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A Scanning and Transmission Electron Microscopic Study of Layered Structure of Wall in Pit Border Region between Earlywood Tracheids in Conifer*

By
Yasuhiko HIRAKAWA and Shigeo ISHIDA**

Introduction

The organization of cell wall in the region of pit border of pits between early wood tracheids in conifers has been investigated by many workers, because the cell wall modification by the presence of a number of pits in radial walls has been thought to have influence on the physical and mechanical properties of wood.

In the previous study for this subject by BAILEY and VESTAL9, it was pointed out that the cellulose of outer layer had distinctively a circular orientation, using the light microscopy.

Afterwards, by the electron microscopic studies with the replica method, the existence of wall layer having concentric microfibrillar orientation in the region of pit border has been confirmed10,16,17).

WARDROP and DADS WELL23) concluded that this circularly oriented layer was formed initially in the process of pit border development, and furthermore WARDROP

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and Davies and Wardrop suggested that this layer should be distinguished from the S1 layer and named it the initial border thickening (B, T). On the other hand, Jutte and Spitt reported that secondary wall layer enveloped continuously from lumen side through chamber side of pit border, microfibrils of which had a streamline fashion in the former and circular orientation in the latter. Harada and Coté examined this region in eight species by the light and the electron microscopy. According to their observation, the initial border thickening having concentric orientation of microfibrils is a thin layer, on the other hand, the S1 layer is considerably thick as compared with that of non pitted region, and the final size of pit aperture is nearly determined by the S1 layer. Murmanis and Sachs concluded that the reason why hardly any two description of pit border structure was identical was the existence of variation of it within same tree species, from the observation on early- and latewoods of Pinus strobus.

Afterwards, the idea of Harada and Coté received a support by some workers. By the suggestion of Imamura and Harada, the diameter of pit aperture was reached its final size until the S1 layer formation, additionally during which microfibrils were generally curved around the pit aperture and some of them extended to the outer surface of developing pit border beyond the pit aperture, resulting in the scale-like texture of pit border was formed.

The existence of the wall layer having concentric microfibrillar orientation in the region of pit border has been discernible by many workers, however, all diagrams of layered structure of pit border as shown by some workers are not identical. The subject having such complex organization as pit border should be studied using various methods. In the previous papers of Hirakawa et al., it was pointed out that SEM was very useful for the study of cell wall formation and structure. Furthermore, it was revealed that the orientation of microtubules reflected the microfibrillar orientation of underlying wall layer by SEM.

The purpose of this study is to clarify the pit border layered structure of cell wall of earlywood tracheids using SEM as well as TEM.

Materials and Methods

Materials for this study were obtained from a thirty year old Todomatsu tree (Abies sachalinensis Mast.), grown in a plantation in Tomakomai Experiment Forest, Hokkaido University.

They were collected at two-week intervals during the active period of the cambium in 1978 and 1979.

Small wood blocks containing xylem, cambial zone and phloem, (about 1 x 1 x 1 cm or 5 x 5 x 5 mm) were plasmolyzed in 0.8 M saccharose in a refrigerator, or fixed initially in 3% glutaraldehyde in 0.05 M sodium cacodilate buffer for 5-6 hrs and postfixied in 2% osmium tetroxide for 2-3 hrs at room temperature. In the former, materials were prepared for the observation on the microfibrillar orientation of wall layer, in the latter for the orientation of microtubules.
After the fixation, for SEM, they were dehydrated in graded series ethanol or acetone, the next dried by the critical point drying method, and then sectioned radially by the razor blade in order to disclose the lumen surface of the cell, or fractured from the middle lamella to reveal the chamber side of pit border. Specimens were shadowed by Pt and then coated Au by the ion sputter coating.

In order to observe the smoothly cut surface of pit border by SEM, some of specimens were embedded with methacrylates, the next they were sectioned transversely with a glass knife, methacrylates were removed by xylen or acetone, and then dried by the c. p. d. method.

The carbon replica film was prepared by the way of direct carbon replica method as described by Côté et al. For the ultrathin sections, they were embedded by spurr, sectioned on LKB ULTRAMICROTOME 8800 and then stained with uranyl acetate and lead citrate on the copper grids.

Observations were made with JSM-2 and JEM-6AS.

**Results**

Firstly, the process of pit border development of differentiating tracheids, i.e., increasing in width and thickness of pit border, was successively examined in a radial file by SEM.

