STRUCTURE OF PIT BORDER IN *PINUS STROBUS* L.

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ABSTRACT

Sections from white pine trees were studied by electron microscopy in a search for the organization of cell wall layers in the pit border. Depending on the developmental stage of the tracheids, or perhaps on technical imperfections, differences appeared in the pit border within the same tree species. From an electron micrograph of a mature latewood tracheid, a diagram was reconstructed that appears to be the most representative structure for the pit border in white pine.

INTRODUCTION

Bordered pits of coniferous tracheids, as a main route for solutes, have attracted considerable attention from researchers. Many publications on their structure have appeared in the literature (Frey-Wyssling, Bosshard, and Mühlethaler 1956; Wardrop and Davies 1961; Jutte and Spit 1963; Liese 1965; Fengel 1966; and Harada and Côté 1967). However, hardly any two descriptions of the structure on the borders in these pits are completely identical. As we will suggest later, the reasons for that may be manifold.

Studying the developmental sequence of the tracheids in *Pinus strobus* L. (white pine), we have seen electron micrographs of the pit borders which, at different developmental stages, would fit one or another of the pit border descriptions already published. Nevertheless, when we scanned a large number of micrographs from mature earlywood and mature latewood tracheid pits, we saw in the pit borders in a majority of cases a slightly different cell wall organization from those so far described.

The purpose of the present work is to show that a variable cell wall organization exists within the pit border of white pine tracheids.

MATERIAL AND METHOD

The present observations were based on thin sections (mainly the transverse) from bordered pits of different white pine trees, collected at various times throughout the whole growth season. Small samples were taken from the tree and fixed immediately in the fixatives used in electron microscopy: $KMnO_4$, OsO_4, glutaraldehyde-OsO_4, and formaldehyde-glutaraldehyde-OsO_4 (Karnovsky 1965). After each type of fixation, the tissue was dehydrated in graded series of acetone and embedded in araldite-epon-DDSA-mixture (according to Mollenhauer 1964). The material was sectioned with a diamond knife on a Porter-Blum ultramicrotome. Grids were examined in an RCA-EMU 3D microscope using 50KV.

It is assumed that after such preparation of material, the artificially induced changes (those caused by drying, for example) should be at the minimum. In the sectioning, it also became apparent that it is difficult to obtain a section which would pass through all the constituent cell wall layers in the border at exactly the same level. Besides, the different wall layers probably exert a different resistance to knife passage. Consequently, wall layers were sometimes missing, at other times present. We found that pits of mature latewood tracheids section easier because of their smaller size and their reduced borders, and, consequently, provide fewer artifacts from that aspect.

RESULTS AND DISCUSSION

Four of the five tracheids shown in Fig. 1 have reached a mature state; the other, in which the plane passes through the tapered end, is still in the process of differentiation,

