and the presentation time and the sensitivity have been studied in terms of the organ. However, there are differences in many aspects of the phenomenon between relatively small and simple primary tissues of herbaceous plants and complicated secondary tissues of large-sized woody plants[45]. Strong indication of IAA as a crucial factor to induce compression wood formation has been repeatedly reported[3,27,29,43,44]. If compression wood formation is caused by a transverse asymmetry of the substances such as IAA in favour of the lower side, and if it takes more time to achieve such an asymmetry in large-sized woody plants than in simple herbaceous plants, the intensity of the stimulus would increase gradually after the inclination. However, lateral migration of the substances from the upper to lower side seems not responsible for the compression wood formation[28] and the time required for the detection of the features of compression wood cells was not different between direct application of IAA in lanoline paste and tilting[29].

WESTING[45] concluded in his extensive review that the compression wood formation under normal conditions is the result of an increased local sensitization to auxin caused by geotropic stimulation. If sensitivity of differentiating cells to auxin increases gradually following inclination, an increase of the severity of compression wood cells would be observed. Although slight difference in the sensitivity of cells to the stimulus was found[50], such a gradual and steady increase has not been reported.

Another possibility lies in the difference in the duration of the stimulation in the cellular level. In the study of compression wood cells, the stimulation should be considered in terms of cells for each structural feature separately. The perception site infered in the present study is only the inner margin of the perceptional zone, and a cell could be stimulated only for the period that the cell takes to pass through the zone. If the zone is wide enough, cells situated within the zone at the inception of the inclination must be stimulated for different periods depending on their position in the zone, namely, cells in the inner side of the zone are stimulated only for a short period and would develop into slight compression wood cells, and those in the outer side are stimulated for a sufficiently long period and would show features of typical compression wood cells. From Figs. 4 and 5 an increase of the cell wall thickness was observed in cell 6 to 14 in the 1st internode and cell 9 to 20 in the 2nd, and therefore, the perceptional zone

for the thick cell wall should be not narrower than 9-cell and 12-cell wide in the 1st and 2nd internode respectively, and assuming the presentation time of ca. 24 hours, further 2 and 3 cells should be added to these values respectively. According to Figs. 15 and 16 in the pervious paper, it takes 4 days for 9 and 12 cells to be newly formed in these internodes respectively, and therefore, 5-day stimulation is though to be enough to induce typical compression wood cells. However, as will be reported elsewhere, inclination of 6 days at 45°, after which the tree was returned to the original vertical position, was not sufficient and actually 8-day inclination was required for the formation of typical compression wood cells.

Conclusions

The site of the perception of each characteristic feature of a compression wood cell estimated in the present study from the anatomical observation on the transitional zone agreed well with that infered in a previous paper from the standard time course. The perception of the stimulus for the thicker wall is thought to occur slightly before the formation of the secondary wall begins, as in the hypothesis of KENNEDY and FARRAR[25], though no account is taken for the presentation time in this hypothesis. However, the site for the disappearance of the S 3 layer and that for the excessive lignification were found in far earlier developmental stages than those expected by the hypothesis. The latter could be explained by the concept of the latent period. The first unusual UV-absorption in the inner region of the S 2 layer and the decrease of the cell wall thickness in the early stage of the transition exceed the basic concept of the hypothesis. Thus, the mechanism of the perception is far more complex than that KENNEDY and FARRAR thought about fifteen years ago and can not be simply explained.

However, the perception could be thought from two different aspects, namely, positive and negative one. Although compression wood has often been regarded as "abnormal" wood by many workers, the wood is formed in some definite conditions through a genetically determined process. Genes responsible for the formation would be switched on depending on the change in environmental or internal conditions. In this respect, compression wood should not be called "abnormal" but a particular type of wood. The characteristic lignin distribution, thicker cell wall, rounded outline and intercellular spaces may be formed by the action of the genes thus switched on. This is the positive aspect in the perception. However, not all the characteristics of a compression wood cell are formed in this manner. Disappearance of the S 3 layer and the initial decrease of the S 2 wall thickness in the tran-

sition, on the other hand, would be a manifestation of the negetive aspect. These characteristics seem to be formed simply by the interruption of the normal wood formation. The genes responsible for the normal formation would be switched off by geotropic stimulation and switched on when the tree recovers its equilibrium position. In this respect, the perception in the true sense of the word can not be said to occur.

