OBSERVATIONS ON MICROFIBRIL ORGANIZATION OF DOUGLAS-FIR BORDERED PIT-PAIR MEMBRANES BY SCANNING ELECTRON MICROSCOPY

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ABSTRACT

Bordered pit-pair membranes of green sapwood Douglas-fir after alteration by pectinase enzymes followed by critical point drying were examined with the scanning electron microscope to confirm and expand results of earlier reported observations with other microscopic equipment. Micrographs of treated bordered pit-pair membranes with various degrees of pectin removal clearly showed the spatial relationship of torus structure. The technique used permits easy cleavage of the torus that, in turn, reveals in great detail the inner organization of microfibrils in the torus sandwich. Indications are that the initial pectinase dissolution of the torus is initiated in regions of plasmodesmata. Elasticity of the microfibrils in water or ethanol is vividly displayed.

Additional keywords: *Pseudotsuga menziesii* var. *glauca*, wood structure, enzymatic degradation.

INTRODUCTION

The structure of the bordered pit-pair membrane in conifer tracheids still draws deliberate scrutiny because of its cardinal importance in wood permeability. Because of the need to understand movement of liquids and gases in or through wood products and of the variability sometimes observed, it is important that we increase our understanding of wood anatomy and the mechanics that control the flow of fluids through wood.

The objective here was to present, as recorded by scanning electron microscopy, observations of the organization of the margo and the torus of Douglas-fir pit membrane. This work represents an extension of an earlier investigation (Tschernitz 1973) in which enzyme action within the bordered pit-pair membrane was used to enhance creosote treatability of poles. The effects of mild dissolution of protopectin (the native pectin substance that gives rise to soluble pectin and pectinic acids or to both on hydrolysis [Wood 1960]) in the bordered pit-pair membrane are investigated. The selective degradation of the torus by the enzyme allows broad aspects of the structural detail of the membrane to become evident.

PROCEDURE AND OBSERVATIONS

Enzyme treatment

Specimens, longitudinal cores ¼-inch in diameter by ½-inch long, of Douglas-fir green sapwood (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) were treated by infusion with one solution composed of 2.0% (volume) Rohm and Haas Pectinol 59L, 1.0% (weight) ammonium oxalate at pH 5.5, and 0.15% sodium benzoate, and held submerged at 30 C (Tschernitz 1973) for varying times. After a period of solvent exchange in ethanol (Sachs and Kinney 1972), the specimens were split radially under ethanol. Splitting the specimens under ethanol after pectin removal with

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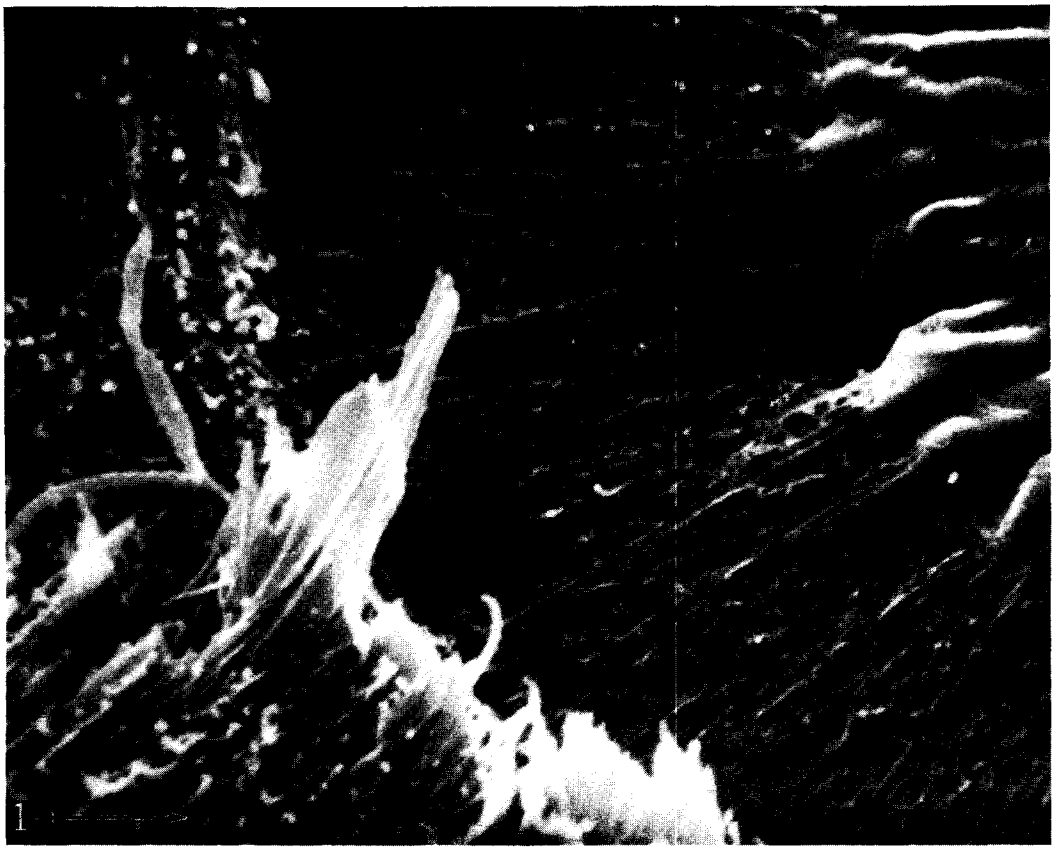