[1] Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

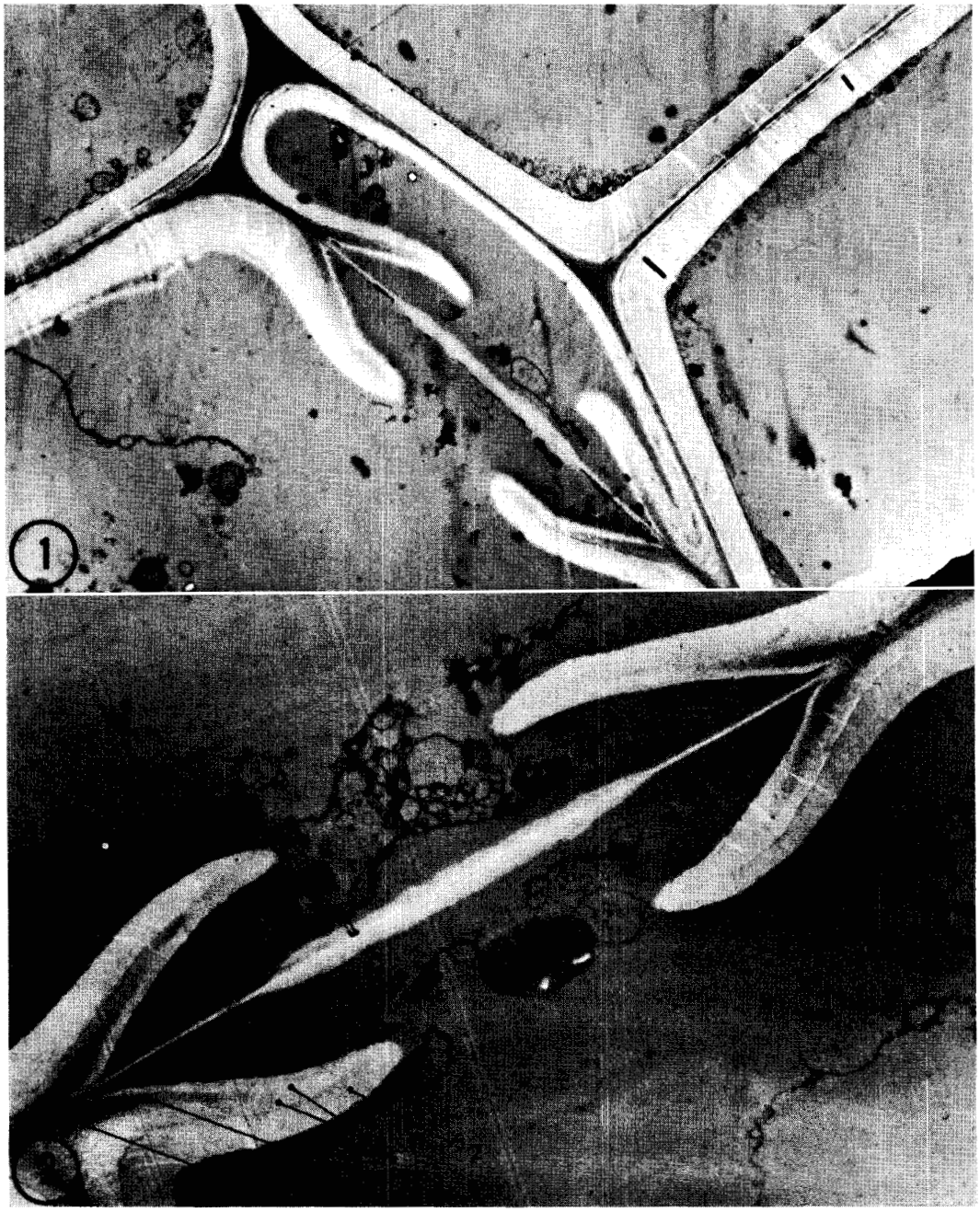


FIG. 1. Cross section of five earlywood tracheids. Bordered pit-pair is located between the tapered end of one tracheid and the more central part of the other tracheid. Collected July 13, 1967; Karnovsky, 3,240×.

FIG. 2. Cross section of bordered pit-pair between two almost mature tracheids. Compound middle lamella (CML); initial pit border (IPB); S_1 layer (S_1); S_2 layer (S_2). Collected July 13, 1967; Karnovsky, 8,200×.