Summary

The transition from normal to compression wood was studied by a SEM-UVM combination method divised for the present study, by which features of the inner surfaces and those found in the cross section of the same cells along a radial file can be shown.

1) The SEM-UVM combination method successfully revealed the nature of the transition from normal to compression wood. The method seems to have a general value for the study of wood anatomy. Matched specimens for SEM and UVM are prepared in the following manner. i) A small piece of a sample is dehydrated and embedded in methacrylate resin mixture and a thin section is cut for the UVM observation. ii) Remainder of the embedded specimen is cut out from the resin block and attached to the top of a slender alminium stub. iii) A radial surface of the specimen is finished on a ultramicrotome to expose the inner surfaces of cells in a radial file adjusting the angle of the specimen following repeated inspections under a metallurgical microscope. iv) A small tangentially flat piece with the finished surface is cut from the specimen to give a new radial surface, the latter is also finished in the same manner. These pieces are soaked in acetone or xylene to remove the resin and dried in room conditions. The rest of the procedure is in common with that of routine methods.

1) The general pattern of the transition is not different among the seasons and sampling positions. The transition occurs in the following order. i) Disappearance of the S 3 layer; the transition layer between the S 2 and S 3 was rarely observed between the cells with and without the apparent S 3 layer. The "hesitative" disappearance (see text) can occur in latewood. ii) Decrease in the thickness of the S 2 layer. iii) Appearance of unusual UV-absorption in the inner region of the S 2 layer, which is found at the bottom of the change in the cell wall thickness. The unusual UV-absorption spreads outwards and finally the outer region of the S 2 layer becomes to show the most strong UV-absorption. iv) Increase in the strength of UV-absorption, the cell wall thickness and the roundness of the outline, followed by the formation of spiral grooves. UV-absorption seems

to increase rapidly and the increase in the degree of the development of the grooves is rather variable. On the other hand, cell wall thickness and the roundness seem to increase in parallel and gradually up to the appearance of intercellular spaces.

3) From these and other evidences, the perception site of the stimulus for each characteristic feature of a compression wood cell was infered and the mechanism of the perception was discussed in comparison with that in herbaceous plants.

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EXPLANATION OF PHOTOGRAPHS

PLATE I.

Photos 1 a and 1 b. A general view of the transitional zone from normal (right) to compression wood (left) formed by artificial inclination in July. The upper half (UV-photograph) and the lower one (SEM-photograph) are matched exactly. Cells of the radial file are numbered; the first cell without the S 3 layer is numbered "1" and the number is increased to the cambial side and decreased to the pith side as "0", "−1", "−2", etc. Unusual UV-absorption is observed faintly in cell 3 and apparently in cell 4. Cell 5 shows roundness in cross section and has spiral grooves and strong UV-absorption in S 2 (L). A typical compression wood cell with an intercellular space is found in cell 11.