FIG. 1. Mature sapwood bordered pit-pair membrane of Douglas-fir after critical point (CO_2) drying. The close spacing of microfibrils indicates the margo has indeed been dried without the presence of a retreating interfacial surface. (No enzyme treatment.) 20,000×. NOTE: In all figures, the arrow is one micrometer long.

pectinase may be the critical part of the treatment. Splitting in the dry condition, even with the weakened torus structure, does not permit as good a cleavage and exposure of the organizational elements of the torus. A modification of the critical point drying technique was used whereby the final solvent, ethanol, was removed by exchange with liquid carbon dioxide followed by an expansive loss of the carbon dioxide as gas (described by Weatherwax and Caulfield [1971]).

Pit-pair structure

Light and electron microscope studies have established that the torus is the thickened portion of the Pinaceae pit membrane and that bordered pits are normally complementary pits in the walls of cells contiguously united in a pair.

The scanning electron microscope reveals that the bordered pit-pair membrane of Douglas-fir before pectinase treatment displays a margo of radiating and interwoven microfibrils extending from the annulus to what appears to be a centrally thickened torus (Fig. 1). Interwoven microfibrils and circularly oriented microfibrils from the torus lend credence to the torus definition of Bauch et al. (1972), that the torus is the central part of a pit membrane that can be distinguished from the margo by the lack of capillary interspaces due to removal of amorphous substances during

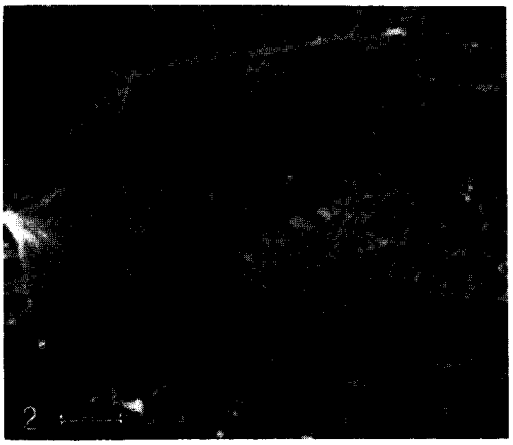

FIG. 2. Enzyme-degraded bordered pit-pair membrane shows a few of the circularly arranged microfibrils intermixed with some supporting microfibrils just under torus. (Enzyme treatment time: 16 h.) 11,500×.