The pit border was developed during the secondary wall deposition. The diameter of pit aperture was gradually decreased with the secondary wall deposition from cells forming the S1 layer to the S2 layer, and its final size was reached nearly during first stage of the S2 layer formation, as the result, it was approximately 4-6 μm in diameter in mature tracheids of earlywoods. The decrease of pit aperture diameter reflected the increasing in width of pit border of differentiating tracheids in a radial file. In each stages of secondary wall deposition containing a transition layer, i.e., the S1, the S12 and the S2, the diameter of pit aperture was 14-10 μm, 10-7 μm, and 6-4 μm, respectively. (Photos 1-6). After the middle stage of S2 formation, it was nearly uniform 4-5 μm in diameter.

In this study, each stages of secondary wall formation was concluded based on the information of the microfibrillar orientation in the non pitted region which was above 10 μm away from the pit aperture circumference but near the pit, and of sites of cells in a radial file.

Subsequently, the thickness of pit border was also measured successively in each stages of secondary wall formation. Photo 7 shows the transversely cut surface of pit border in the stage of S1 or S12 layer formation. The thickness of pit border is as same as of double walls of non pitted region. Untill the first stage of S2 layer formation, the width of the pit border increased keeping nearly same thickness. (Photo 8). After the diameter of pit aperture was reached its final size in the S2 layer formation, it became more thicker by the deposition of S2 layer of lumen side. (Photo 9).

Secondly, the orientation of depositing microfibrils in the region of pit border
was examined in both lumen and pit-chamber sides by the replica method and SEM. In the case of the study of chamber side of pit border, wood specimens were fractured radially from the middle lamella, thus each stages of secondary wall formation in the lumen side can not be shown generally, however, in this study it can be easily decided by the diameter of pit aperture and site of cells in a radial file.

In the initial stage of pit border formation, microfibrils having two different kinds of orientations were deposited simultaneously, the one is oriented in a streamline fashion, the other concentrically, in the lumen surface of inner primary or first stage of S1 layer as shown in Photo 10 in which the protuberance of the pit border was still little visible. The pit border was protruded to the lumen with the deposition of the S1 layer. Photos 11 and 12 show the pit border in the stage of S1 layer formation in which microfibrils are running transversely in a streamline fashion in the lumen surface, on the other hand, circularly in the chamber side. (Photos 11 and 12) Photo 13 reveals the SEM micrograph in the same stage in which microfibrils having circular orientation in the chamber side are not joined to the layer of lumen surface.

In the stage of S2 layer formation, microfibrils were oriented at an angle of about 5°–10° in non pitted region, and in a streamline fashion in the pitted region of lumen surface as shown in photo 14. Photo 15 reveals disturbed microfibrils of S2 layer near the tip of pit border in the lumen side, and below these microfibrils the layer having concentric orientation of microfibrils is found. This micrograph indicates that microfibrils of S2 layer are oriented along the pit aperture outline. In the chamber side in the same stage, microfibrils are oriented concentrically on the base of pit border as shown on the lower left of photo 16, on the other hand, in a scrollwork-like fashion near the pit border tip as shown in photo 17. In the latter, microfibrils are running to the pit border tip.

Photo 18 shows the lumen surface of the cell forming the S3 layer in which microfibrils are oriented concentrically in the region of pit border tip, in a streamline fashion near the pit aperture and straight in other region. In the chamber side, its orientation was as same as that of S2 layer formation.

It is somewhat remarkable that when the microfibrillar orientation of lumen surface was examined in all stages of secondary wall formation, concentrically oriented microfibrils were frequently found in the region of pit border tip, i.e., in the region of an intersection of pit aperture circumference and a line through the center of aperture, parallel to the microfibrillar orientation of each stages of secondary wall formation. (Photos 11, 14 and 18)

Thirdly, the layered structure of pit border was also examined in split specimens by SEM, and in the ultrathin section. Photos 19, 20 and 21 show the materials which were fractured from the middle lamella. The separated wall layer as a ring is often found as shown in photo 19, additionally below this wall layer as a ring, the S1 and the S2 layer of lumen side can be visible, where it is apparent that the S2 layer forms the pit aperture outline. (Photos 20 and 21)
This ringed wall layer of chamber side is considerably thick one as shown in photo 21.

Photo 22 reveals the lumen side of the cell forming the S12 layer in which the S1 and S12 layer are mostly torn off, and as the result only the ringed wall layer remains. Similarly, in the lumen side of the cell forming the S3 layer, the circular wall layer can be visible below the separated secondary wall layers as shown in photo 23.