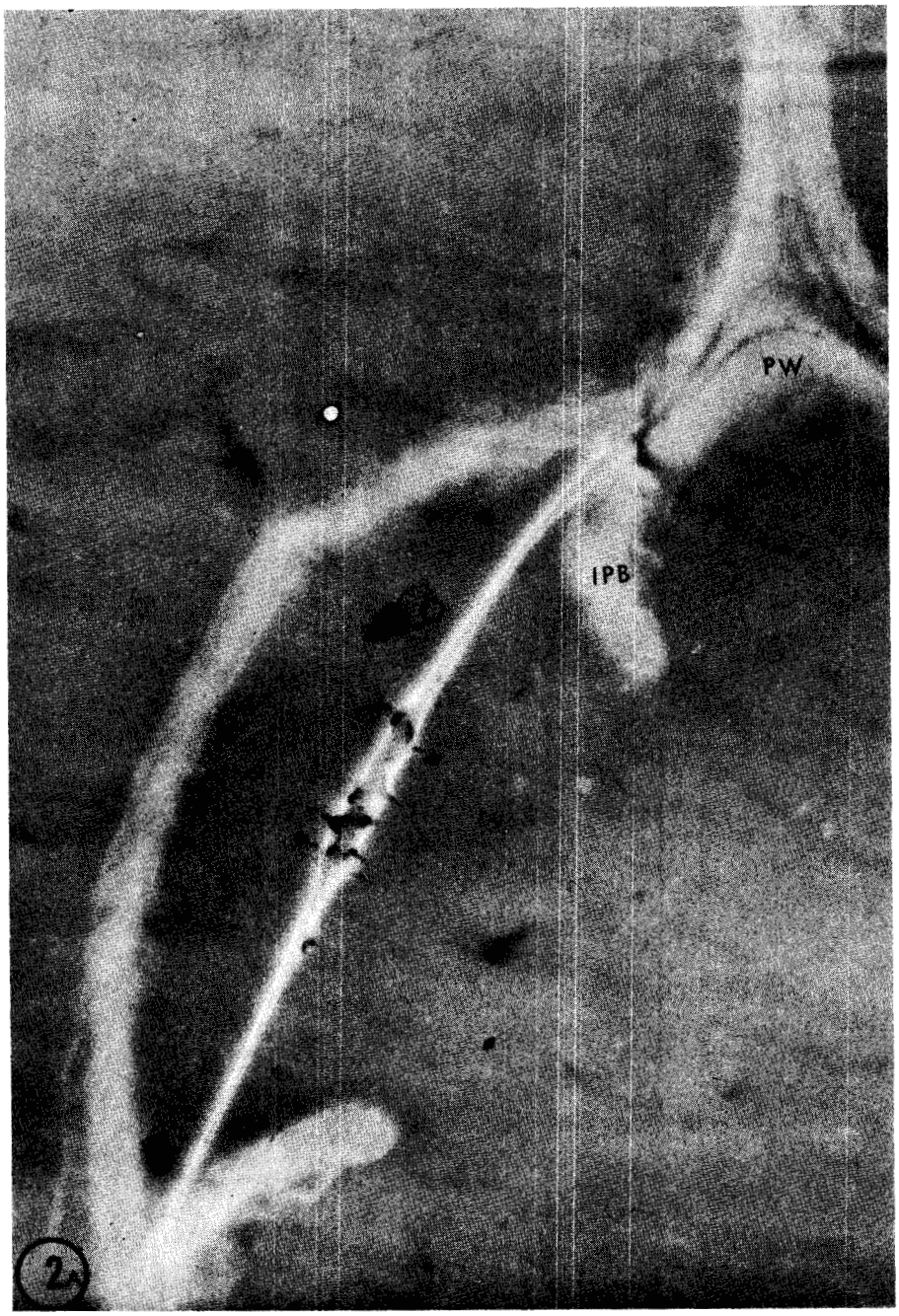


FIG. 2A. Cross section of a young, radially expanding tracheid at a time when bordered pits first appear on the radial walls. Initial pit border (IPB); primary wall (PW). Collected July 13, 1967; Karnovsky, 18,000×.

FIG. 3. Bordered pit-pair in differentiating earlywood tracheids (tangential section) showing the contrasting initial pit border (IPB); the pit membrane (PM) with no torus yet; the S_1 layer (S_1); and some S_2 layer (S_2) on its lower side. Collected July 23, 1964; $KMnO_4$, 17,000×.

FIG. 4. Differentiating tracheids with differentiation progressing from right to left. The last cell at right is a cambial cell (CC). Tangential section. Collected July 23, 1964; $KMnO_4$, 3,060×.

as judged by its thinner secondary wall. This illustrates the progression of the surface growth of cells in the direction toward the cell tip. The tip border of this developmentally less advanced tracheid shows a central contrasting zone which, in our opinion, corresponds to the initial pit border. It is also obvious that the initial pit border is surrounded by a layer of a lower electron opacity. This layer corresponds to the S_1 and a portion of the S_2 layer; in this tracheid at this developmental

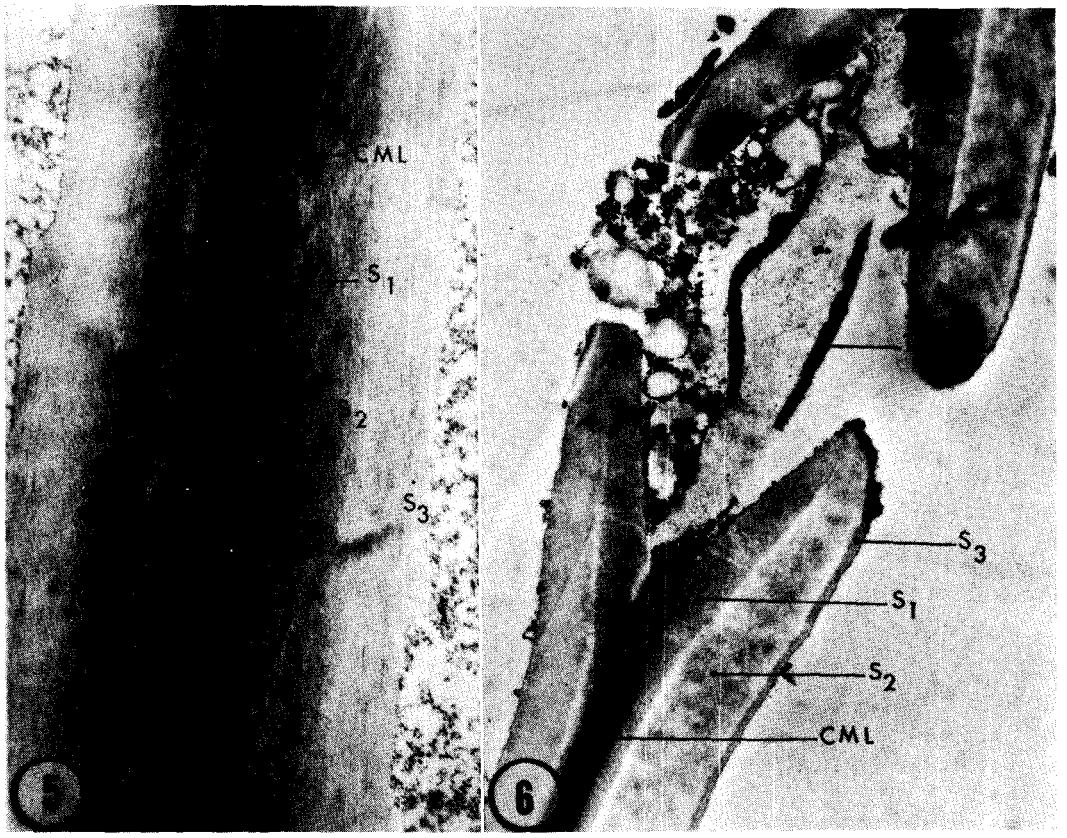


FIG. 5. Tangential section of a mature earlywood tracheid cell wall. Compound middle lamella (CML); S_1 layer (S_1); S_2 layer (S_2); S_3 layer (S_3). Collected July 23, 1964, $KMnO_4$, 15,300×.