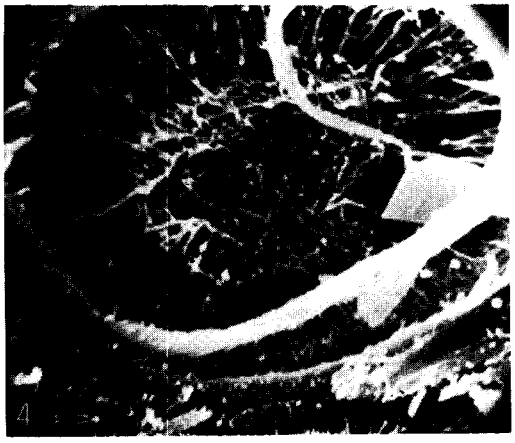

FIG. 4. With one torus removed in its entirety from pit-pair membrane during specimen preparation, orientation of microfibrils between pit-pair tori can be seen. (Enzyme treatment time: 16 h.) 5,000×.

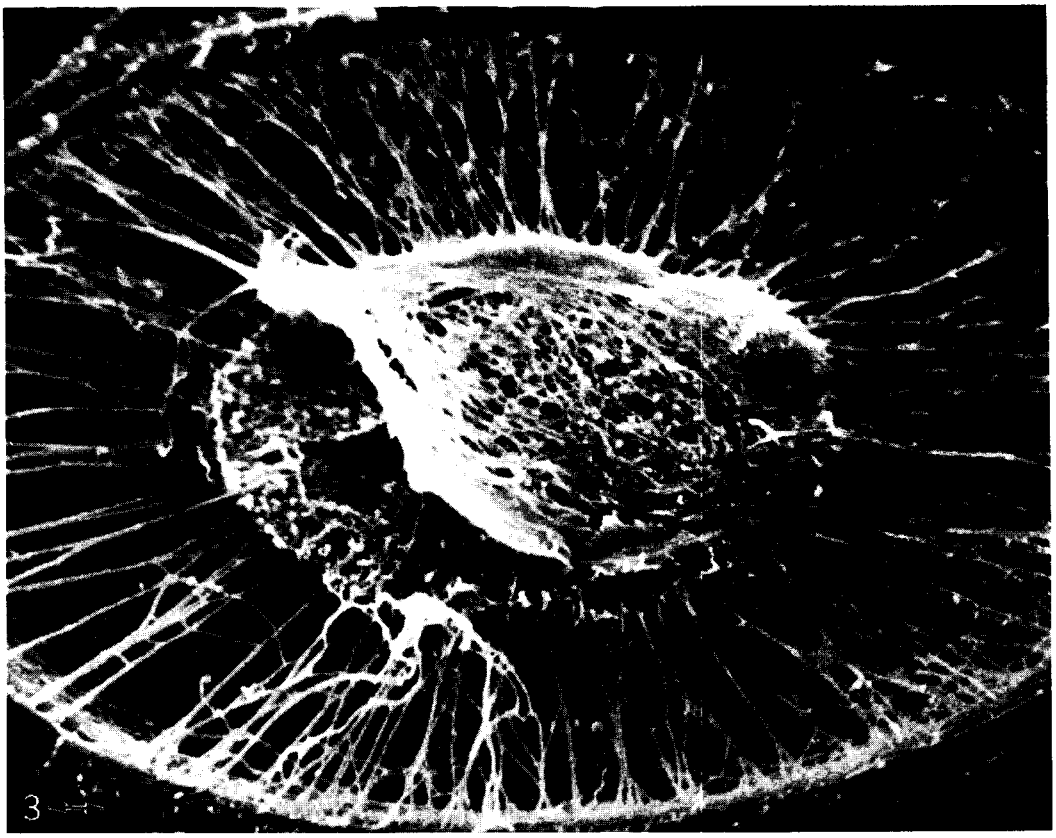

FIG. 3. The torus raised by specimen preparation opens to show microfibrils, margo, and primary wall or just microfibrils and margo sandwiched between pit-pair tori. (Enzyme treatment time: 16 h.) 7,700×.

