

# THE ULTRASTRUCTURE OF SOUTHERN PINE BORDERED PIT MEMBRANES AS REVEALED BY SPECIALIZED DRYING TECHNIQUES<sup>1</sup>

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## ABSTRACT

The study of the mature bordered pit membrane from three species revealed that there were no major differences in ultrastructure. The pit membrane consists of an imperforated torus and a margo with considerable variation in porosity. Springwood pit membranes from the sapwood were rarely incrustated, whereas summerwood pit membranes from both the sapwood and heartwood were heavily incrustated.

The use of specialized drying techniques, specifically solvent-exchange, critical-point, and freeze-drying techniques, prevented pit aspiration and permitted a detailed study of the pit membrane. Since the margo structure was not altered to any substantial degree by the different drying systems, it appears that the surface tension of the evaporating liquid is important only with regard to pit aspiration. A study of trees from several age classes showed the decrease of the number of margo microfibrils to be related to the age of the wood rather than to the time of formation. The mechanism of the deterioration process was not determined.

## INTRODUCTION

Many of the relationships which exist between wood anatomy and the physical and chemical properties of wood are well known. For example, it has been shown that softwood permeability is controlled largely by inter-tracheid pitting. However, a review of the literature revealed little information with regard to the pit ultrastructure of the southern pines. Because of the importance of this group of species to the southeastern region and to the rest of the nation and because of their diversified utilization, a study of the bordered pit membrane ultrastructure was conducted. Increased knowledge of pit membrane ultrastructure may ultimately lead to more efficient methods of drying, treating, or pulping.

The pioneer work on the ultrastructure of bordered pit pairs was performed by Liese and Fahrenbrock (1952). Their results supported Bailey's (1913) concept of a perforated margo and an imperforate

torus. Additional work by Frey-Wyssling and co-workers (1956), Harada (1956), and Côté (1958) supported and clarified the earlier findings. The results of these workers indicated that in the majority of cases the central area of the pit membrane is occupied by a thickened portion called the torus. The torus is supported by strands of microfibrils radiating from the torus to the edge of the pit, much like the spokes of a wheel. In many cases the radiating strands appear to consist of aggregations of a number of microfibrils. The radiating strands which make up the margo are arranged in such a manner that they cross over one another and leave spaces between the strands so that the membrane can be considered porous. A detailed study by Liese (1965) revealed considerable structural variation of the bordered pit membrane in longitudinal tracheids among the various softwood genera. He has proposed five basic types based on the number and density of microfibrils within the margo and the presence or absence of a well-defined thickened torus. He defined the *Pinus* type as one with a definite thickened torus supported by a moderately dense margo with fairly large openings.

In the southern pines, bordered pit pairs interconnect (1) longitudinal tracheids,

<sup>1</sup> Paper No. 2780 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina. Paper presented at the Utilization of the Southern Pine Symposium held November 6, 7, and 8, 1968, at Alexandria, Louisiana.



FIG. 1. A non-aspirated, springwood bordered pit membrane from the solvent-dried, outer sapwood of longleaf pine. (The bar in lower right corner of each micrograph represents one micron.)

(2) ray tracheids, and (3) longitudinal tracheids to ray tracheids. The detailed characterization of the bordered pit membranes which follows is concerned with the longitudinal tracheid bordered pit pairs.

#### MATERIALS AND METHODS

The three species of southern pine selected for study were loblolly (*Pinus taeda* L.), pond (*Pinus serotina* Michx.), and longleaf (*Pinus palustris* Mill.). For each of the species studied, three trees were sampled. From each tree a cross-sectional disk was removed at breast height. Portions of each cross-sectional disk were allowed to air-dry to a moisture content of 5%, while the remainder of the disks were retained in the green state by storage in a 30% methanol solution.

Through the application of solvent-exchange drying techniques, Thomas and

Nicholas (1966) were able to depict bordered pit membranes in the non-aspirated state. The prevention of pit aspiration permits a more detailed study of the pit membrane since the margo microfibrils are more easily detected. Thus, the solvent-exchange drying procedure as well as two other methods, freeze drying and critical-point drying, which also prevent pit aspiration (Thomas 1967) were utilized in this study.

The solvent-exchange drying was accomplished by sequentially extracting the specimens with methanol, acetone, and pentane in a soxhlet apparatus. A period of 24 hr elapsed between changes of the liquids. After removal from pentane, the specimens were dried at 65 C for 15 min, then stored in a desiccator over calcium chloride until utilized.

