

THE ULTRASTRUCTURE OF DIFFERENTIATING AND MATURE BORDERED PIT MEMBRANES FROM CYPRESS (*TAXODIUM DISTICHUM* L. RICH.)¹

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ABSTRACT

The bordered pit membrane of cypress is characterized by a margo and torus composed of a dense network of microfibrils oriented in a predominately radial direction with amorphous materials deposited on the torus. Incomplete border pit aspiration often results, presumably because of the angular shape of the inner side of the pit aperture and the fact that the torus is only slightly larger than the aperture. Differentiating pit membranes are characterized by a decidedly thickened torus formed prior to secondary border formation. Removal of the matrix substance from the margo and thinning of the torus occur simultaneously. After matrix removal, the margo is perforated and the torus attains its mature dimension.

INTRODUCTION

The bordered pit membrane has been defined as the part of the intercellular layer and primary cell wall that limits a pit cavity externally. The central, thicker portion of the pit membrane is termed the torus. (IAWA 1964). The German version further defines the pit membrane by calling the marginal unthickened portion the margo (IAWA 1964).

Although the literature contains numerous references to the bordered pit membrane structure of various species in *Pinaceae*, few citations are noted for other gymnosperm families. Liese (1965) reviewed existing literature and made a systematic investigation on species from more than forty-five genera and noted that the gymnosperm pit membranes differ in the number of margo microfibrils as well as in the presence or absence of a torus. As a result he classified gymnosperm pit membranes into five main types. The presence of a torus was noted in only the *Pinus* and *Araucaria* types with the *Pinus* type torus formed by apposition and the *Araucaria* type not due to apposition, but only as

matrix substance at the central region. Apparently the term "apposition" refers to the additional microfibrils deposited in a circular pattern around the periphery of the torus as in *Pinus*. Liese (1965) also states that only the *Pinaceae* and the *Cephalotaxaceae* families always possess a margo with a distinct torus and that species of *Gingoaceae*, *Taxodiaceae* and *Araucariaceae* which were investigated have a membrane of densely packed microfibrils in a radial orientation with the central part sometimes occluded with incrustations. Circularly oriented microfibrils as a secondary apposition in the central region do not exist in these latter families. Although this description does not specifically state the presence of a torus as defined by the International Association of Wood Anatomists, Liese's definition of the *Araucaria* type within which he places the *Taxodiaceae* indicates the presence of a torus. Liese defined the *Araucaria* type bordered pit membrane as follows: "microfibrils in a radial pattern more densely packed with narrower spaces; torus not due to apposition, but only as matrix substance at the central region."

The low ratio of sapwood to heartwood permeability reported by Stamm (personal communication 1971) for bald cypress

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(*Taxodium distichum* L.) implies that either heartwood pit aspiration was not effective or did not occur. The question of ineffective pit aspiration due to the lack of a distinctly thickened torus was raised.

Although the *Taxodiaceae* family has two genera, *Sequoia* and *Taxodium*, only the genus *Taxodium* was selected for this study. Preliminary studies on *Sequoia sempervirens* revealed a very high degree of incrustation on the pit membranes such that structural details were obscured. This fact, plus the availability of permeability data on bald cypress, but the lack of data on cypress bordered pit anatomy and the ease of obtaining never-dried samples resulted in the selection of this species for additional studies. Thus the objectives are to describe 1) the ultrastructure of bordered pit membranes of the *Araucaria* type and the effectiveness of their aspiration and 2) the differentiation of these pit membranes.

MATERIALS AND METHODS

Sapwood and heartwood specimens of bald cypress were removed from cross-sectional disks of a 220-year-old tree and a 39-year-old tree. Additional sapwood specimens were removed from the outermost portion of two trees 50 years old. From each tree, some specimens were air-dried to a moisture content of 5%, while others were either freeze-dried, solvent-exchanged dried, or dried by the critical point method from liquid carbon dioxide. Earlier work (Thomas and Nicholas 1966; Thomas 1967; Thomas 1969) has shown that compared with drying from water, the other listed drying techniques reveal considerably more margo detail because the pit membrane remains in the nonaspirated condition.