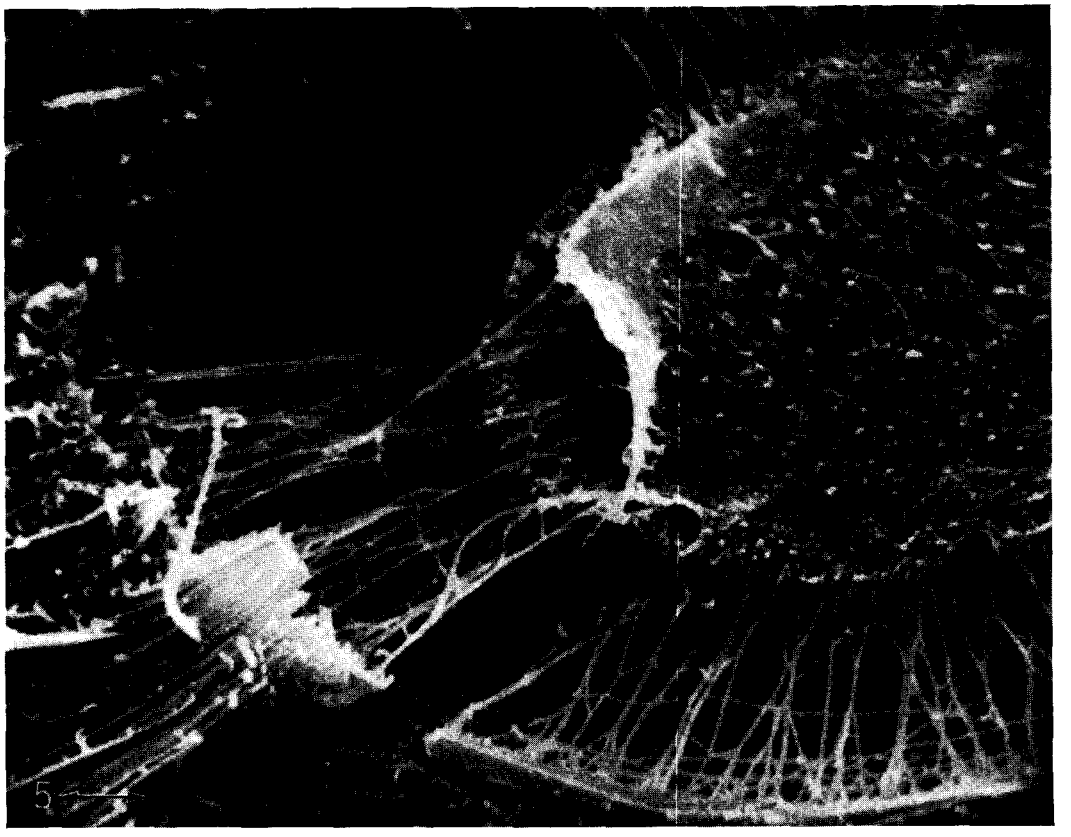

FIG. 5. Raised torus discloses that some spokelike microfibrils of margo extend into and are a part of microfibrils sandwiched between pit-pair tori. (Enzyme treatment time: 16 h.) 11,250×.

cell maturation. Frey-Wyssling et al. (1956) showed that the circularly directed microfibrils of the torus are laid down during differentiation of the tracheid wall of *Pinus sylvestris*. In the altered mature torus of Douglas-fir, the circularly arranged bundles of microfibrils become evident in a band form approximately 1 to 2 μm in width after dissolution of torus matrix materials (Fig. 2). It is obvious that the circularly oriented microfibrils are not merely supported by the underlying microfibrils, but additionally there is some interweaving of the ring microfibrils with the supporting microfibrils (Fig. 2).

Studies have shown that the chemical composition of the bordered pit-pair membrane consists of cellulose, hemicellulose, pectin, and lignin. Trying to allocate the chemical components to morphological units of the bordered pit-pair membrane, Nicholas and Thomas (1968), Bauch et al. (1968), Jutte and Levy (1971), Tschernitz (1973), and Meyer (1974) used various enzymes and observed that in the sapwood of some conifers pectinase degrades the torus, cellulase ruptures the microfibrils of the margo, and the margo is not affected by hemicellulase. However, this selective breakdown of the pit membranes was not possible in dried Douglas-fir because the sapwood contained phenolic compounds (Bauch et al. 1970; Bauch and Berndt 1973). In the work reported here using green sapwood, phenolic compounds if present did not interfere with the treating solution as they normally do in heartwood.