FIG. 6. Cross section of a pit-pair between the latewood tracheids. Compound middle lamella (CML); S_1 layer (S_1); S_2 layer (S_2); S_3 layer (S_3); torus (T). Collected May 29, 1964; Glutaraldehyde, 11,020×.

stage, the S_2 layer has only about one-third of the thickness of the adjacent tracheid.

Fig. 2 shows a higher magnification of a bordered pit at a later developmental stage. Here the S_2 deposition is close to its completion. This micrograph also shows the initial pit border as a separate zone, surrounded now by the S_1 and S_2 layers. The S_2 layer is wide on the lumen side of the border but quite thin on the pit chamber side.

Jutte and Spit (1963) observed a contrasting zone in the pit border of three coniferous species (*Araucaria*, *Aghatis*, and *Picea*) studied. According to their interpretation, the dark zone corresponds to the S_1 layer; however, we think this to be the primary wall, classified so by its distinctness from the S_1 layer, rather than the time of its deposition (Fengel 1966). Its higher electron opacity probably results from the lignin incrustation, which has already started in the compound middle lamella but not yet begun in the secondary wall layers (Wardrop 1965).

When the initial pit border first becomes visible, both the primary wall and the initial pit border have a very low and a comparable electron opacity (Fig. 2A). From this we conclude that the initial pit border develops before the secondary wall deposition has started, although after deposition of the primary wall. Consequently, the initial pit border appears as a

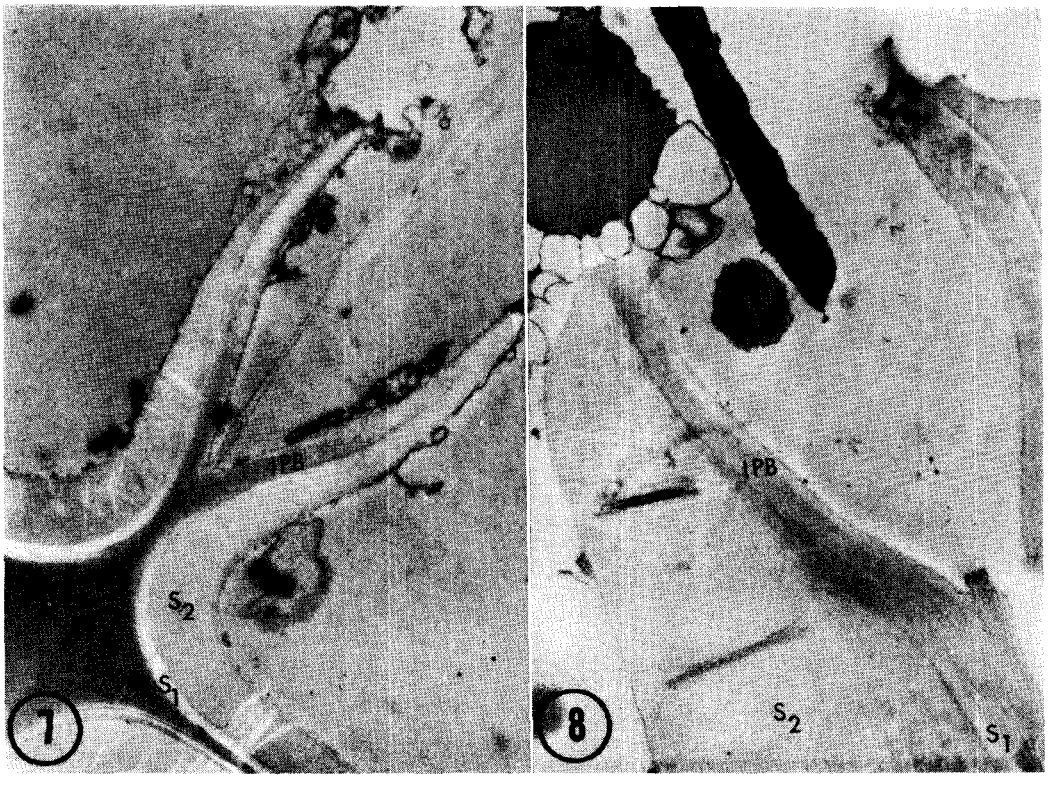


FIG. 7. Cross section of bordered pit-pair from differentiating earlywood tracheids. Initial pit border (IPB); S_1 layer (S_1); S_2 layer (S_2). Collected July 13, 1967; Karnovsky, 6,120×.