Additionally, the layered structure of pit border was also shown by the ultrathin transverse section. Photo 24 shows the cell forming the S2 layer in which the thickness of S2 layer is still same as that of the layer of chamber side (B.T.), and the S1 layer is visible in the middle of pit border.

Lastly, the orientation of microtubules in the region of pit border was observed in double-fixed materials by SEM, because it reflects that of depositing microfibrils.

Photo 25 shows the inner surface of plasmamembrane of the cell forming the S1 layer. In this cell, microtubules are almost running in a streamline fashion along the pit aperture. In the cell forming the S2 layer, they are oriented axially in a streamline fashion in the lumen side as shown in photo 26, and some of them are running concentrically in the region of pit border tip in photo 27. (arrow) In the cell forming the S3 layer as shown in photo 28, they are oriented straight and in a streamline fashion near the pit aperture. In the chamber side, microtubules were oriented circularly or in a scrollwork-like fashion, but they are not present in line in an orderly way as compared with that of lumen side. (Photos 29 and 30)

**Discussion and Conclusion**

The observation on pit border formation and structure was made by SEM as well as TEM in differentiating tracheids in order to clarify the layered structure of it.

Although the organization of pit border region has been studied by many workers, there are no agreed opinions for this question with the exception of a few examples. It may be implied that the discrepancy of interpretations is caused by difference of studying methods, therefore the choice of the method seems to be in itself a problem in this type of investigation. The availability of SEM which was used also in this study, has been already indicated by the previous paper of the authors\(^{12}\), but the SEM study for such subject has been little undertaken in detail\(^{12,14}\).

In view of some variability in reported results, it should be discussed, whether the layer which determines the final size of pit aperture in differentiating tracheids is the S1\(^{15,16}\) or the S2 layer\(^{15,24}\) the layer having concentric microfibrillar orientation in chamber side of pit border is distinguished from the secondary wall layer of lumen side\(^{23}\) or not\(^{15,18}\) and such concentric layer is thick\(^{24}\) or thin\(^{9}\). Additionally, the most significant point in this study seems to be that each stages
of secondary wall formation is exactly discerned in non pitted region. In this study, based on the information of the previous paper of the authors, they indicated that depositing microfibrils were oriented almost regularly in the short distance of a differentiating tracheid, the orientation of microfibrils was examined in the region which was above $10 \mu m$ away from the pit aperture circumference but near the pit. Additionally, as also indicated in the previous paper, the secondary wall can be divided into five different kinds of layers by their microfibrillar orientation in non pitted region, i.e., S 1, S 12, S 2, S 23 and S 3 layer.

Firstly, increasing of pit border width was shown in differentiating cells by SEM. When the diameter of pit aperture was measured in each stages of secondary wall formation as shown in photos 1-6, it was reached its final size in the first stage of S 2 layer formation. The diameter of it is nearly uniform in earlywood of an annual ring of mature tracheids, thus the decreasing of aperture size reflects the increase of width of pit border. Even if the secondary wall is divided into only three different kinds of layers by the way of Dunning's work, i.e., the S 1 layer may contain the S 12 layer named by HARADA et al. and other workers, increasing in width of pit border lasted also in the S 2 layer formation. A number of differentiating tracheids were investigated by SEM, but there were no variations in pit aperture size. The role of S 2 is confirmed in split specimens as shown in photos 20 and 21. This result is not consistent with that of HARADA and CÔTE, and IMAMURA and HARADA, on the other hand, only in this point of view this is agreement with that of JUTTE and SPITT, Murmanis and Sachs, and WARDROP and DAVIES.

Subsequently, measurements of thickness of pit border were made in each stages of secondary wall formation by SEM as shown in photos 7-9. The thickness of pit border was uniform from cells forming the S 1 layer to the first stage of S 2 layer, and it is more thicker as compared with that of non pitted region until the first or middle stage of S 2 layer formation. From above observation, it is implied that the secondary wall of pitted region is thickened peculiarly (JUTTE and SPITT, HARADA and CÔTE, IMAMURA and HARADA, and Murmanis and Sachs), or the pit border is thickened by the deposition of both secondary wall of lumen side and other layer of chamber side. (WARDROP and DAVIES) This problem should be discussed on the basis of observation on the microfibrillar orientation of differentiating tracheids.