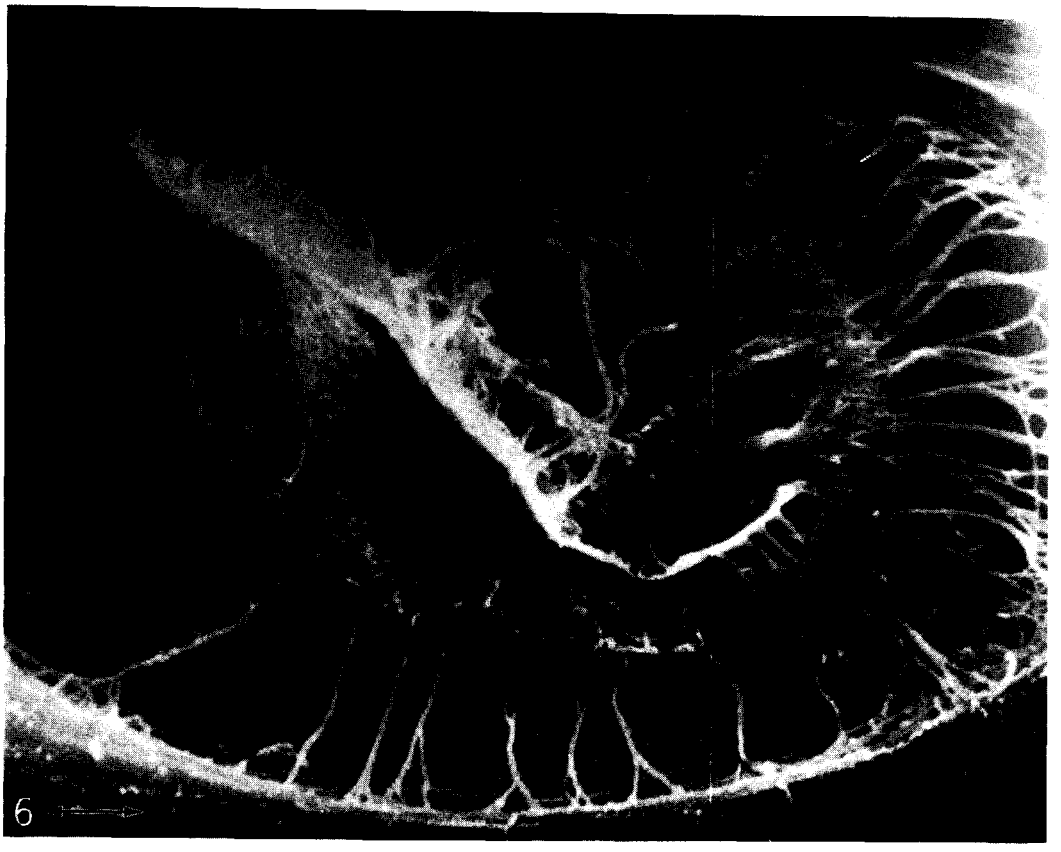

FIG. 6. Spokelike microfibrils of margo appear to extend from torus to annulus. (Enzyme treatment time: 4 h.) 14,000×.

Sandwich construction of torus

After 16 h of enzyme (pectinase) treatment, the lamellate structure of the torus became distinct; four lamellae were evident. Nicholas and Thomas (1967) demonstrated that steaming altered torus structure in a manner so that the swelling forces induced by methacrylate embedding caused a delamination of the pit membrane into three distinct layers. In this study, four distinct layers of the torus were observed. Two layers of randomly arranged microfibrils, indistinguishable from primary walls or remnants thereof, are sandwiched between the tori-pair (Figs. 3 and 4).