FIG. 8. Cross section of bordered pit-pair from mature latewood tracheids. Initial pit border (IPB); S_1 layer (S_1); S_2 layer (S_2). Collected January 23, 1964; OsO_4, 14,000×.

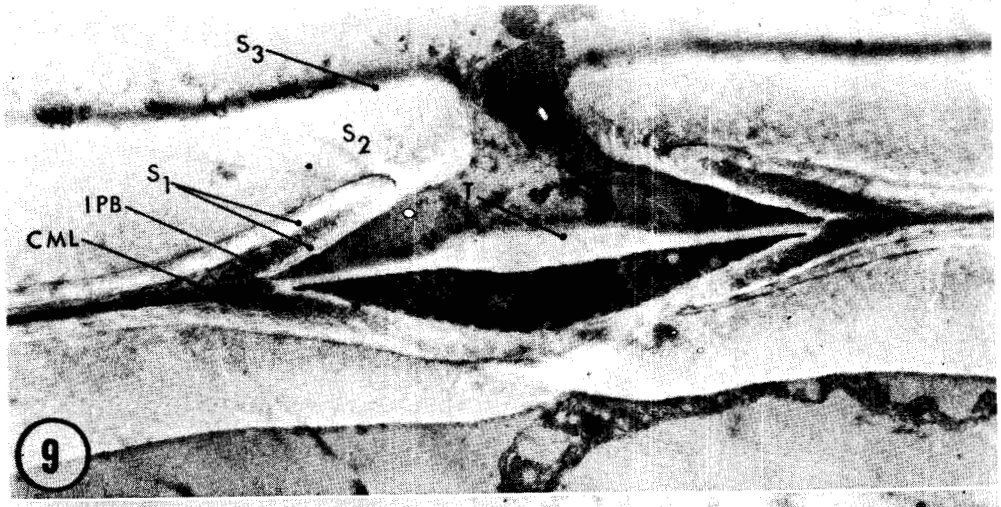
separate layer, as also noticed by Wardrop and Dadswell (1957).

According to Fengel (1966), the initial pit border (Hofanlage) up to a certain developmental stage can be seen as an "individual" layer. Later, when the S_1 layer deposition begins, the S_1 overlays the initial pit border and the two cannot be separated anymore. Harada and Côté (1967) also do not consider the initial pit border as a layer distinct from the S_1 layer. On the other hand, Wardrop and Dadswell (1957) state that the initial pit border and the S_1 are two distinct layers. Frey-Wyssling et al. (1956) and Jutte and Spit (1963) consider the initial pit border to be a part of the S_1 layer.

The contrasting zone in the pit border is especially evident after the permanganate fixation (Fig. 3). The lighter wall layer around the contrasting zone is mainly the S_1 layer, with a little S_2 showing on the lower side.

Fig. 4 is included to show that the secondary wall formation in differentiating tracheids occurs progressively; in this case from the right to the left. Fig. 5 is a higher magnification micrograph of the cell wall. It is included to confirm that the dark central layer corresponds to the compound middle lamella; it also shows all the cell wall layers characteristic of a mature tracheid.

Some of our electron micrographs of bordered pits also exhibited the structural organization described by Harada and Côté (1967). For example, in our Fig. 6, as in work of Harada and Côté, the initial pit