Both replication and ultrathin sectioning techniques were utilized to prepare specimens for study with a Siemens 1A electron microscope. Replicas of split-radial specimens were prepared by the direct carbon method (Côté et al. 1964). Specimens for ultrathin sectioning were embedded either in methacrylate (3 parts methyl to 7 parts

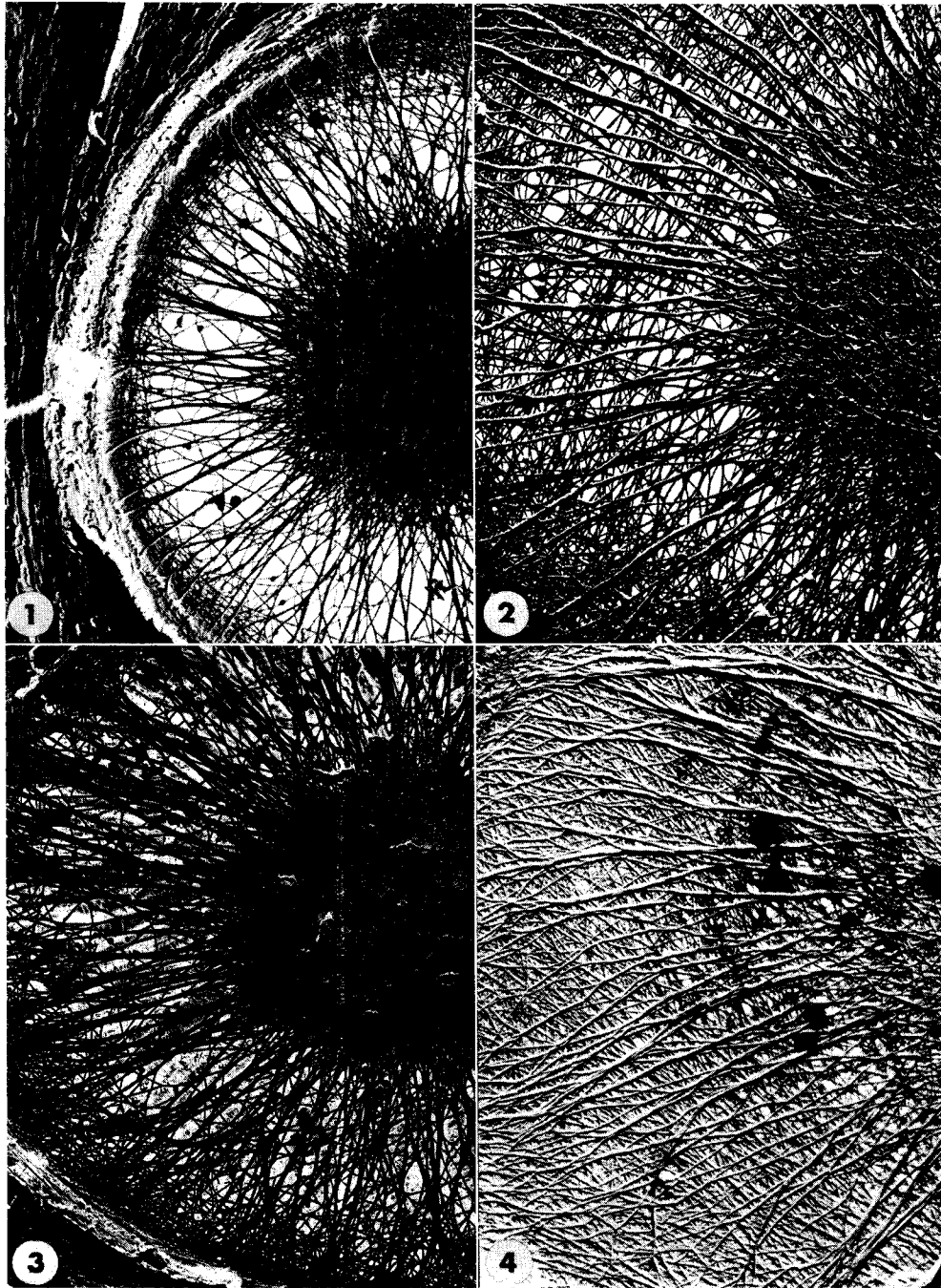
n-butyl) or Epon 812. After sectioning, the methacrylate was removed with xylene fumes and the sections shadowed with platinum from a 20° angle. Sections prepared from epon embedded specimens were stained with uranyl acetate.