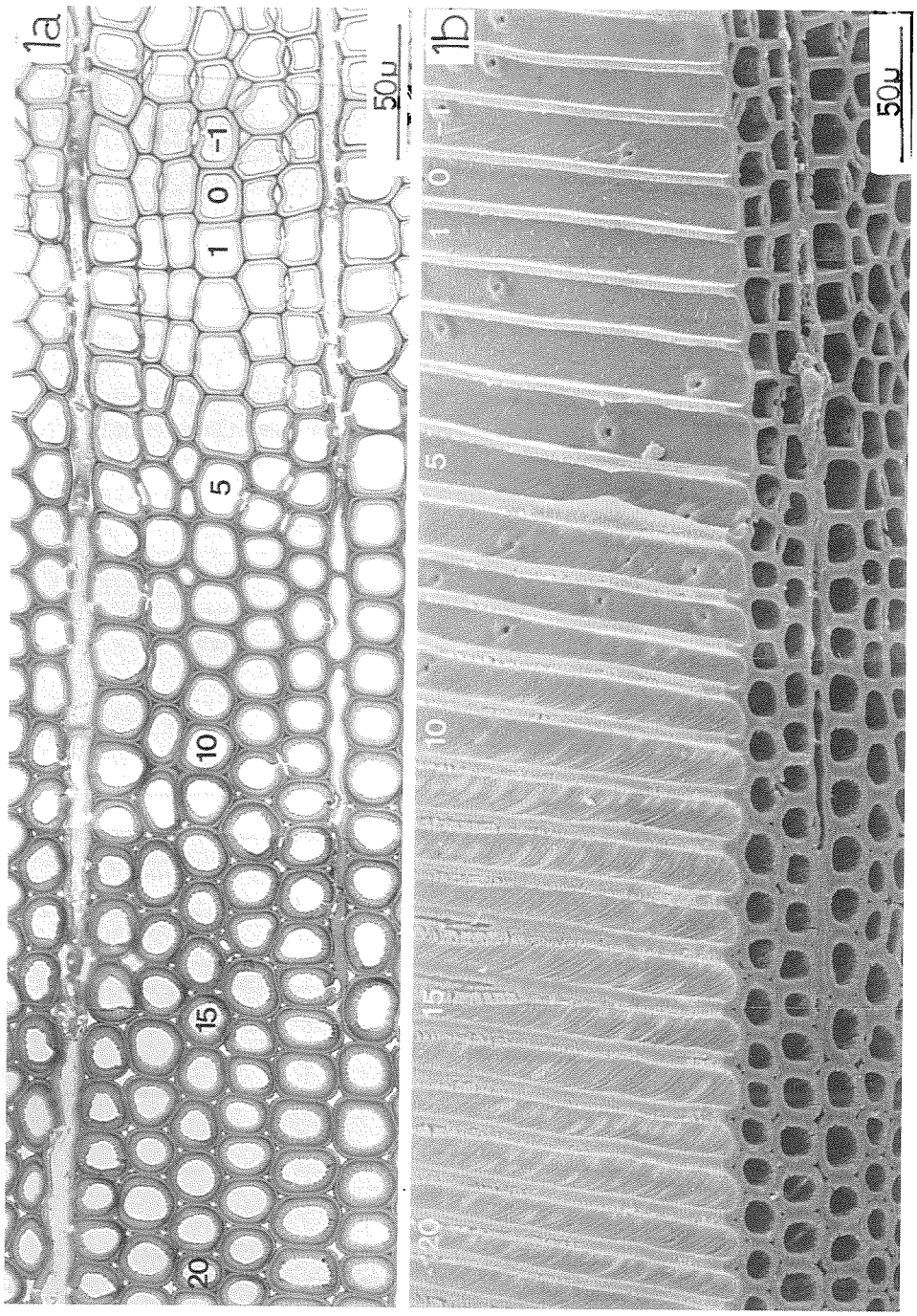

1a
50μ
1 0 -1
5
10
15
20
1b
50μ
-1 0 1
5
10
15
20

PLATE II. Disappearance of the S 3 layer

Photos 2 a, 2 b and 2 c. Normal disappearance of the S 3 layer found in the transitional zone formed in July. The disappearance in SEM-photograph (2 c) is confirmed also by polarizing microscopy (2 a). No changes in UV-photograph (2 b) is found accompanying the disappearance.

Photo 3. The "hesitative" disappearance of the S 3 layer found in the transitional zone formed in September. The S 3 layer disappeared once in cell −1 and reappeared in cell 0, then redisappeared in cell 1. Such a "hesitative" transition is common in September. The inner most layer of cell −2 is reminiscent of the transition layer between S 2 and S 3.

