THE BORDERED PIT MEMBRANE IN DIFFERENTIATING BALSAM FIR

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ABSTRACT

Bordered pit membranes in the cambial zone of balsam fir [*Abies balsamea* (L.) Mill.] were studied via surface replica and transverse section. An initially solid membrane is gradually perforated at tracheid maturity to yield a peripherally permeable margo bounded by a denser annulus and centrally thickened torus. Large, radially directed microfibrils in the margo are present at an early stage of pit development and do not arise as a result of pit aspiration. Relevance of findings to recent literature and viewpoints is discussed.

Additional keywords: *Abies balsamea*, wood structure, electron microscopy.

INTRODUCTION

A limited number of recorded observations are available for judging the ultrastructure of differentiating membranes in softwood bordered pits. Perhaps those of most value are the recent descriptions of Thomas (1968, 1972) and Fengel (1966, 1972). These authors also provide an excellent review of earlier pertinent literature, which abounds with valid speculation but offers little documentation. Apparently this paucity of micrographs depicting bordered pit membrane development stems from the fact that suitable replicas for transmission electron microscopy of cambial-zone pits are extremely difficult to prepare.

Bordered pits in longitudinal tracheids occur predominantly on the radial cell face (Panshin and deZeeuw 1970). While cambial derivatives are expanding, the radial face is very narrow in comparison to the tangential cell dimension. In addition, the developing tracheids are tenuously thin-walled, unlignified, and tend to collapse easily upon drying. As a result, satisfactory replicas of the intercellular pits prove very tedious, if not impossible to achieve by conventional methods (e.g., Côté et al. 1964).

In efforts to maintain mature as well as developing pit membranes in their natural state, specialized drying procedures have been employed (Thomas 1969, 1972; Sachs and Kinney 1972). Also, humid preparations have been attempted (see Fengel 1972). Still, controversy exists as to the true formation process; that is, what forces and/or growth phenomena contribute to the final morphology of the mature membrane? Despite differences in opinion, there are some convergent data.

At a very early stage of tracheid ontogeny, probably starting in the first cell inward from the cambial zone, the wall region (composed of the compound middle lamella) in which future pits are to be developed becomes noticeably thicker (Fengel 1972). At this time the membrane is completely solid and is comprised of primary wall microfibrils and incrusting matrix substances (Fengel 1966; Thomas 1968, 1972). These latter materials are probably various "polyoses" and pectins (Fengel 1972). The membrane then apparently remains imperforate until cell-wall deposition is completed (Fengel 1966; Thomas 1968). An initial pit border of primary wall is noticed at an early stage of tracheid differentiation (Murmanis and Sachs 1969), but the bulk of the overarching border is laid on at the inception of secondary wall thickening (Fengel 1966).

The central region of the developing membrane in a great many conifers (primarily the Pinaceae) becomes noticeably thicker by accumulation of additional cellulose microfibrils and amorphous materials.

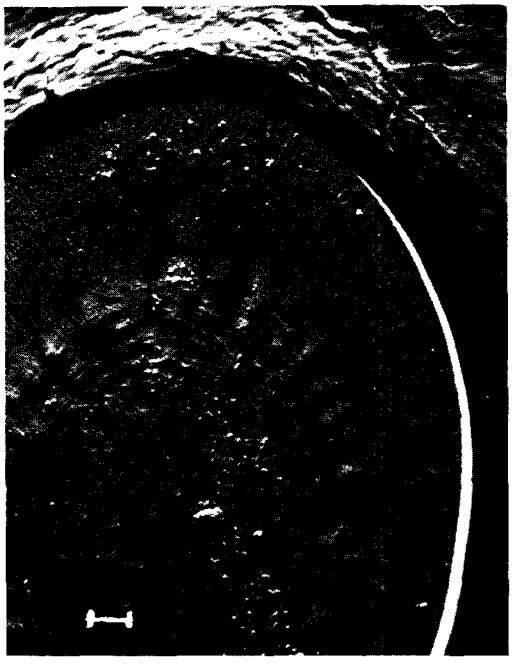

FIG. 1. Bordered pit membrane at inception of secondary wall thickening. View from lumen side of tracheid. (Line on micrographs represents 1 μm).