RESULTS AND DISCUSSION

Pit membrane structure and aspiration

Figures 1 through 4 depict the typical bordered pit membranes encountered in the springwood tracheids of the outermost sapwood and illustrate the range of margo porosity. Most of the margo regions had a microfibrillar density similar to those shown in Figs. 2 and 3. The margo microfibrils are in a radial pattern and in most instances densely packed. Thus the margo openings are, on the average, smaller than those found in membranes of the genus *Pinus*. Note also that in most instances the larger radiating microfibrils are on the surface of the smaller, randomly arranged microfibrils. However, in some cases, the smaller diameter microfibrils near the outside of the margo are branches of the large radiating microfibrils (Fig. 3).

The existing torus structure, as revealed in surface replica views (Figs. 1 through 4) consists of amorphous materials and numerous microfibrils. Note that the large margo microfibrils diverge into many small microfibrils in the torus portion of the membrane (Figs. 1 and 2). Although the amount of amorphous materials is variable, the torus microfibrils are rarely obscured completely. The nonsoluble nature of the amorphous substances in organic solvents was indicated by the fact that specimens dried from either solvents or water revealed essentially the same amount of amorphous material. Although a secondary deposition of microfibrils in the central region of the membrane is lacking, this region is thicker than the margo portion. Figures 5 and 6 illustrate cross-sectional views of bordered pit membranes and clearly indicate the presence of a central thicker portion, thus, by definition, a torus. The torus structure results from the intersecting of the many



Mature sapwood, nonaspirated, springwood bordered pit membranes from solvent-dried specimens.

FIG. 1. 6,460 \times

FIG. 2. 11,000 \times

FIG. 3. 6,750 \times

FIG. 4. 12,000 \times

margo microfibrils at the center of the membrane, the presence of amorphous type material, and perhaps the middle lamella. Although evidence of the presence of the middle lamella in the torus of mature membranes was not detected, indications of its presence was often noted in differentiating membranes.

Pit membrane aspiration in the living tree is a continuing process directly related to the reduction of the moisture content to the fiber saturation point. In the trees examined, all of the pit membranes in the outermost 5 to 10 annual increments were in the nonaspirated state. In the adjacent zone of 20 to 30 growth rings, both aspirated and nonaspirated pits existed. Often pits were aspirated in one tracheid and not in a contiguous tracheid. To the inside of this zone, nonaspirated pit membranes were rarely detected. The above general description of pit aspiration in the standing tree applies to the springwood regions. Throughout the tree, summerwood pit membranes were for the most part in the nonaspirated condition.

One of the most interesting aspects of this study was the high incidence of incomplete pit membrane aspiration in specimens dried from water. Figures 7 and 8 depict aspirated pit membranes with the torus and a portion of the margo surrounding the torus in the elevated position. Approximately 20–30% of the pit membranes were detected in this condition throughout the 39-year-old tree and in the outermost 150 growth increments of the 220-year-old tree.