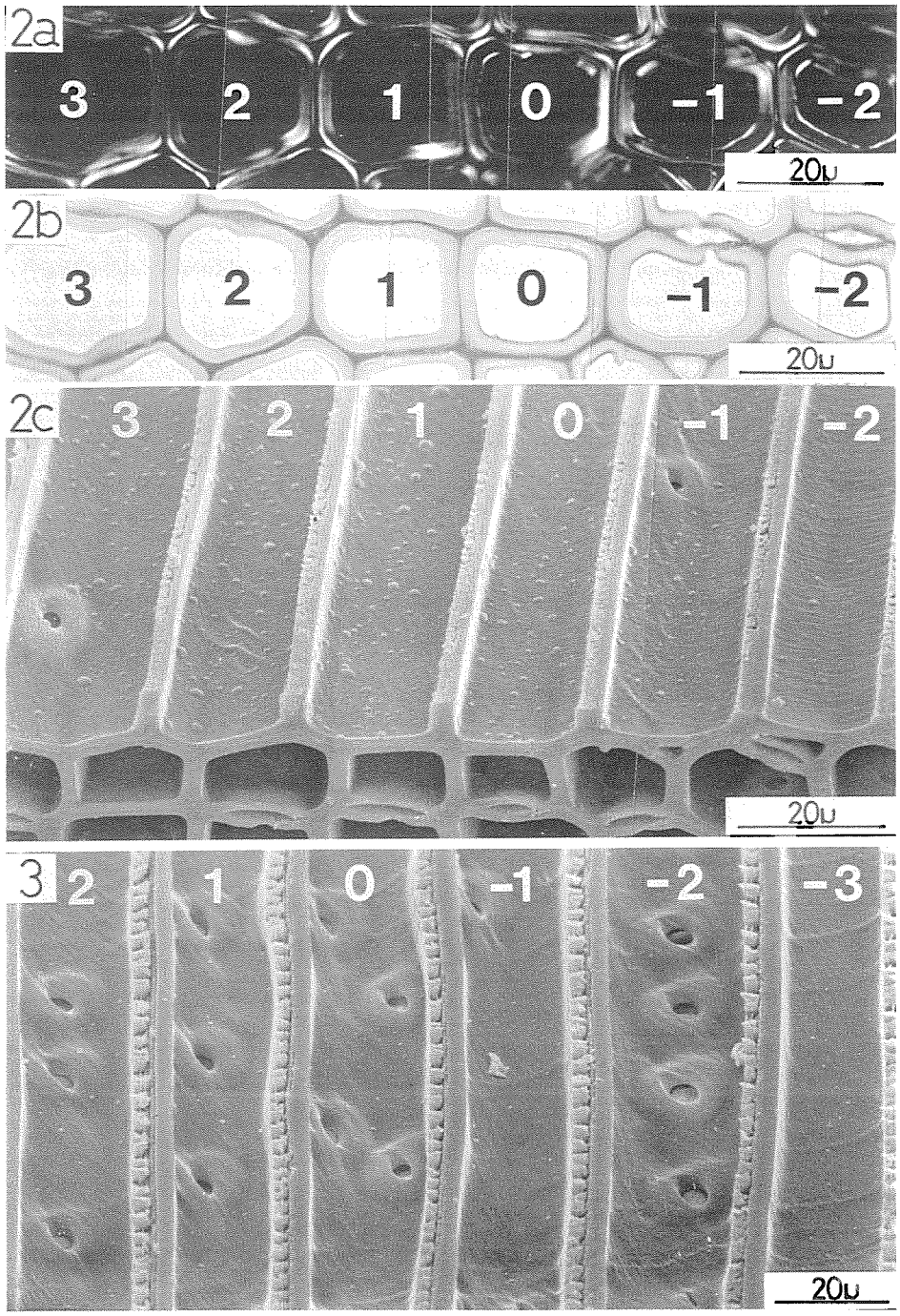

2a
3
2
1
0
-1
-2
20μ
2b
3
2
1
0
-1
-2
20μ
2c
3
2
1
0
-1
-2
20μ
3
2
1
0
-1
-2
-3
20μ

PLATE III.

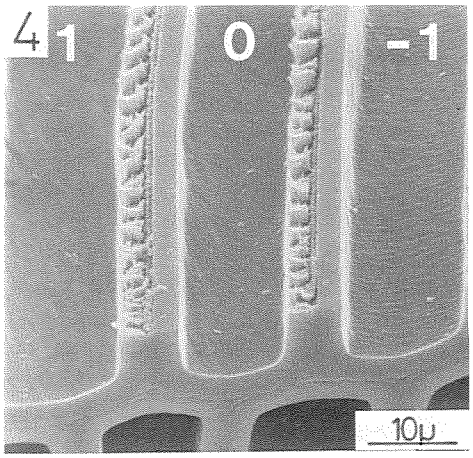
Photo 4. The transition layer found in a cell between cell 1 without S 3 layer and cell −1 with apparent S 3 layer. The occurrence of such a cell is very rare and only once observed as a convincing case through the scrutiny of ca. 60 radial files.

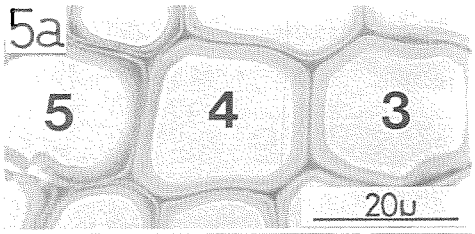
Photos 5 a and 5 b. Appearance of unusual UV-absorption in the inner region of the S 2 layer, faintly seen in cell 3 and apparently in cell 4. Photo 5 b shows the inner surfaces of the same cells.