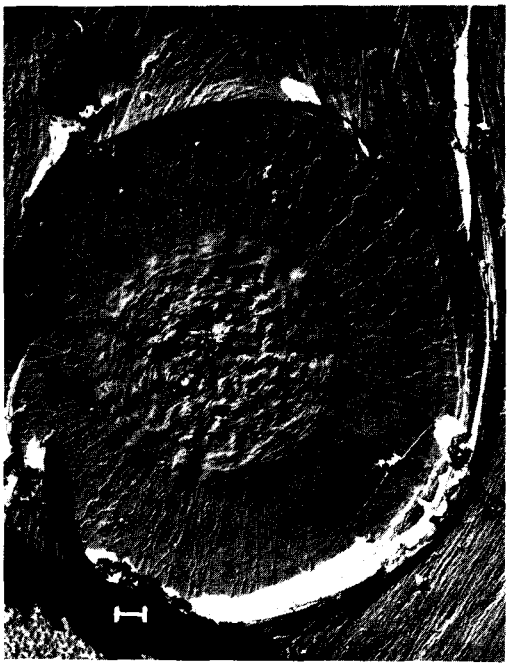

FIG. 2. Membrane of bordered pit in an almost fully differentiated cell. Note loosely apposited microfibrils in the margo. Warty layer is visible in adjacent tracheid.

This region (designated the torus) eventually becomes lens-shaped in transverse section, although considerable deviation from this pattern has recently been observed (Bauch et al. 1972; Fujikawa and Ishida 1972).

At tracheid maturity, perforations in the pit membrane begin to appear in the region (designated the margo) between the central torus and pit edge. These holes seem to occur initially near the torus and then rapidly progress to the membrane periphery, often leaving a fairly dense, narrow rim of microfibrils (the annulus) in the latter region (Thomas 1968). Ultimately, in those species in which the pit membranes imply this type of development, the perforation process gradually transforms the solid membrane into one consisting of a peripherally permeable, net-like margo with a flattened, disk-, or lens-shaped torus.

It is generally believed that margo perforation results from enzymatic action of the cell protoplast, although there are still questions on the exact nature and specificity of the enzymes involved (Fengel 1972; Thomas 1968; O'Brien 1970). In any event, this process is apparently one of the last functions of the living tracheid.

The controversy over the mechanisms that determine the ultimate morphology of bordered pit membranes still awaits reconciliation. Fengel (1972) likens the *in vivo*, undisturbed membrane to that of a ray tracheid with a loose network of microfibrils throughout. He contends that large, radially oriented microfibrils and large openings in the margo arise only after membrane perforation and as a consequence of stretching induced at pit closure (aspiration). On the other hand, Thomas (1968–1970, 1972), through gentle drying procedures, has revealed pit membranes in the unaspirated state showing the same structural characteristics as aspirated ones. He

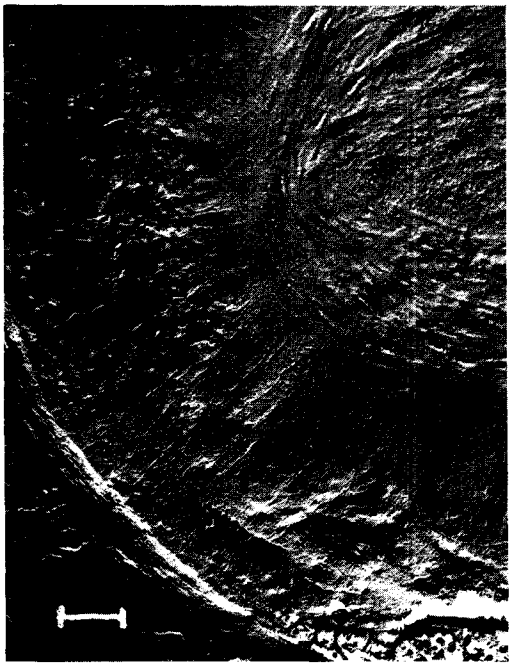

FIG. 3. Partially aspirated pit at tracheid maturity.