The deposition of microfibrils of pit border was firstly made on the inner primary or the S 1 layer, and microfibrils having two different kinds of orientations were depositing nearly at the same time. (Photo 10) Concentrically oriented microfibrils are forming, as will be mentioned later, the border thickening (B.T.), but the other in a streamline fashion can not be distinguished whether that of the inner primary wall or the S 1 layer. Although the S 1 layer possesses microfibrils with angular dispersion predominantly as compared with this lamella, both seem to be one of the first stage of S 1 layer formation because it is impossible to consider that microfibrils are depositing making like a circle during the surface growth.
This lamella having concentrically oriented microfibrils were named the initial border thickening by Wardrop and Davies.\textsuperscript{24} Since it seems to be not formed initially from the above observation, therefore, it should not be named the initial border thickening as already indicated by Imamura and Harada\textsuperscript{13}. Also as shown in photo 13, such oriented microfibrils were present independently on the wall. This evidence also indicates the peculiarity of pit border development in comparison with that of non pitted region of cell wall. If lamellae are deposited uniformly in the pitted region, the pit border can not be protruded peculiarly. Imamura and Harada\textsuperscript{13} stated that the reason why the pit border was thickened peculiarly was that the S1 layer was partially extended to the chamber side, however, such oriented microfibrils were not detected by the authors as shown in photos 12 and 13. Additionally, from their conclusion, microfibrils of chamber side must be deposited much more in the region of an intersection of pit aperture circumference and a line through the center of aperture parallel to striation of S1, however, the layer of chamber side, in other words, the border thickening was developed nearly with an uniform thickness. (Photos 7 and 8)

The microfibrillar orientation was almost a streamline fashion in the lumen surface, on the other hand, circular in the chamber side in cells forming from S1 to S3 layer as shown in photos 10-18. Such orientations of both sides were also discernible by the examination of microtubules of cellular organelle on the plasmamembrane as shown in photos 25-30. The orientations of them are more clearly detected by SEM. They were not oriented continuously from lumenside through chamber side.

Each secondary wall layers is named by its microfibrillar orientation, thus the thick layer having concentrically oriented microfibrils of chamber side should be distinguished from the other secondary wall layers. In this study it is named the border thickening, for convenience sake. The pit border is formed by the deposition of different layers having differently oriented microfibrils in each sides, therefore, the pit border is thickened much more than the wall of non pitted region of differentiating tracheids untill the first or middle stage of S2 layer formation. This seems to be consistent with the result of Okumura et al.\textsuperscript{19} by the polarizing microscopy, because the much more thickened border thickening as above mentioned reflects the concentric orientation in the pit border. On the other hand, Jutte and Spitt,\textsuperscript{20} and Murmanis and Sachs\textsuperscript{15} concluded that this layer (B.T.) was part of secondary wall layers in spite of their observation on the circular orientation. Although this layer and secondary wall layers of lumen side were deposited nearly at the same time, microfibrillar orientations are apparently distinct each other, and the evidence of the continuity of microfibrils from lumen side to chamber side is not present. Additionally, if the S2 layer of lumen side envelopes successively the pit border, the layer of chamber side must be thickened untill the same thickness of both sides in maturing cells, however, as shown in photo 9 this layer is more thinner than the S2 layer of nearly mature tracheids.
In the region of pit border tip, i.e., the boundary between the chamber and the lumen side, circularly oriented microfibrils were formed as shown in photos 11, 14 and 18. In the existence of such microfibrils on pit border tip of differentiating cells the difficulty seems to arise in the interpretation of the region of pit border. It was found that in the chamber side microfibrils were oriented concentrically on the base and in a scrollwork-like fashion near the pit border tip as shown in photos 16 and 17. In view of above observations, it seems reasonable to suppose that microfibrils having orientation in a scrollwork-like fashion in chamber side are joined to ones of the pit border tip, and they form the pit aperture outline, on the other hand, microfibrils on the base thicken the pit border chamber side. The pit border is developed with nearly uniform thickness by the deposition of both secondary wall layers in lumen side and B.T. in chamber side until the first stage of $S_2$ layer formation, the next only the $S_2$ layer is thickened extremely in the lumen side, and during which border thickening is a little thickened. Thus the scale-like texture which was shown by Harada and Cote, and Imamura and Harada appears to be not formed by the thick $S_1$ layer, but by the trace of pit border development during increasing in width, because microfibrils forming the pit aperture outline are deposited repeatedly on the pit border tip. (Fig. 1) After the pit aperture diameter is nearly reached its final size, the microfibrillar deposition seems to be made a little in the chamber side untill the cell matured.