In Fig. 5, the raised torus after enzyme preparatory treatment, reveals that some of the spokelike microfibrils of the margo are of the network of microfibrils sandwiched between the tori. Sachs and Kinney (1972) suggested that the spokelike appearance of the margo may result from a rapidly drying interface. Before drying occurs, the margo is a dense network of microfibrils similar in density to those found between the tori (Fig. 1). That some microfibrils do not break and form a network of radiating strands may be due to the overlay and interweaving of the circularly arranged microfibrils, whose presence may prevent or slow the dislocation of the microfibrils during drying. In addition, as the tori become separated by pectinase treatment, linking microfibrils appear to be extending from the circularly oriented microfibrils of the torus to the

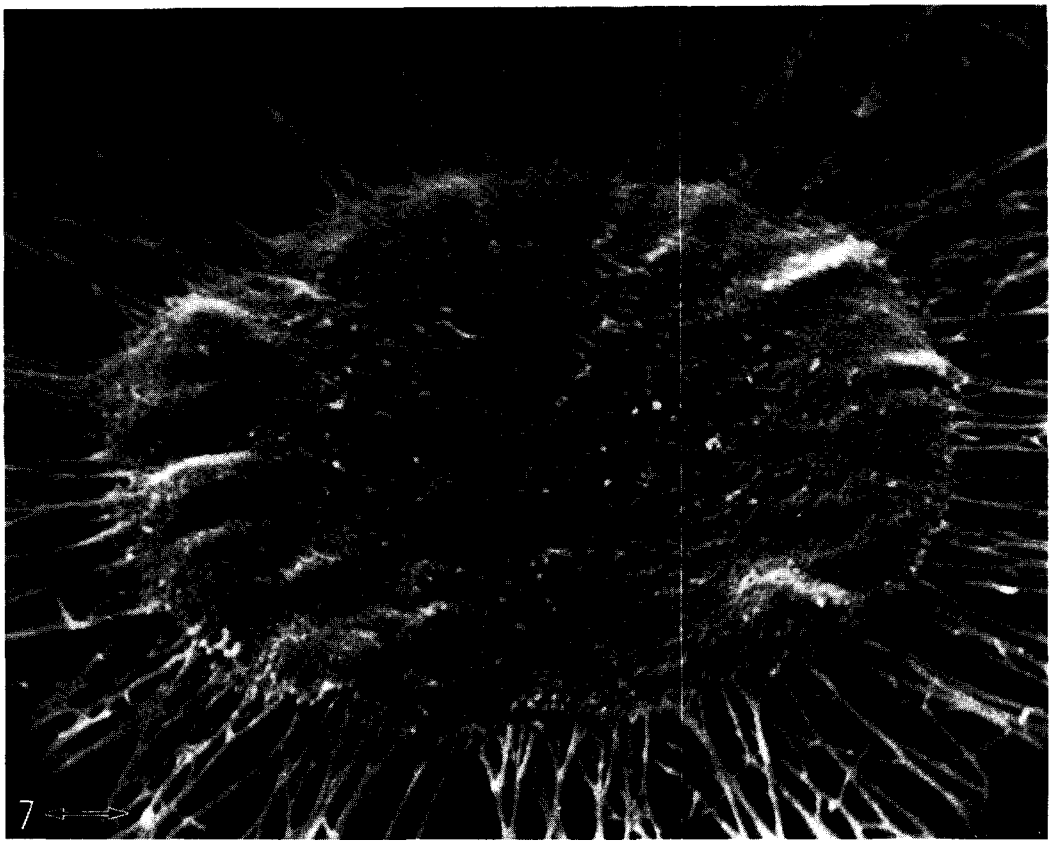

FIG. 7. With partial pectin removal, plasmodesmata (arrow) show enlargement as primary point of enzyme attack. (Enzyme treatment time: 2 h.) 12,000×.

annulus (Fig. 6). The sandwich construction of the torus plus the interwoven arrangement of microfibrils tends further to support the contention that the torus may be a composite structure (Sachs 1963).

Speculation on plasmodesmata removal

Interspersed in the tori are a number of openings approximately 80 nm in diameter (Fig. 7). These may be the location of former plasmodesmata that penetrate the primary wall in its early development and are later still visible in the pit membrane. Plasmodesma equals cytoplasm plus cisterna (Esau 1965).