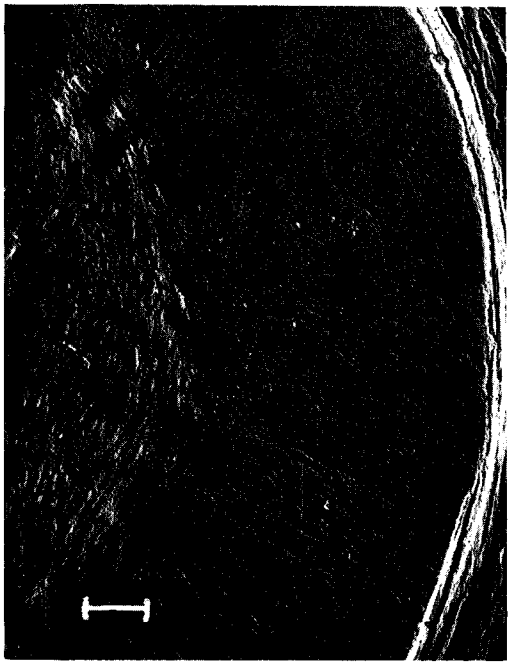

FIG. 4. Typical unaspirated pit membrane just before perforation. Note radially oriented microfibrils in the margo.

thus contends that the large, radiating microfibrils in the margo are deposited in that orientation and form and are not a result of pit closure.

A recent study by Sachs and Kinney (1972) employing a very gradual schedule of critical-point drying implies that the margo of *in vivo* mature pit membranes is actually a dense, close-mesh net of numerous microfibrils. These authors propose that the large openings in the classically pictured margo are formed by interfacial stresses due to air or water exchange. They suggest that these holes appear first near the torus and spread toward the annulus, causing disposition of the microfibrils to a more open, netlike margo with pores most numerous near the torus. Within- and between-species variability was not reported in this latter study. Therefore, from the existing literature, it is still uncertain whether the classical image of the *in vivo* pit membrane can be regarded as artifact or whether it actually does exhibit structural variability as depicted from other drying procedures.

Additional information on developing membranes of coniferous bordered pits is provided by the present study via a technique that facilitates preparation of extensive, intact replicas of the cambial zone.

EXPERIMENTAL

Cambial tissue was harvested during the month of June from the dbh region (12 inches) of an erect balsam fir [*Abies balsamea* (L.) Mill.] growing in central Wisconsin. The outer bark of a selected area was scraped away, and a sharp chisel and single-edged razor blades were used to obtain 1-cm cubes that included the cambium as well as smaller, 1-mm radial slices through the cambial zone. These latter slices were placed immediately into fixative, transported to the laboratory, and subdivided while still in the fixative to a size suitable for embedment. Two primary fixative mixtures in sodium cacodylate

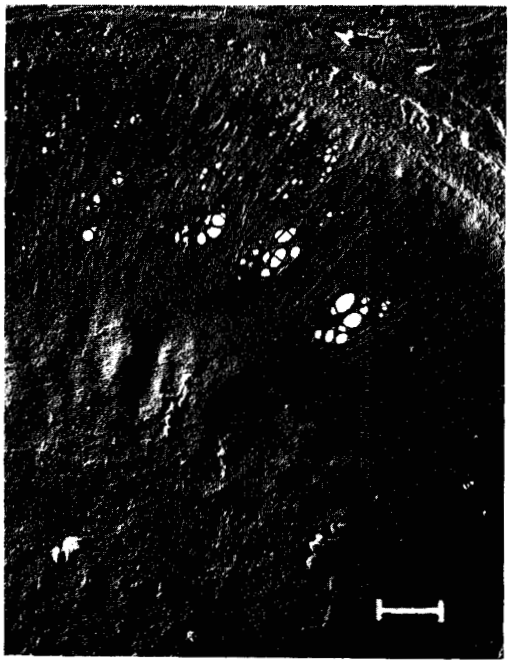

FIG. 5. Initial perforation of the differentiating pit membrane. Note small holes at margo periphery.