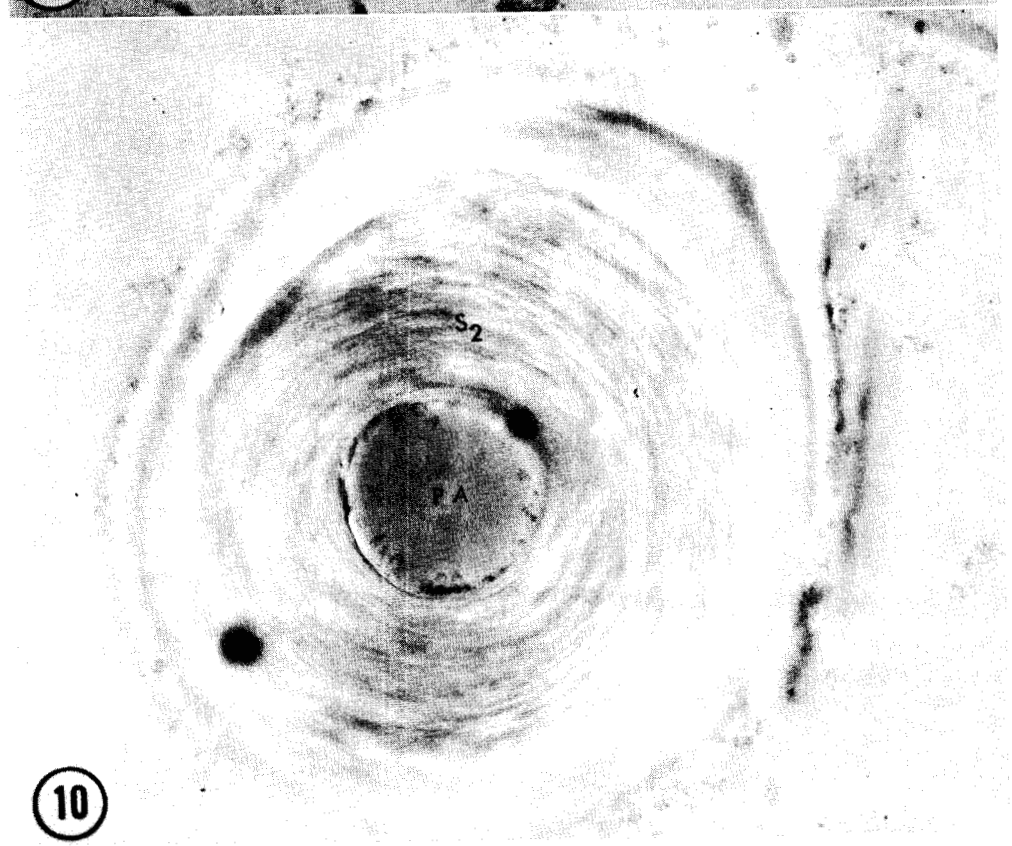


FIG. 9. Pit-pair of mature latewood tracheids. Compound middle lamella (CML); S_1 layer (S_1); S_2 layer (S_2); S_3 layer (S_3); initial pit border (IPB); torus (T). Collected September 6, 1967; Karnovsky, 6,800×.

FIG. 10. Radial section of bordered pit from a latewood tracheid. Pit aperture (PA); S_2 layer (S_2). Collected March 20, 1964; OsO_4, 14,450×.

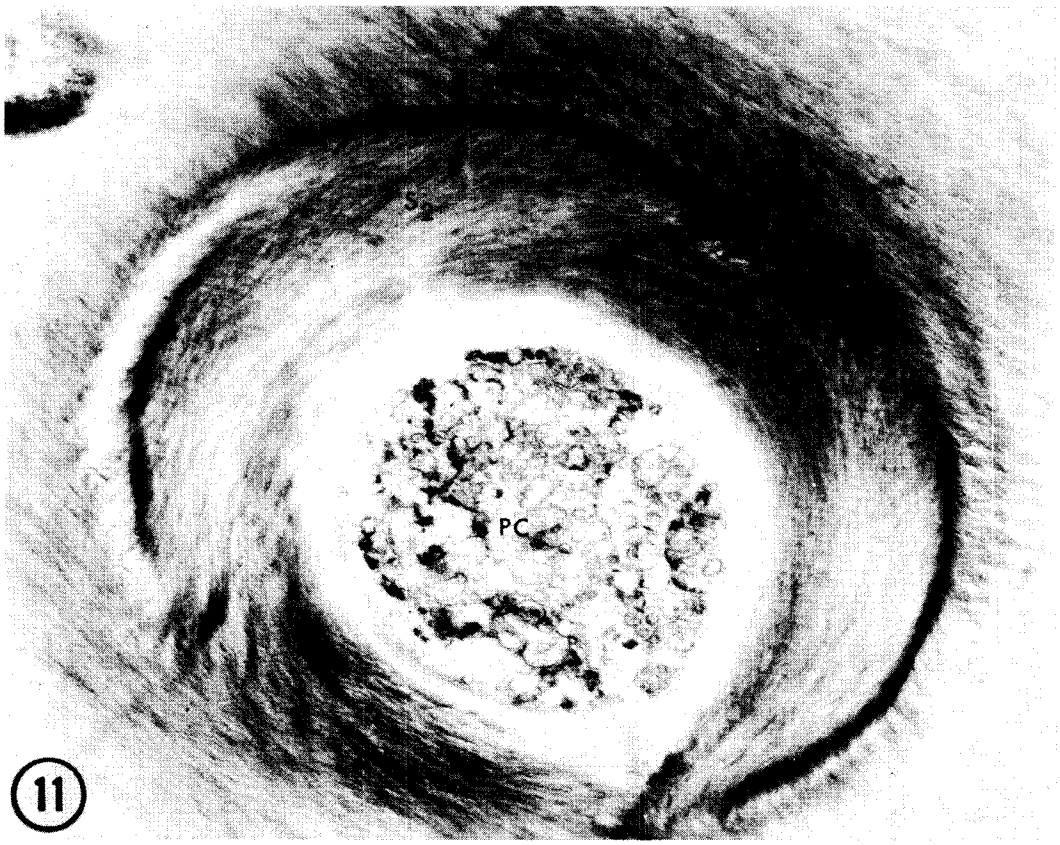


FIG. 11. Radial section of bordered pit from an earlywood tracheid. Pit chamber (PC); S_2 layer (S_2). Collected July 23, 1964; $KMnO_4$, 10,200×.