![Fig. 1. The formation of scale-like texture of pit border.](image)

It seems to be very important that the layered structure of the wall which has been already formed is compared with the result of the study of depositing wall layer and discussed. The layered structure of pit border was also examined in split specimens by SEM. (Photos 19-23) As shown in photo 19, the pit border of chamber side was often separated as a ring, as pointed out by Wardrop and Davies. This ring appears to be the border thickening. The reason why this ring is often visible is that the border thickening has circularly oriented microfibrils which were deposited distinctively in chamber side of pit border.

It is no doubtful that the $S_2$ layer and border thickening formed finally the
pit aperture outline as shown in photos 20 and 21, and this is consistent with the result obtained by the study of microfibrillar deposition as shown in photos 1-6, and 15.

In photo 21, it is clearly shown that the border thickening is considerably thick layer. Harada and Côté,9) and Imamura and Harada15) concluded that this thick layer consisted of both the S1 and B. T., however, from their conclusion it can not be explained that the thick layer is separated as a ring. Also, if the pit border is enveloped by each secondary wall layers as suggested by Jütte and Spitt,16) and Murmanis and Sachs,17) they can not be found in split specimens, although shown in photos 20 and 21. Additionally, the border thickening also can be found in the lumen side as shown in photos 22 and 23. It is apparent that circularly oriented microfibrils which are distinguished from the S1 layer, form the thick layer. In the TEM micrograph of photo 24, the scale-like texture which was indicated by Harada9) and the other workers,15) is not visible, but it was often discernible in others. The layered structure of pit border which was shown in split specimens is entirely in agreement with the result above mentioned. The thickness of each layers of it is written as follows, the S2 layer is most thickened, the border thickening is the next, the S1 and the S3 layer in order of thickness.

On the basis of above information, the authors propose the diagram of pit border layered structure in bordered pits between earlywood tracheids in conifer (Fig. 2). Diagrams of pit border have been proposed by many workers,5,6,9,13,14,15,16) however, they are not identical each other.

The result of the authors is similar to that of Wardrop and Davies,24) notwithstanding the studying method was different in each other. But, the initial border thickening which was named by them should be named only "border thickening" in our opinion. The pit aperture final size is nearly determined by the S2 and B. T.

According to the interpretation of Jütte and Spitt,16) and Murmanis and Sachs,17) circularly oriented microfibrils of chamber side are joined to the secondary wall of lumen side. When this layer of chamber side having concentric orientation which was named as the secondary wall by them, is distinguished from wall in lumen side as indicated in this study, their diagram is nearly consistent with that of our study.

According to the conclusion of Harada and Côté,9) and Imamura and Harada,15) the S1 layer is very thick and the B. T. is thinner, however, the evidence of the existence of thick S1 layer was not observed in a Todomatsu tree.
But, the scale-like texture which was shown by them, can be explained also by
our observation as shown in Fig. 1.

MURMANIS and SACHS¹⁵ stated that differences appeared in the pit border
within the same tree species, however, in this study the layered structure of pit
border was identical in all examined tracheids. Although the layered structure of
pit border in a Todomatsu tree is not variable within the same species, it may be
noted that differences in the pit border organization appear between tree species.

The orientation of microtubules on the inner surface of plasmamembrane has
already been reported in the non pitted region of cells forming the secondary wall
using SEM by the authors¹¹,¹². To discuss the relation between microtubules and
microfibrils, from the point of view of the orientation and the arrangement, it
seems available to observe them in the region of pit border, particularly using
SEM, because they are oriented in a particular and complicated fashion. From
this study on the pit border region, as same as the previous study,¹² it was
clearly shown that various orientations of microtubules were consistent with that
of microfibrils in cells forming from S1 to S3 layer as shown in photos 25–30.
According to the interpretation of ROBANDS and HUMPHERSON,²⁰ in the bordered
pit of vessel elements, although microtubules are more or less parallel to the micro­
fibrils being laid down, there is no direct evidence for the involvement of micro­
tubules in the orientation of microfibrils. Although the parallel arrangement of
microtubules and microfibrils was only revealed and the other cellular organelles
were not observed in the previous and current study, it seems reasonable to suppose
that microtubules have an important function for the microfibrillar orientation
during the secondary wall formation because the orientation of microtubules was
regularly changed in a radial file, in cells forming from S1 to S3 in both pitted
and non pitted regions. Additionally, when the orientation of microtubules, thin
and long, is observed by TEM with the ultrathin sections, particularly in the pit
border region which is complicated in organization, it seems to be not discerned
precisely. In any event, in view of some variability in the reported results²⁰ with
regard to the function of microtubules, the relation between microtubules and
microfibrils should be discussed based on the informations from use of SEM and
the replica technique, in future.