FIG. 7. Removal of matrix substance from pit margo and torus. Membrane is fairly dense throughout margo region.

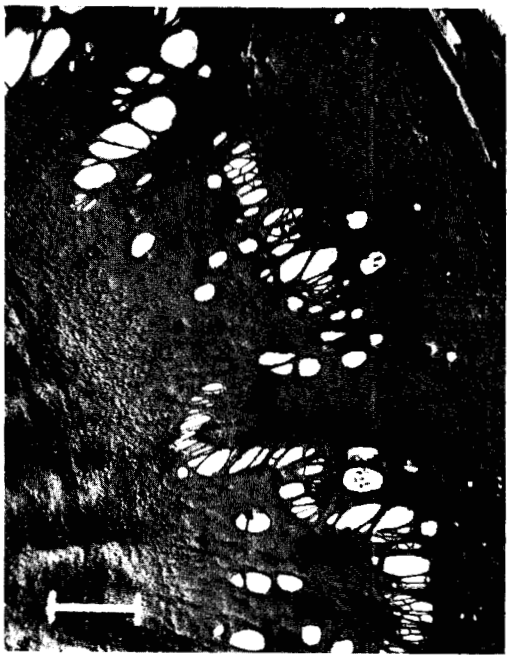

FIG. 6. Progressive perforation of the margo region. See text for explanation of dark spots.

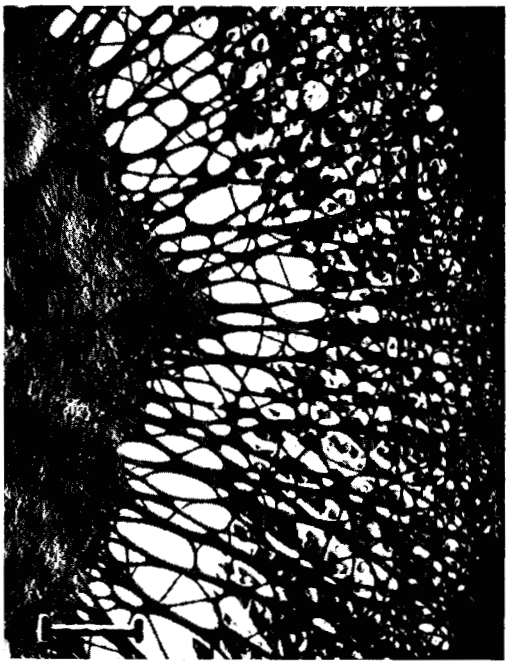

FIG. 8. Pit membrane just prior to completion of differentiation.

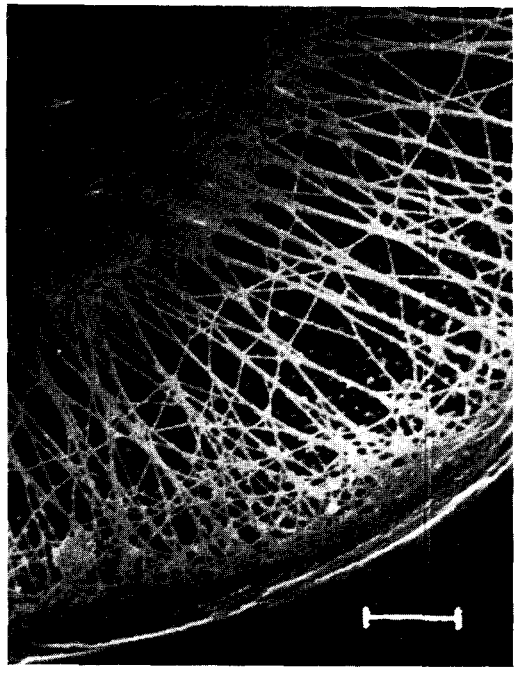

FIG. 9. Typical, fully mature membrane in earlywood of balsam fir. Scanning electron micrograph.