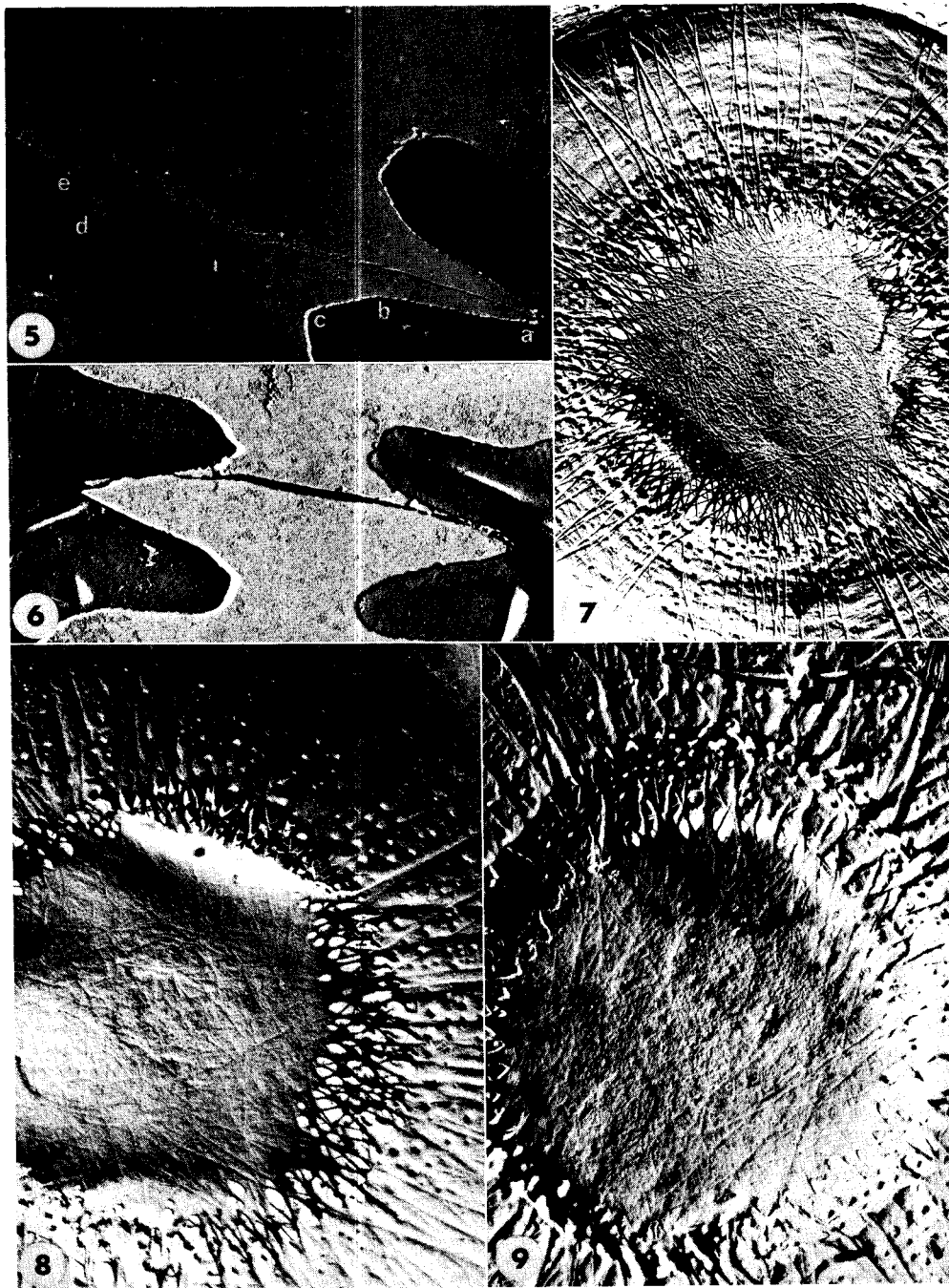
Another condition that was rather prevalent (10–20%), when the specimens were dried from water, was the separation of the torus from the margo microfibrils (Fig. 9). Note that the torus has receded into the aperture to such an extent that openings have been created. Such openings were still present in the innermost growth rings of the 220-year-old tree. The remaining 50 to 60% of the pits were aspirated completely with the aperture effectively sealed.

The lack of an effective seal between the torus and border in many of the aspirated

pits is reflected in permeability measurements of cypress. Measurements of the maximum pit pore radius determined from the air pressure required to displace water result in a value of $0.22\ \mu\text{m}$ for the sapwood and $0.066\ \mu\text{m}$ for the heartwood (Stamm personal communication 1971). Since flow volume is proportional to the fourth power of the radius, an indication of flow volume differences can be established by comparing the fourth power of the sapwood to heartwood ratios from various species. A comparison of the cypress value (123.4) with the ratios published by Stamm (1970) for six other softwood species showed only redwood with a smaller value (33). Values for the other species ranged from 1,065 to 124,000. The relatively small difference between cypress sapwood and heartwood permeability can be attributed to the large amount of ineffective pit aspiration.

The two anatomical characteristics responsible for ineffective pit aspiration in the springwood tracheids of cypress are the small torus diameter relative to the aperture and the shape of the pit border. The torus diameter (5.6 to $6.4\ \mu\text{m}$) is only slightly larger than the aperture diameter (3.5 to $4.1\ \mu\text{m}$). In hemlock the average difference in diameters is $3.2\ \mu\text{m}$ (Thomas and Scheld 1967) and in southern pine species the difference is usually in excess of $5\ \mu\text{m}$ (Thomas 1972), more than twice the difference found for cypress. Thus, in cypress, any slight movement of the torus to either side of the aperture, or rupture of margo microfibrils with the subsequent movement of the torus through the aperture (Fig. 9) will result in openings.

A characteristic, abrupt change in the shape of the border near the aperture is illustrated in Figs. 5 and 6. Points b and c in Fig. 5 clearly define the area of change. Between points a and b capillary tension forces cause aspiration of the membrane rather readily. However, the force required to pull the membrane tightly against the border between points b and c is substantially larger since the membrane must elon-



Aspirated and nonaspirated bordered pit membranes from the heartwood and sapwood of cypress.

