SCALARIFORM PERFORATION-PLATE FINE STRUCTURE¹

R. W. Meyer

Department of the Environment, Canadian Forestry Service, Western Forest Products Laboratory, Vancouver, British Columbia

and A. F. Muhammad

Biology Department, McGill University, Montreal, Quebec

(Received 22 September 1971)

ABSTRACT

Microfibrillar webs were frequently observed to connect adjacent bars of scalariform perforation plates of secondary xylem from a variety of tree species. When present, these webs occur most often in the lateral extremities of scalariform openings, where the microfibrils are in somewhat random alignment and hence are named reticulate microfibrillar webs. The number of strands increases and they appear more interwoven in openings near ends of plates. As the number of strands increases, reticulate webs in end scalariform openings grade into typical intervessel pit membranes composed of randomly oriented microfibrils. In certain species, microfibrils may also occur at fairly regular intervals across scalariform openings and exhibit a preferred orientation perpendicular to the bars. These were named orthogonal microfibrillar webs.

INTRODUCTION

Wood anatomists (Panshin and de Zeeuw 1970), plant anatomists (Esau 1965), and most other published evidence indicate that openings in scalariform perforation plates are unobstructed by any substructure. However, evidence that there might be substructures within scalariform openings of primitive plants was first obtained as early as 1833 (Witham) from studies of fossilized remains of lycopods found in coal. References to studies of lycopods as found in coal balls have been summarized by Fry (1954) and Barghoorn and Scott (1958).

Electron micrographs of lycopod perforation-plate structure were published by Wesley and Kuyper (1951) and Fry (1954). Although these fossils are vastly different from modern angiosperms, Barghoorn and Scott (1958) obtained evidence that such structures also existed in some fossil *Platanus* and living *Euptelia polyandra* Seib. et Zucc. Ishida (1970) and Ohtani (1970), utilizing scanning electron microscopes, observed similar structures in the scalariform perforation plates of katsura (*Cercidiphyllum japonicum* Sieb. et Zucc.), which is a woody tree of 20–40-m height native to western China and grown in North America as an ornamental.

This study deals with scalariform perforation-plate structure in a variety of modern angiosperms, and suggests that some modification of current concepts of perforationplate structure is necessary. A short review of perforation-plate formation is germane to the present discussion. Esau (1965) refers to earlier research by herself on perforationplate development, in which it was observed that the site of a future opening becomes thicker than neighboring portions of primary cell wall. After completion of cell-wall growth and during lysis of the cytoplast, a perforation is formed by dissolution of thickened portions of a cell wall, which are then eroded away by the transpiration stream (Yata, Itoh, and Kishima 1970). As pointed out by O'Brien (1970), it is not yet known whether cell walls are enzymatically removed or whether they are mechanically removed by action of the transpiration stream. Secondary thickening of scalariform bars with overhanging borders has been shown by Yata et al. (1970) and Panshin and de Zeeuw (1970).

¹ Paper presented at the 25th annual meeting of the Forest Products Research Society. Technical assistance of A. E. Bramhall is gratefully acknowledged.

SPECIMEN PREPARATION

Woody tissues were taken from two primary sources: 1–3 year-old twigs from living trees, and wood specimens from both the Western Forest Products Laboratory and the personal collection of the senior author. These species, their families, and common names were:

Alnus rubra	Betulaceae	red alder
Bong. Betula alle-	Betulaceae	yellow birch
Britton		
japonicum	ceae	katsura
Cornus nuttallii Audubon	Cornaceae	Pacific dogwood
Ilex opaca Ait.	Aquilifoliaceae	American holly
Liquidambar styraciflua L.	Hamamelidaceae	red gum
Nyssa sylvatica Marsh.	Nyssaceae	black gum

Green twigs were dried in air at room temperature from 2-methyl butane after dehydration in acetone. This drying technique lessens tendencies for drying artifact formation associated with drying from liquids with large surface tension. The drying history of the herbarium material is uncertain, but it was probably air dried from the green state. Direct carbon replicas (Côté, Koran, and Day 1964) were prepared and specimens were examined in an Hitachi HU-11E electron microscope. Other brief observations were made with a scanning electron microscope.

RESULTS AND DISCUSSION

The microfibrillar webs shown in Fig. 1 are typical of those most commonly observed, which are found in the lateral extremities of perforation-plate openings. These webs are more prevalent in openings towards the ends of perforation plates and in the last openings of the plates often appear to resemble intervessel pit membranes (Fig. 1). Those webs in lateral portions of perforations appear to grade into lateral intervessel pit membranes (Fig. 3). This type of arrangement was found in each species examined. The microfibrils are arranged in a reticulate fashion, hence the names reticulate microfibrillar web or reticulate web to describe them.