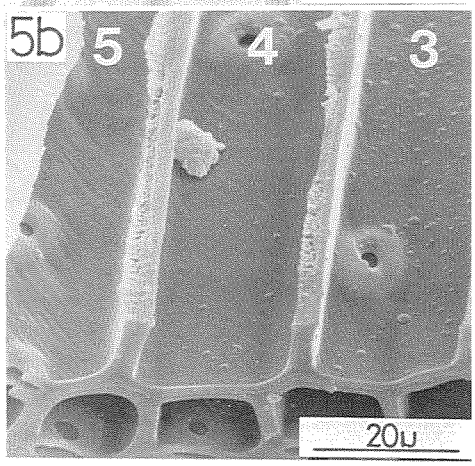
Photo 6. Appearance of the unusual UV-absorption in latewood (inclined in September). The unusual UV-absorption in the inner region of the S 2 layer is clearly shown.

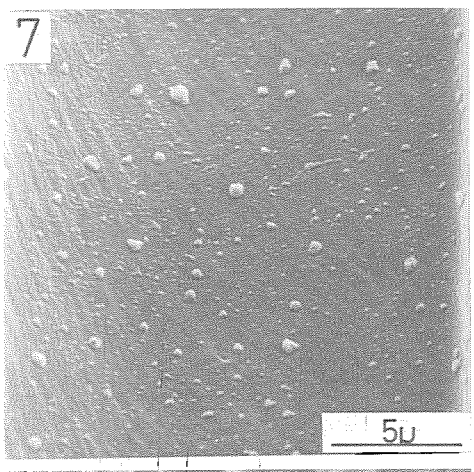
Photo 7. Pebble-like deposits found on the inner surface of a cell in the early stage of the transition. The specimen was not prepared by the SEM-UVM combination method but finished on a freeze microtome (see text), therefore, they were not caused by insufficient removal of the embedding resin. Such deposits are also seen in other photographs.

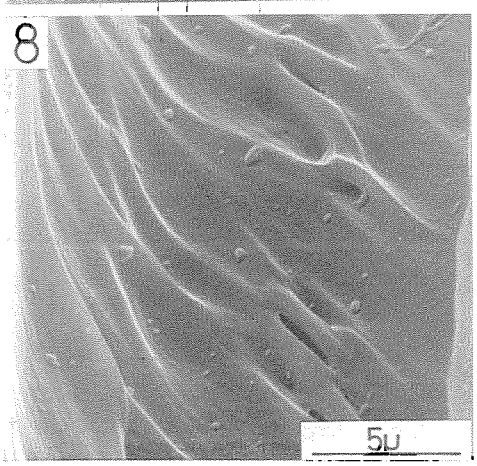
Photo 8. An extreme case of anomalous modification of the S 2 layer found in early stage of the transition. Such modification is also seen in Photo 2 c and is not necessarily restricted in this region.


4
1
0
-1
10μ


5a
5
4
3
20μ


5b
5
4
3
20μ


7
5μ


8
5μ

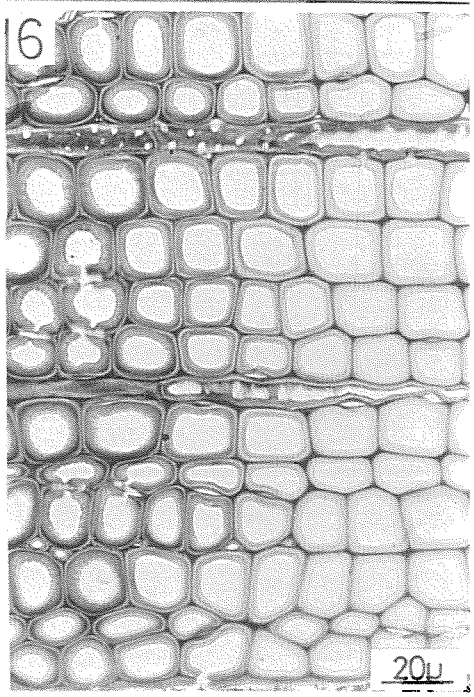

6
20μ

PLATE IV.

Photos 9 a and 9 b. Later stage of the transition. A case of abrupt change in the degree of the development of spiral grooves found in the 2nd internode of a sample tree inclined in July. Cell 10 is the first cell with the grooves and the next cell 11 shows already fully developed grooves as those found in later formed cells.