FIG. 5. Sapwood, springwood. 4,500 \times

FIG. 6. Heartwood, summerwood. 4,500 \times

FIG. 7. Sapwood, springwood dried from water. 7,500 \times

FIG. 8. Heartwood, springwood dried from water. 12,000 \times

FIG. 9. Heartwood, springwood solvent-dried. 12,000 \times

gate considerably. In many cases the membrane resists the forces of capillary tension and does not aspirate completely (Figs. 7 and 8). In other cases the capillary tension forces exceed the membrane strength and the margo ruptures near the torus (Fig. 9). Also contributing to the openings around the torus is the fact that the torus diameter (Fig. 5) is only slightly larger than the measured aperture (c-d) and is smaller than the effective aperture (b-e). Thus aspiration does not create an adequate seal.

The detection of incrusting materials on the pit membrane surfaces occurred in about the 20th growth increment from the outside. The incrustations do not block the major openings adjacent to the torus in partly aspirated membranes as they tend to occur only on the torus and that portion of the margo adpressed against the border (Fig. 8). Thus incrusting materials in the heartwood have little or no impact on permeability insofar as the bordered pit is concerned.

Pit membrane differentiation

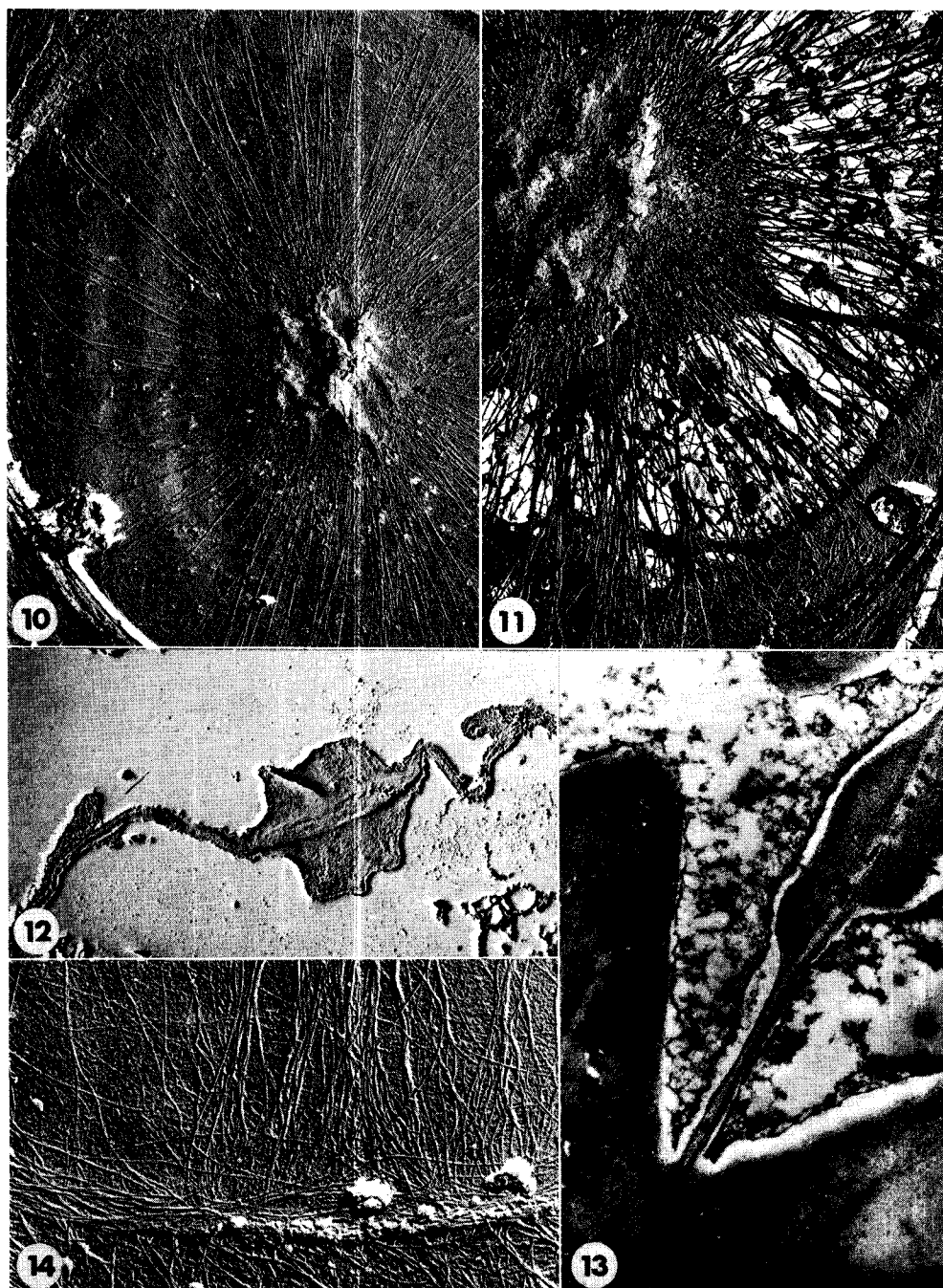
The differentiation of the bordered pit membrane proceeds in essentially the same manner as described for southern pine (Thomas 1968). In the early stages the membrane does not contain openings in the margo region (Fig. 10). As the tracheids mature, the matrix substance is removed (Fig. 11) resulting in a membrane with openings in the margo region. One obvious difference in pit membrane differentiation between the southern pines and cypress is the presence of a greatly thickened torus in an early stage of cypress membrane differentiation (Figs. 10 through 13). During the removal of the matrix substance, the material that constitutes the greatly thickened torus is also removed, leaving a torus only slightly thicker than the margo.