Another distinct arrangement of microfibrillar strands is typically found in katsura (Fig. 4) and red gum. These strands occur at more or less regular intervals across the scalariform openings and are oriented perpendicular to the bars forming rectangular or orthogonal openings. These structures have, therefore, been named orthogonal microfibrillar webs or, more simply, orthogonal webs. In some cases the microfibrils are very numerous, as shown in Fig. 5. Often associated with the perpendicular strands are a smaller number of more randomly oriented microfibrils, some of which are oriented nearly parallel to the bars (Fig. 5). Reticulate webs are also found in species with orthogonal webs; however, the orthogonal webs are distinguished by their regular perpendicular strands and rectangular openings in contrast to the randomshaped openings in reticulate webs.

The term "microfibril web," as used here, refers to microfibrils located within scalariform perforation-plate openings. The term "fimbril" coined by Barghoorn and Scott (1958) is not used because it is confusing for the accepted "microfibril." A microfibrillar web is a fitting description of those microfibrils forming a web within a scalariform opening.

The inside edge of reticulate microfibrillar webs often takes the form of the outside edge of a scalariform opening (Fig. 6). The close resemblance between the two edges suggests that web microfibrils are formed during enlargement of vessel elements at a time when boundaries of future

FIG. 1. Gradation from web to intervessel pit membrane in yellow birch. Fig. 2. Typical reticulate microfibrillar web in scalariform perforation plate of katsura. Fig. 3. Gradation from web to lateral intervessel pit membranes in red alder. Fig. 4. Orthogonal microfibrillar web in katsura. Note the parallel microfibrils perpendicular to the scalariform bars. Some microfibrils, or their replicas, were broken prior to observation.





scalariform openings are being formed. If a vessel element enlarges further, or if an actual opening becomes slightly larger than this original template, then first-formed microfibrils would lie within openings. While this argument is entirely plausible in the case of reticulate arrangements, it does not account for the orientation of orthogonal microfibrils. These microfibrils are predominantly parallel to each other and, therefore, perhaps should be considered as a specialized part of primary walls. Their possible function cannot be explained.

The larger strands are aggregates of smaller microfibrils, as is evident in Figs. 4 and 5. In this respect, these structures are analogous to radially aligned margo microfibrils of *Pinus*-type bordered-pit membranes. The openings between strands vary in size, depending on the number of strands present in a given area. In Fig. 5, for example, the web superficially resembles a margo. A microfibrillar web is multilayered, as can be seen from Fig. 7. Total thickness of web is equivalent to that of a compound middle lamella, as is a coniferous margo.

Radially oriented margo microfibrils are deposited at an early stage in cell-wall development, probably late during primary wall growth or at its completion (Frey-Wyssling, Bosshard, and Muhlethaler 1956). Since the spatial organization of a mature scalariform microfibrillar web so closely resembles a coniferous margo, it is not unreasonable to assume that these microfibrils likewise may be deposited during the last stages of primary wall growth. Their location relative to overhanging secondary walls (see Figs. 2, 4, 7) indicates that they are formed prior to secondary wall deposition and should, therefore, be considered as primary cell-wall growth phenomena. In this context, it is of course extremely difficult to distinguish between late primary and early secondary wall formation at edges of openings in cell walls, especially when the structures in question pass from walls into openings.

Apparently anomalous formation of scalariform perforation-plate bars was observed in several samples. One particularly interesting example is shown in Fig. 6, where the secondary wall microfibrils sweep around the short, stubby bars, suggesting that bar stubs were formed during primary wall formation, but were not included in the secondary wall organization of the complete perforation plate.

In a few random instances, a membrane was found in the plane of web microfibrils (Fig. 8). It is felt that membranes are composed of original primary cell-wall matrix material and surround web microfibrils. These membranes do not appear to be extraneous material because they form a coherent layer, and are not granular as were those deposits tentatively defined as extraneous material. Neither the chemical nature of membranes nor their function could be determined. The membranes are very sensitive to sintering during replica prepa-Frey-Wyssling, Muhlethaler and ration. Moor (1956) have presumed that infrared radiation occurring during evaporation of replicating materials may cause labile portions of a substrate to "sinter." In our laboratory, we have observed that materials sensitive to sintering are generally low in cellulosic components and high in hemicellulosic or pectinaceous material. The addition of lignin to a matrix increases its resistance to sintering, but amorphous substances are still much more sensitive than is cellulose. The membranes are probably remnants of primary cell walls that were originally present in vessel end walls. The theory for end wall degradation and removal proposed by Yata et al. (1970) suggests that after enzymatic degradation cell walls may be eroded away by the transpiration stream passing

FIG. 5. Orthogonal web with numerous microfibrils in katsura. Fig. 6. Comparison between edges of web and scalariform perforation opening in yellow birch. Note microfibrils sweeping around intervessel pit to include pit, bar stub, and web microfibrils. Fig. 7. Multilayered appearance of reticulate web in red alder. Fig. 8. Primary wall remnants present as a membrane between web microfibrils in black gum. The membrane was sintered during replica preparation.

through perforations. Incomplete erosion could account for the presence of these residual membranes.