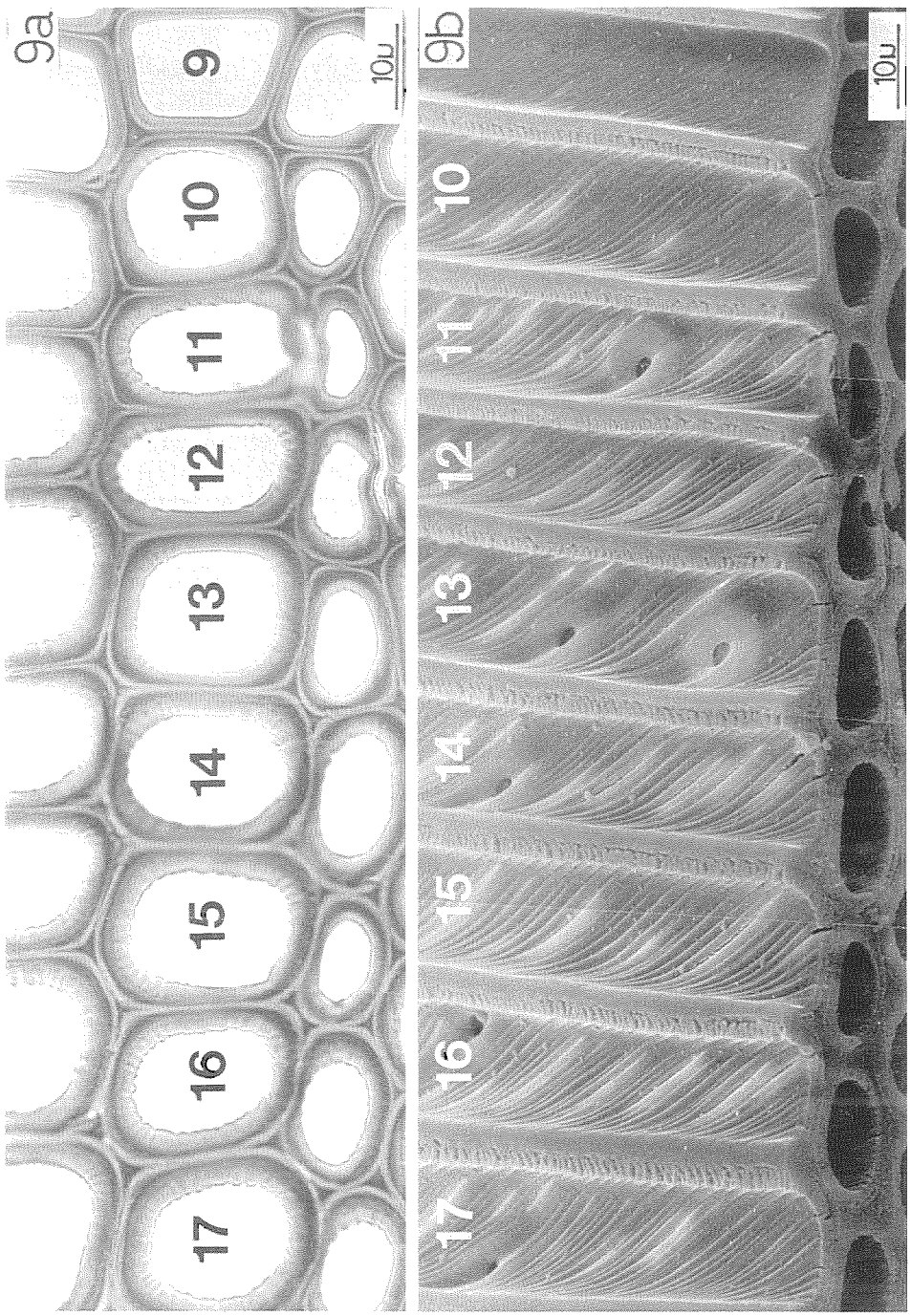

9a
9
10
11
12
13
14
15
16
17
10μ
9b
10
11
12
13
14
15
16
17
10μ

PLATE V.

Photos 10 a and 10 b. A case of gradual change in the degree of the development of spiral grooves found in the 1st internode. Grooves first appear in cell 5 and do not fully develop up to cell 9. The typical grooves are found in cell 10 or 11, the latter is the first cell with an intercellular space.