Since the entire membrane is, at this state of development, composed of cellulose microfibrils, matrix substance, and perhaps a middle lamella, a system for the removal

of the matrix substance and middle lamella in the margo region only is required. Assuming that the removal system is enzyme-controlled, some mechanism must operate to prevent enzymatic action in the future torus region. In southern pine species, the secondary deposition of cellulose microfibrils on the surface of the differentiating membrane could block enzymatic action in this region. In cypress, the lack of a secondary deposition of microfibrils on the central part of the membrane may be compensated for by the addition of the abundant amount of amorphous type material on the future torus site. It is not known whether this amorphous material is removed by the same enzyme and protects the torus region as a result of the increased amount of material that must be removed, or whether a separate enzyme system is responsible for its removal. Figure 13 suggests that the substance constituting the thickened torus is the same as the matrix substance in the margo region. This fact plus the observation that the thickened torus appears to be removed simultaneously with matrix removal from the margo (Figure 11) tends to support the concept of a single enzyme system. The above-suggested role for the thickened torus during pit membrane differentiation is speculative. Additional evidence to support this hypothetical role might be obtained by examining other species that do not have a secondary deposition of microfibrils as part of the torus structure.

The fact that the intercellular layer, or middle lamella, is absent from the margo of the mature pit membrane indicates that the current IAWA (1964) pit membrane definition is not applicable to gymnosperms. In addition, some doubt exists as to the presence of the complete primary cell wall in the margo area (Thomas 1968, 1970). Thus, a modification of the definition to read "that portion of the cell wall that limits a pit cavity externally" would result in a more accurate statement.

The greatly thickened torus and the margo microfibrils are formed prior to



Differentiating bordered pit membranes.

- FIG. 10. Springwood, solvent-dried 6,000 \times
FIG. 11. Springwood, solvent-dried 7,500 \times
FIG. 12. Springwood, methacrylate-embedded 6,000 \times
FIG. 13. Summerwood, epon-embedded 14,250 \times
FIG. 14. Springwood, solvent-dried 24,000 \times

border formation. Figure 12 depicts the beginning of border formation on one side of the membrane and the almost complete absence of a border in the adjacent cell. Note that in Fig. 13, border formation is complete, yet the margo matrix substance and thickened torus still persist. Figure 14 presents a surface view of the outermost region of the margo and illustrates the presence of radiating microfibrils and the initiation of border formation. In this case, the path of failure crossed the double cell wall and the lumen, thus exposing an unaltered differentiating border and margo.

CONCLUSIONS

The mature, springwood, bordered pit membrane of cypress consists of a fairly dense margo region and a torus slightly thicker than the margo. The torus is composed of amorphous materials and microfibrils continuous through the margo region. A secondary deposition of circularly oriented microfibrils in the central portion of the membrane such as occurs in southern pine species is lacking.

A large amount of incomplete pit aspiration was detected. The anatomical features responsible for the incomplete pit aspiration are the small difference in the torus and aperture diameter and the pit border shape near the aperture.

Pit membrane differentiation was similar to southern pine species, except that a

greatly thickened torus is formed early in the differentiation process. As matrix substance is removed from the margo, the torus thickness is also reduced until it attains its mature size.

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