Microfibril webs are essentially indiscernible on ultrathin sections because there are not enough microfibrils per unit volume to generate coherent images in an electron microscope. Such is often the case when examining a *Pinus*-type pit membrane. where the torus appears to be unsupported by a margo. All published observations of these webs have been made using carbon replicas in transmission electron microscopes or with direct observation of wood sections with light and scanning electron microscopes. These observations have demonstrated the rather coarse microfibrils common to katsura (Ishida 1970; Ohtani 1970) or Platanus and Euptelia (Barghoorn and Scott 1958), but they were limited by the resolution of microscopes used. The finer structures shown here apparently have not been demonstrated previously. Although they are of a size allowing observation with a scanning electron microscope, their clarity would be much less than has been obtained here with a transmission electron microscope.

Close examination of specimens revealed that many web microfibrils had become "aspirated," or were laterally displaced from their normal position in the plane of the compound middle lamella. These aspirated strands were more difficult to observe bccause of lack of contrast between strands and overhanging secondary wall, just as aspirated margos are lacking in contrast in coniferous woods. Where strands stretch over perforation openings, they are easily visible but are also easily broken (Fig. 4). These reasons probably account for the nearly complete lack of published observations of microfibrillar webs within openings of scalariform perforation plates, even though they appear to be of quite common occurrence.

Many specimens examined in this study were twigs containing only about three annual increments. An estimate of the frequency of occurrence of web structures in mature wood, therefore, could not be made. When the structures were found, they were present on 5 to 50% of the perforation plates examined. The short length of vessel elements results in many end walls and hence many possible obstructions in only a few centimeters of wood.

The presence of microfibrillar webs on scalariform perforation plates could retard movement of fluids through vessels and so influence wood permeability. Such an effect would be dependent on the frequency of these structures throughout a piece of wood, and would decrease permeability by collecting bubbles of air from the permeating fluid, as described for conifers by Kelso, Gertjejansen, and Hossfeld (1963). Further obstructions to fluid flow could occur if membrane remains are found to be more commonly present.

CONCLUSIONS

The microfibrillar webs described here were found in tree species from six different plant families. Their relatively common occurrence in this rather small sample suggests that they should be examined further for their existence in other species with scalariform perforation plates. The structural similarity between scalariform openings containing reticulate webs with closely spaced microfibrils and intervessel pit membranes at the ends of scalariform perforation plates may reveal a close connection between the two structures. It could also indicate developmental similarities between scalariform perforation plates and intervessel pit membranes.

REFERENCES

- BARGHOORN, E. S., AND R. A. SCOTT. 1958. Degradation of the plant cell wall and its relation to certain tracheary features of the Lepidodendrales. Am. J. Bot. **45**(3): 222–227.
- Côré, W. A., Z. KORAN, AND A. C. DAY. 1964. Replica techniques for electron microscopy of wood and paper. TAPPI, 47: 4477–4484.
- ESAU, K. 1965. Plant anatomy, 2nd ed. Wiley, New York.
- FREY-WYSSLING, A., H. H. BOSSHARD, AND K. MUH-LETHALER. 1956. Die submikroskopische Entwicklung des Hoftüpfel. Planta, 47: 115– 126.

FREY-WYSSLING, A., K. MUHLETHALER, AND H. MOOR. 1956. Electronenmikroskopische Präparationsartefacte dünner Cellulosemembranen. Mikroskopie, 11: 219–224.

TON N. W.

- FRY, W. L. 1954. A study of the carboniferous lycopod, *Paurodendron*, gen. nov. Am. J. Bot., 41(5): 415–428.
- ISHIDA, S. 1970. [Research on wood structure by scanning electron microscopy II; in Japanese]. Wood Ind., 25(12): 560–564.
- KELSO, W. C., R. O. GERTJEJANSEN, AND R. L. HOSSFELD. 1963. The effect of air blockage upon the permeability of wood to liquids. Univ. Minn. Agr. Exp. Sta., Tech. Bull. 242, St. Paul.
- O'BRIEN, T. P. 1970. Further observations on hydrolysis of the cell wall in the xylem. Protoplasma, 69: 1-14.

- OHTANI, J. 1970. [Representation summary of the 20th meeting of the Japanese Wood Research Society; in Japanese]. Hokkaido Univ., Sapporo, Japan. p. 70.
- PANSHIN, A. J., AND C. DE ZEEUW. 1970. Textbook of wood technology, v. 1. McGraw-Hill, New York.
- WESLEY, A., AND B. KUYPER. 1951. Electronmicroscopic observations on the xylem elements of a fossil plant. Natur., 168: 137–140.
- WITHAM, H. T. M. 1833. The internal structure of fossil vegetables found in the carboniferous and oolitic deposits of Great Britain. Adam and Charles Black, Edinburgh. (orig. not seen; reported in Barghoorn and Scott 1958).
- YATA, S., T. ITOH, AND T. KISHIMA. 1970. Formation of perforation plates and bordered pits in differentiating vessel elements. Wood Res., 50: 1-11.

PAGE CHARGE INCREASE

We would like to call contributors' attention to the increase in page charges from \$25 per page to \$40 per page, effective 1 January 1972.

Papers received before that time will be charged at the \$25 rate, even though the article may not actually appear in print until after the increased rate has gone into effect.