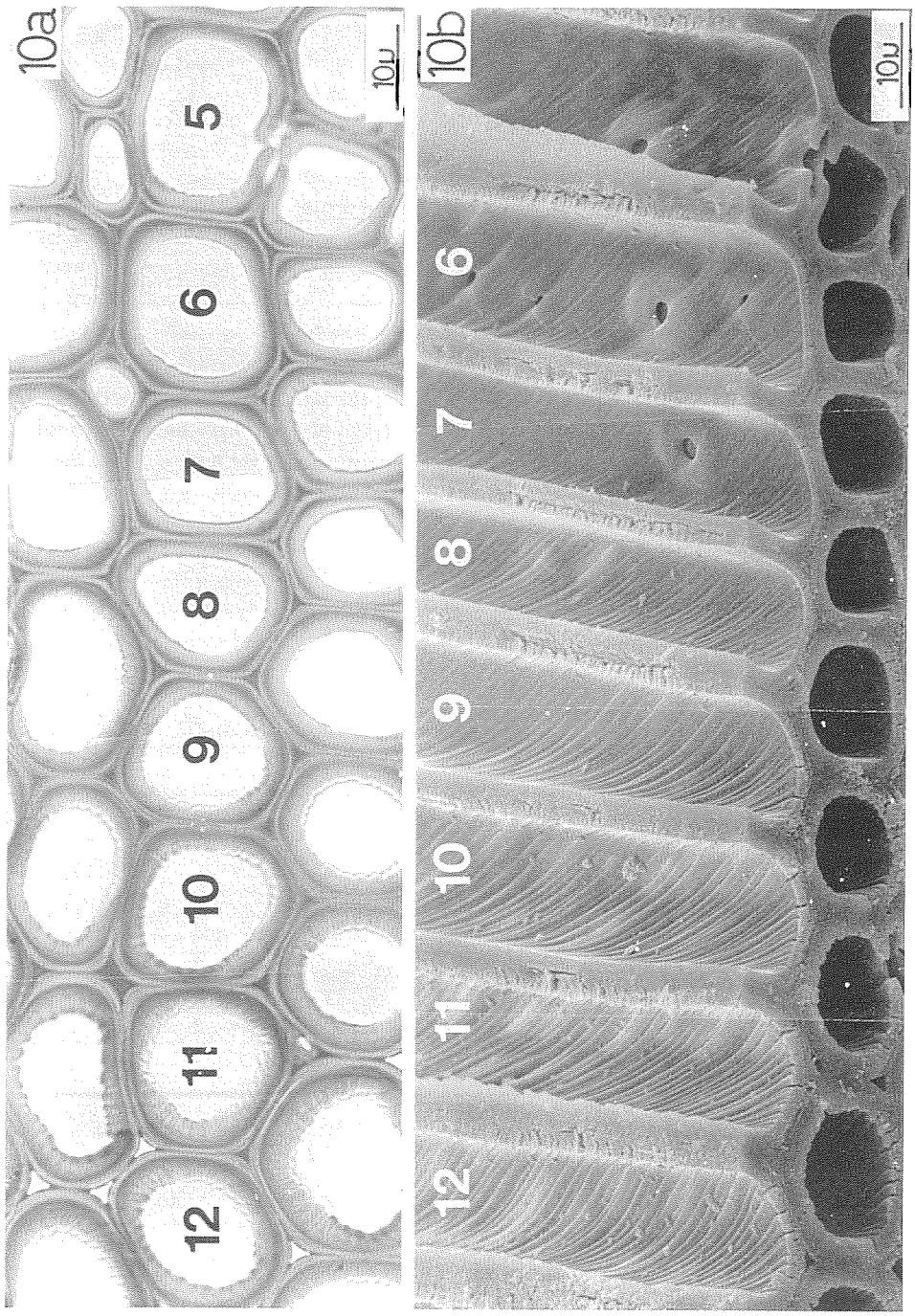
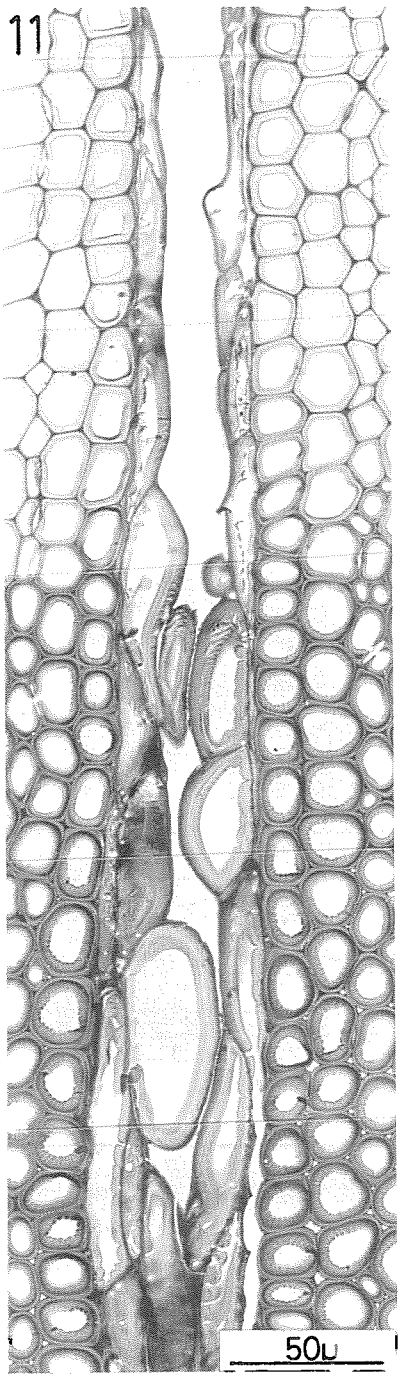