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要　約

トドマツ早材仮道管の半径壁に存在する有縁壁孔 (T-T) における壁孔縁 (pit border) の壁層構成を明らかにするために, その形成過程と, それに関連する細胞小器官 (ミクロチュービュール) 及び形成後の壁層を, SEM 及び TEM で観察した。

得られた結果は次のとおりである。

1) 分化中の仮道管内表面において, 壁孔縁の影響を受けない領域 (ここでは, 壁孔口の輪郭から 10 μm 超を超えた部分とし) でのミクロフィプリル配向の観察から判断された 2 次壁形成の各 stage と付近の壁孔口の直径を対照した結果, 壁孔口が成熟仮道管にみられるような最終的な大きさになるのは, S2 の初期段階であることが示された (Photos 1〜6)。ここでの S2 形成段階は, ミクロフィプリルが, Z-helix, 5°〜10° のほぼ一定の傾角で配向し, 典型的な交差構造のラメラを持つない段階をいう 13)。

さらに S2 の初期段階までは, 壁孔縁の壁の厚さは, 壁孔縁の影響を受けない領域のそれより明らかに厚い (Photos 7〜9)。

壁孔縁領域における堆積中のミクロフィプリル及びミクロチュービュールの配向の観察から以下の事が示された。ミクロフィプリルは, 分化中の仮道管の内腔側の壁孔縁領域では, S1〜S3 まで壁孔口を迂回するように流線形を描くように配向し, 他方, 壁孔室側においては円状及びうす巻状に配向していた (Photos 10〜18)。ミクロチュービュールの配向も同様であった (Photos 24〜30)。壁孔室側に存在する円状のミクロフィプリル配向を持つ層は, 一般的に呼ばれている 2 次の壁の各層 (S1〜S3) とは別個の層 (B.T.) として区別されるべきものであると考えられる。またこの層は, 内腔側での S2 の中期形成段階までは, ほぼ形成を終えると思われる。

2) 既に形成された壁孔縁を引裂いて SEM で観察した結果及び超薄切片による壁層構成の観察結果も上記のそれを良く一致した (Photos 19〜23)。即ち, 壁孔縁は壁孔室側の円状配向のミクロフィプリルからなる厚い層と内腔側の流線形を描くような配向のために若干の厚さの変動があると思われる 2 次壁により構成される。

3) 壁孔縁の壁層構成は Fig. 2 で示された。これは, Wardrop と Davies が既に示した結果と類似しているが, 彼らが Initial border thickening とした層は単に Border thickening と呼ぶべきものと考えられる。その理由は, 円状のミクロフィプリル配向をもつ層 (B.T.) と内腔側の 2 次壁とはほぼ同時に形成が続いていくことによる (Photos 10〜18)。Jutte, Spitt と Murmanis, Sachs は, ここで B.T. と呼んだ層を内腔側の 2 次壁の連続と考えている。しかし, 著者らの観察によれば, この連続は認められず, ミクロフィプリル配向の違い及びその形成時期の違いからみて, これは別個の独立の層として区別すべきものであると思われる。これに従って彼らの壁孔室側の層を, B.T. とみなせば本研究の図を良く一致する。Harada, Côte
Explanation of photographs

Note: The cell axis is vertical in all SEM photographs. The SEM photographs were taken at an angle of 45° of specimen tilt with the exception of photos 7, 8 and 9 which were taken at 0° of specimen tilt, thus the true length of specimens is shown only horizontally. In photos 7, 8 and 9, the true length of specimens is shown in all directions. The axial direction of tracheids in photographs of the replica is shown by the arrow.

Key words: B.T, the border thickening. S12, the transition layer from S1 to S2 layer.

Plate 1: Increasing in width of pit border in each stages of secondary wall formation.

Photo 1. The pit aperture in the cell forming the S1 layer. The diameter of it is about 14 μm. (c.f. photos 3 and 5)

Photo 2. The microfibrillar orientation of the S1 layer in non pitted region of the cell as shown in photo 1.