After 2 h in the pectinase solution with partial pectin removal, some of the openings appear empty (Fig. 7). The openings increase in diameter with time in the enzyme solution so that after 16 h, they appear larger (Fig. 8). The presence and the proximity of these openings suggest that the plasmodesmata integrity or architecture is destroyed by the action of the enzyme treatment. Perhaps as the protopectin is hydrolyzed by enzyme action, the plasmodesmata drop away because the support for them is eliminated. This would indicate that the plasmodesmata may be surrounded or embedded in protopectin.

Elasticity of microfibrils

The elasticity of the microfibrils in water or ethanol becomes fairly evident by observing a calcium oxalate crystal that was derived from the enzyme treating solution that had become entrapped in the margo (Fig. 9). Calcium oxalate crystals that

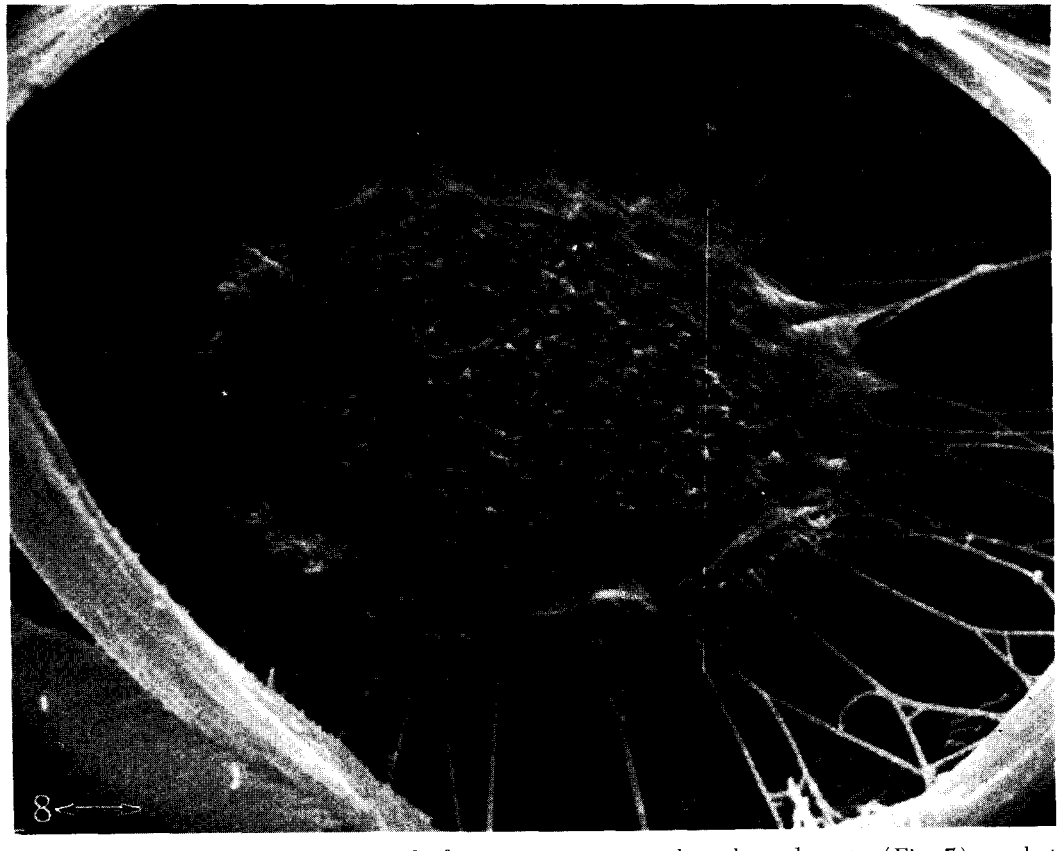

FIG. 8. Voids in torus from removal of protopectin suggest that plasmodesmata (Fig. 7) are lost. (Enzyme treatment time: 16 h.) 11,250×.

occurred as an impurity in the treating reagent were identified by X-ray diffraction (D. F. Caulfield, personal communication, 1972). Many microfibrils in the figure —including exceedingly fine microfibrils— remain intact and appear stretched over the broad surface of the crystal. The crystal support apparently prevented dislocation of the fine microfibrils of the margo while they were subjected to interfacial drying stresses.