border and the S_1 layer are confluent; the S_2 layer appears to end at the tip of the border; the S_3 layer covers the border on the lumen side and terminates at the border tip. However, it is apparent that in our Fig. 6 the plane of section is not median, and this is responsible for the structural organization in the pit border seen in this picture. However, we must add that the tracheid in our Fig. 6 is a latewood tracheid; whereas in Fig. 7 of Harada and Côté, it is a "representative of micrographs made from several species," and is an earlywood tracheid. Fig. 7 shows a differentiating tracheid whose bordered pit is almost identical to a figure representing the bordered pit of a European spruce in Jutte and Spit's article. In our Fig. 7, the initial pit border cannot be distinguished from the S_1 layer. We also found pictures of white pine bordered pits (Fig. 8) which correspond to the diagram for coniferous bordered pits given by Wardrop and Davies (1961). Our picture does not show the S_3 layer, but we often find it lacking in thin sections.

Fig. 9 shows bordered pits of mature latewood tracheids. They reveal the presence of the same cell wall layers as do earlywood pit borders, but the layers are more distinct in the latewood tracheid. For this reason, the latewood tracheids will be used for the generalized description of the pit border, as we visualize it in the perspectives of our present data. As seen from Fig. 9, the center of the border contains the initial pit border, recognizable as a zone distinct from the S_1 layer. The initial pit

border is enveloped by the S_1 layer, which thus is found on the inner and outer sides of the initial pit border. The S_1 is overlaid by the S_2, which is thick on the lumen side but thin on the pit chamber side. In the S_2 layer the microfibrils curve around the tip of the border; a few microfibrils extend beyond the tip of the border and overlay the S_1 layer on the pit chamber side. The S_2 on the lumen side is covered by S_3 which, as far as we could judge from our micrographs, ends at the tip of the border.

A warty layer was not seen in any of the tracheids examined, but there are always some protoplasmic constituents present, usually in the pit chamber. Neither did we find in white pine the veil in the pit aperture found by Jutte and Spit (1963) in the coniferous species they examined. If Jutte and Spit consider this veil to be a remnant of the plasmalemma, it would mean that the protoplast has been retracted from the pit chamber to the pit aperture. Normally, the protoplasmic components fill the pit chamber and the plasmalemma outlines the pit chamber.

Radial sections, although so useful for replicas, do not reveal much of the different wall layer organization in the border region and are considerably more difficult to interpret. Figs. 10 and 11 are radial sections of bordered pits at different levels through the pit chamber. Fig. 10 shows the aperture of the pit chamber, surrounded by the S_2 layer; in Fig. 11, the plane of section passes through the pit chamber at a deeper level—between the pit membrane and pit aperture. Both Figs. 10 and 11 show that in the immediate vicinity of the pit aperture, the microfibrils run in a streamline fashion. The microfibrils at the outer zone of S_2 unite with the S_2 layer of the rest of cell wall. The pit chamber is filled with cellular contents.

REPRESENTATION

To summarize the results, the diagram of the white pine pit border (Fig. 12), reconstructed from Fig. 9, is compared with diagrams given by Harada and Côté (1967), Jutte and Spit (1963), and Wardrop and Davies (1961). When we compare the four schemes, we see that they all differ in one way or another. It is also interesting to remember that Jutte and Spit (1963) stated that their scheme for coniferous bordered pit does not agree either with that given by Trendelenburg (1939) or those given by Bucher (1957) and Wardrop and Davies (1961). Similarly, Harada and Côté (1967) stated that their concept for bordered coniferous pits differs from those of Jutte and Spit (1963) and Wardrop and Davies (1961).

According to Wardrop and Davies and Harada and Côté, the secondary wall deposition stops at the tip of the border, the border on the pit chamber side being covered only by the initial pit border. From our observation we assume that the S_1 and the S_2 layers of the secondary wall continue to be deposited around the tip of the border, and thus the pit chamber is laid out by S_1 and S_2 layers. Our scheme, probably, comes closest to that of Jutte and Spit; the main exception is that in their pictures, the S_1 layer and the initial pit border appear as one inseparable layer, and the S_3 layer is a continuous layer covering the border along the pit chamber side and along the lumen side. In Fengel's (1966) model for a coniferous bordered pit, the S_2 layer ends at the tip of the border and the S_3 covers the border on the lumen side and on the pit chamber side. Our electron micrographs show that the S_3 layer ends at the tip of the border. However, we would think that by logical sequence of the cell wall formation, the S_3 layer would follow the other secondary wall layers (S_1 and S_2) and cover the pit border on the pit chamber side. Jutte's and Spit's and Fengel's electron micrographs show that such an assumption is not unreal. Of course, our study is not based on the analysis of the microfibrillar orientation in each of these layers. On the other hand, it is not certain how precisely this can be done by the present methods available. In short, we, like other workers, found structural variations in the coniferous pit border. It appears that in some cases in white pine, these variations resulted from different developmental stages of the tracheids used. Some-