Photo 3. The pit aperture in the cell forming the S12 layer. It is about 10 μm in diameter.

Photo 4. The microfibrillar orientation of the cell as shown in photo 3. Microfibrils are oriented at an angle of about 45° in non pitted region.

Photo 5. The pit aperture in the cell forming the S2 layer. It is 5 μm in diameter.

Photo 6. The microfibrillar orientation of the cell as shown in photo 5. Its direction is nearly parallel to the cell axis.

Photos 7-9; Increasing in thickness of pit border in differentiating tracheids. These specimens embedded with methacrylates were cut with a glass knife and then methacrylates were removed.

Photo 7. The transversely cut surface of pit border in the cell forming the S1 layer. The thickness of pit border is as same as of the double wall of non pitted region.
Photo 8. The transversely cut surface of pit border of the cell in the first stage of S2 layer formation. The pit border is a little thicker as compared with that of photo 7, but the width is increasing extremely.

Photo 9. The transversely cut surface of pit border of the cell in the last stage of S2 layer formation. The pit border is considerably thickened. The boundary line between the lumen and the chamber side can be visible. (arrow)

Photos 10-18: The orientation of depositing microfibrils in the region of pit border of differentiating tracheids.

Photo 10. The microfibrillar orientation of initial stage of pit border formation. Concentrically oriented microfibrils (under arrow) and ones in a streamline fashion (upper arrow) are depositing at the same time. They are not joined to each other.

Photo 11. The lumen surface in the region of the pit border of the cell forming the S1 layer. Microfibrils are oriented mostly in a streamline fashion and concentrically in the border tip.

Photo 12. The circular orientation of microfibrils in the chamber side of the cell forming the S1 layer.

Photo 13. SEM micrograph showing the lumen surface of the cell forming the S1 layer. Concentrically oriented microfibrils (white arrow) in the chamber side and on the pit border tip are not joined to ones of S1 layer.

Photo 14. The lumen surface of the cell forming the S2 layer. Microfibrils are oriented in a streamline fashion. The circular orientation is also visible in the region of pit border tip.

Photo 15. The disturbed microfibrils in the region of pit border tip. The underlying layer having the circular orientation of microfibrils is the border thickening (arrow). The lamella of the S2 layer forms the part of pit aperture outline.

Photo 16. The microfibrillar orientation of chamber side in the cell forming the S2 layer. The circular orientation in the region of the base of pit border (left hand) is visible.

Photo 17. The orientation of microfibrils in a scrollwork-like fashion of chamber side of the cell forming the S2 layer. Microfibrils are running from the base to the pit border tip.

Photo 18. The microfibrillar orientation of lumen side in the cell forming the S3 layer. In the region of border tip (arrow), microfibrils are oriented concentrically.

Plate 4: The layered structure of the pit border.

Photo 19. The split layer as a ring of chamber side of the cell forming the S2 layer.
The layered structure of pit border from the chamber side. Below the split layer (B.T.), the S1 and the S2 layer of the lumen side are visible. The latter forms the pit aperture outline.

The thick layer of border thickening. The pit aperture outline is formed by the S2 layer of lumen side.

The circular striation of border thickening in the lumen side of the cell forming the S12 layer. The S1 and the S12 layer are, for the most part, torn off.

The circular striation of border thickening (arrow) in the lumen side of the cell forming the S3 layer. In the left hand, the secondary wall layers are separated from the pit border region.

The transverse section of the cell forming the S2 layer by TEM. The border thickening, the S1 and the depositing S2 layer are clearly shown.

The orientation of microtubules on the plasmamembrane in the region of pit border.

The orientation of microtubules in the lumen side of the cell forming the S1 layer. They are running along the pit aperture outline.

The orientation of microtubules in a streamline fashion of lumen side of the forming the S2 layer (c.f. photo 14).

The circular orientation of microtubules in the region of border tip (arrow). The streamline fashion is also found in the other region.

The orientation of microtubules in the lumen side of the cell forming the S3 layer. They are oriented in a streamline fashion near the pit aperture, on the other hand, straight in the upper and under side of this photograph.

The circular orientation of microtubules in the chamber side of the cell forming the S2 layer. They are often crisscrossed. The tubular membrane is visible (arrow).

The circular orientation of microtubules in the chamber side of the cell forming the S2 layer. The scrollwork-like fashion is visible near the border tip.