SUMMARY

Enzyme pretreatment of green sapwood of Douglas-fir in combination with techniques of scanning electron microscopy has resulted in the following:

1. Clear delineation of the spatial relationship of the microanatomy of the bordered pit-pair membrane.

2. Indication that splitting under water or ethanol provides easy cleavage of the torus in conjunction with enzyme treatment.

3. A detailed visual demonstration of microfibril orientation within the torus structure that confirms the presence and the manner of distribution of pectin in the torus.

4. Reconfirmation and elaboration of the sandwich structure of the pit-pair membrane.

5. Although by no means proved, the photomicrographs strongly imply that the initial perforation of the torus is in and about the plasmodesmata.

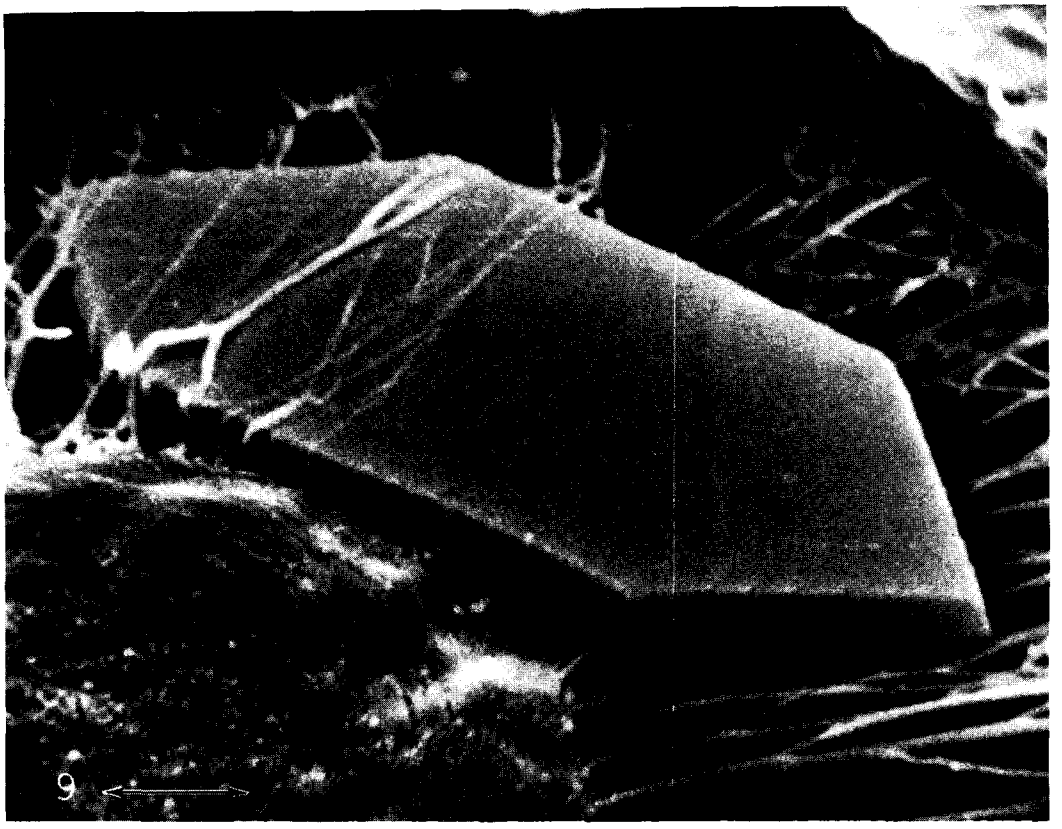

FIG. 9. Calcium oxalate crystal (similar crystals identified by X-ray diffraction) entrapped in margo of bordered pit-pair membrane illustrates elasticity of fine microfibrils. (Enzyme treatment time: 1 h.) 18,250×.

6. By using the critical point technique, microfibrils of the margo appear elastic in water or ethanol.

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