10a
5
6
7
8
9
10
11
12
10μ
10b
6
7
8
9
10
11
12
10μ

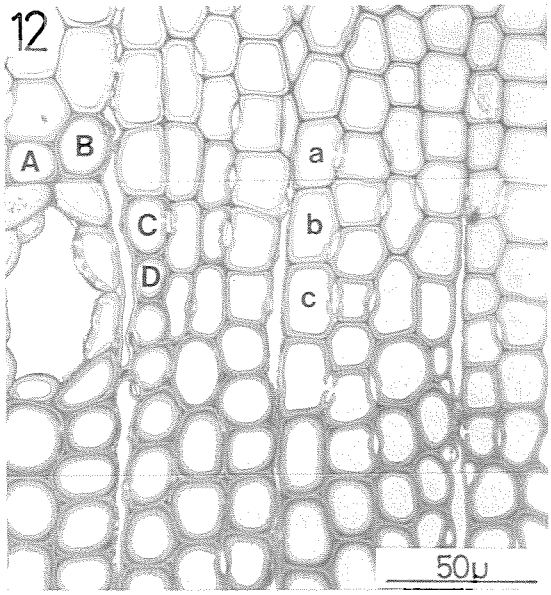
PLATE VI.

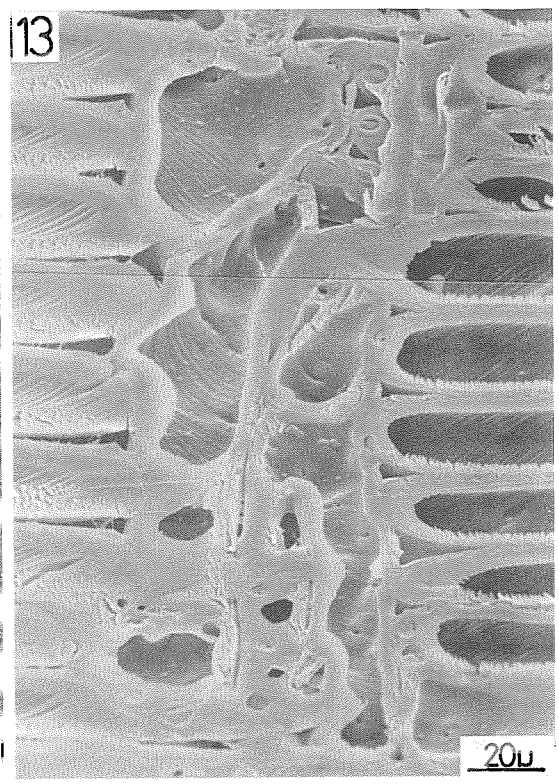
Photo 11. The change of the epitherial cells of a horizontal resin canal accompanying the transition from normal to compression wood. Flattened epitherial cells with relatively thin walls are found in normal wood and expanded thick-walled ones in compression wood. These change accompanying the transition, however, does not always occur.

Photo 12. The occurrence of a vertical resin canal in the early stage of the transition. The transition is shifted towards the pith side. Cells A to D show strong UV-absorption in S 2 (L) and some of them have apparent spiral grooves, on the other hand, cells a to c situated in the similar radial position do not.

Photo 13. "Compression ray tracheids" found in a specimen. The degree of the development of the grooves are variable.


11
50μ


12
A
B
C
D
a
b
c
50μ


13
20μ