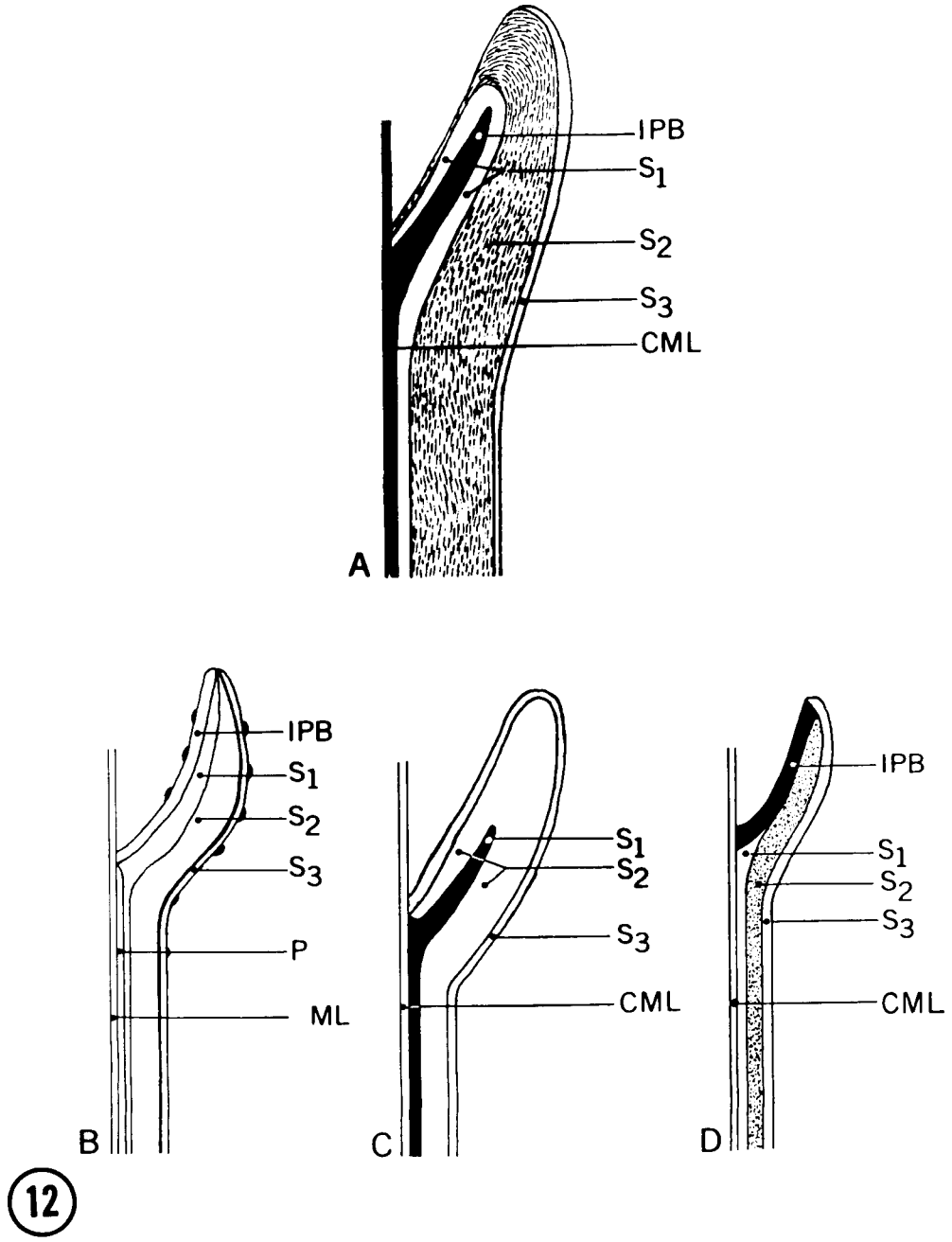


FIG. 12. Comparison between diagram representing our concept (A) and those of other researchers (B, C, D). A, Diagram for a bordered pit of white pine, reconstructed from the latewood tracheid of Figure 9; B, Harada and Côté; C, Jutte and Spit; D, Wardrop and Davies. Compound middle lamella (CML); initial pit border (IPB); middle lamella (ML); primary wall (PW); S_1 layer (S_1); S_2 layer (S_2); S_3 layer (S_3).

times, no doubt, they resulted from technical imperfections, that is, the plane of section not passing through all cell wall layers at the same level, or, when some of the layers were lacking because of the compression in sectioning. Besides, it was stated by Bailey and Vestal (1937) that the arrangement of cellulose (microfibrils) in the outer and central layers of the secondary wall "varies more or less from specimen to specimen, from tracheid to tracheid, and in different parts of the same cell." This probably would explain why sometimes the initial pit border and the S_1 layer of the secondary wall are seen as separate layers, sometimes not. Deviations from the prevailing orientation of microfibrils in any tracheid are especially evident in the pitted parts of the wall as had been noted by Bailey and Vestal (1937) and other workers. Thus it seems logical that, for all these reasons, variations could exist in the organization of cell wall layers in a pit border region of tracheids in conifers.

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