FIG. 10. Transverse section of a balsam fir pit at the completion of secondary wall deposition. Note residual cytoplasm and solid margo region.

buffer at pH 7.2 (3 hr at 25 C) were used —2% formaldehyde, 2% glutaraldehyde, 2% acrolein, 0.5% glucose, and 1% glutaraldehyde, 2.5% acrolein, 0.5% glucose. Post fixations by either $KMnO_4$ or OsO_4 were followed by a soak in uranyl acetate. Despite varied fixation procedures, no significant advantage was noted for any particular schedule. The specimens were then dehydrated via ethanol and propylene oxide before embedment in Spurr's "hard" epoxy (Spurr 1969). Ultrathin transverse sections were stained with $KMnO_4$ and/or lead citrate.

The 1-cm cubes were dehydrated in an ethanol:ether sequence and embedded in collodion according to Johansen (1940). They were sectioned radially at about 100 μm with a sliding microtome. The collodion was dissolved with ether:ethanol (1:1), and the sections were air-dried from ether at room temperature while sandwiched between glass slides. The sections were then shadowed in the fiber direction with platinum at 45°, carbonized, and processed according to the replication procedures of Côté et al. (1964) and Dunning (1968). Some dried sections were also coated with carbon and a 60:40 mixture of Au/Pd (about 20 nm of each) for scanning electron microscopy with a JSM-U3 at 15–25 kV. Replicas and ultrathin sections were examined with an RCA EMU-3F transmission microscope at 50 kV.

RESULTS AND DISCUSSION

During an intermediate stage of cell differentiation, the bordered pit membrane between longitudinal tracheids (earlywood) of balsam fir appears as in Fig. 1 as seen from the cell lumen. The beginning of a border is apparent, and through the large pit aperture an imperforate membrane can be observed to exhibit already a distinct architecture. Torus formation is essentially complete with the presence of circularly oriented microfibrils at its periphery, these latter microfibrils presumably arising as

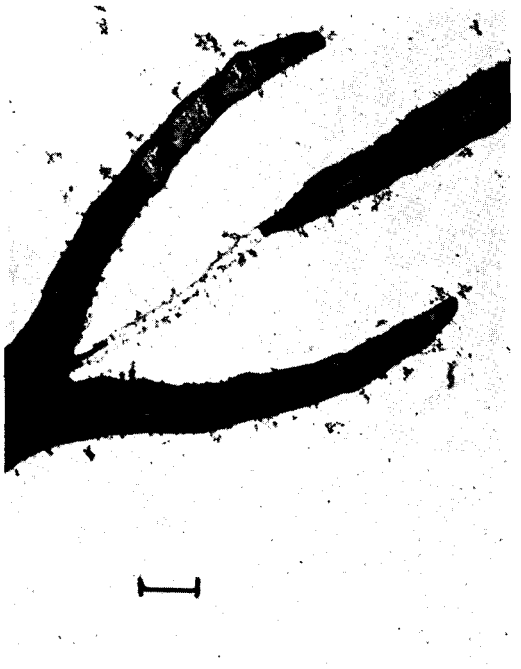

FIG. 11. Transverse section of balsam fir pit after cell autolysis. Note the apparently perforated margo and dense annulus at the membrane periphery.

secondary structures on the membrane (Thomas 1968). These, as well as radially directed microfibrils in the margo region, are seen more clearly at a slightly later stage in Fig. 2. Note here the appearance of a warty layer in the adjacent tracheid.

Because of an incrusting matrix substance, only very few circularly or randomly oriented microfibrils can be discerned in the developing pit margo. The only easily resolvable microfibrils are the larger, seemingly loosely apposed and radially oriented ones that appear to lie on the surface of the matrix material. These radiating microfibrils could be manufactured simultaneously with the rest of the membrane, but it appears in most instances that they are deposited appositionally as suggested by Thomas (1970) onto earlier formed membrane structure. In any event, they are definitely not an artifact produced by pit aspiration.

A small portion of the underlying, wart-covered border of the pit (partially aspirated) in Fig. 3 is viewable through a torn but imperforate membrane. This observation confirms the supposition that the pit membrane is still imperforate at the completion of cell-wall deposition. Figure 4 illustrates the condition of a typical, unaspirated, intertracheid membrane just before perforation.

Loss of matrix substance from the membrane and initial perforation of the margo region are illustrated in Fig. 5. Although the larger holes are often found predominantly near the torus, they can occur initially anywhere in the margo (Fig. 6). The perforation process itself most likely involves a combination effect of enzymatic action by the living tracheid and later physical erosion via the intercellular transpiration stream, and appears to begin in the cell at about the same time as overall cell autolysis.

On all replicas of perforated membranes, electron-dense spots arranged in a pattern identical to the one formed by the perforations were found displaced to one side of the actual holes. These dark areas represent regions where the platinum shadowing metal passed through the perforations and onto the underlying pit border. This shadowing artifact obscured some detail but did confirm that the membranes so affected were unaspirated.

As more matrix substance is removed from the pit membrane, numerous microfibrils become discernible in the margo and torus (Fig. 7). The densest microfibrillar portion of the margo remains consistently near the membrane periphery with larger holes usually nearer the torus. However, a few membranes observed during their early perforation period did possess a fairly dense web of microfibrils in the latter zone.

The pit membrane in Fig. 8 is almost completely differentiated, and residual matrix material is slight. Note the largest holes near the torus. A fully mature membrane is depicted in Fig. 9, clearly demonstrating a torus, margo, and annulus region. This latter micrograph is probably repre-

sentative of the *in vivo*, unaspirated, mature pit membrane in balsam fir.

Indirect evidence of membrane perforation was gained from transverse sections of differentiating tracheids. Figure 10 shows a pit which still contains some cytoplasm, though the secondary wall and warty layer have already been deposited.

At this stage the margo still appears solid throughout, further indication that membrane perforation is one of the last actions of cell development. Later, after perforation has taken place (Fig. 11), the margo in cross section is seen to consist of only two thin layers of microfibrils with a dense annulus at the edge.

SUMMARY AND CONCLUSIONS

The obviously permeable margo in mature, unaspirated pits of balsam fir results from gradual perforation of an initially solid membrane. Holes appear to grow between the larger, radially oriented microfibrils by loss of an incrusting matrix substance with perhaps some damage to smaller, randomly oriented microfibrils. It appears irrefutable that this perforation process transpires in the differentiating cell, but the condition of the photographed pit replica may also be influenced somewhat by unavoidable drying stresses or perhaps even by sintering effects during vacuum evaporation of the replica materials. Therefore, from the present data, it is difficult to speculate as to whether the *in vivo*, mature margo of coniferous bordered pits is universally as dense as is believed by some researchers (Fengel 1972; Sachs and Kinney 1972), or, as is shown in this work and in recent others (Thomas 1969, 1972), whether it is commonly quite porous with large holes. It is our contention that both of these views are valid to some extent, and that reasons for differences in observed membrane structure of similarly dried pits lie predominantly in the variability of this structure between species, between earlywood and latewood, and even between pits in the same tracheid. But only further research on wood drying procedures and documentation of wood variability will resolve this enigma.

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