The critical-point drying procedure as

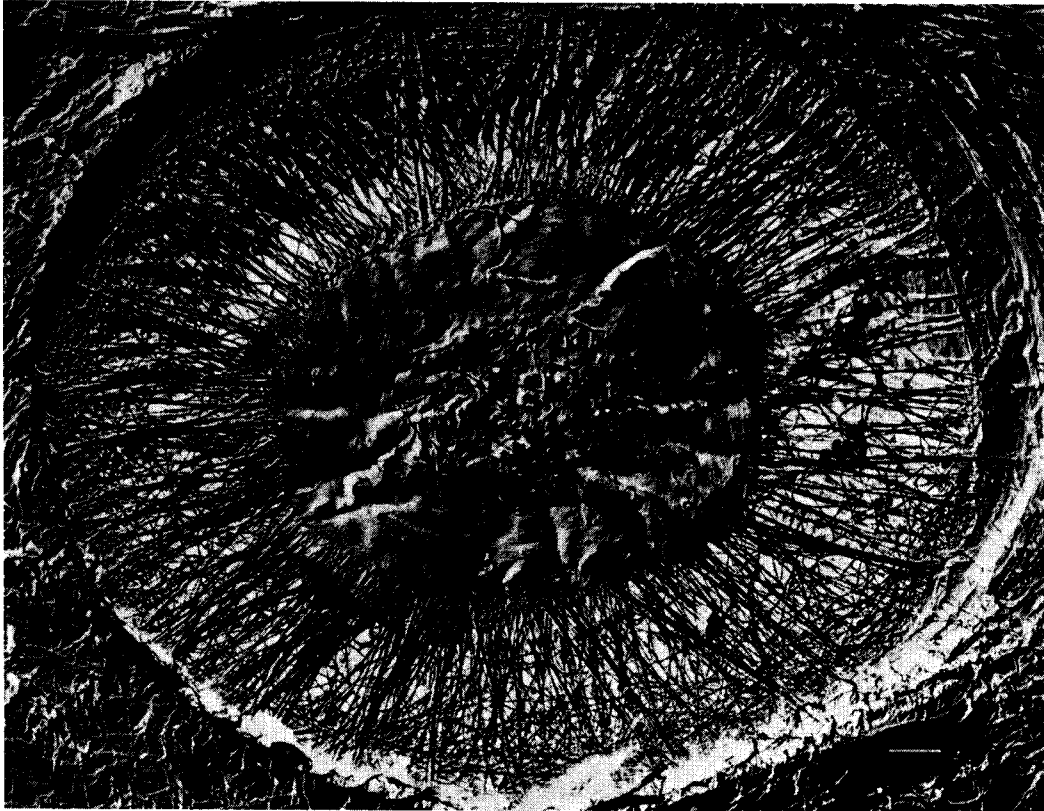


FIG. 2. A non-aspirated, springwood bordered pit membrane from critical-point dried outer sapwood of longleaf pine.

described by Anderson (1951, 1953) takes advantage of the fact that the interface between fluid in equilibrium with its own vapor disappears at temperatures above the critical point. Thus, specimens were impregnated with methanol, amyl acetate, and finally liquid carbon dioxide. At this point, the temperature was raised above the critical point (34 C), converting the liquid carbon dioxide to a gas which was allowed to escape slowly. Specimens dried in this manner are not subjected to liquid surface tension forces and should thus reveal structures with little or no alteration.

For freeze drying, water-saturated specimens were placed in a freezer set at -20 C for a period of 24 hr. After freezing, the samples were dried in an American Sterilizer, pilot-type freeze drier for approximately 15 hr. Radiant heat was supplied

from a 50 C platen under a vacuum of 50 microns throughout the drying period.

Replicas of split-radial sections were prepared by the direct carbon method outlined by Côté and co-workers (1964). Either platinum or chromium, applied at an angle of 45°, was used for shadowing. The replicas were studied and photomicrographs taken with a Siemens Elmiskop II electron microscope.

#### RESULTS AND DISCUSSION

Although specimens from all three species were examined with regard to characterizing the longitudinal tracheid bordered pit membrane, most of the specimens were from the longleaf and pond pine species. Thus, most of the micrographs illustrated are from these two species. However, sufficient observations were made on loblolly pine to ascertain that there were no major

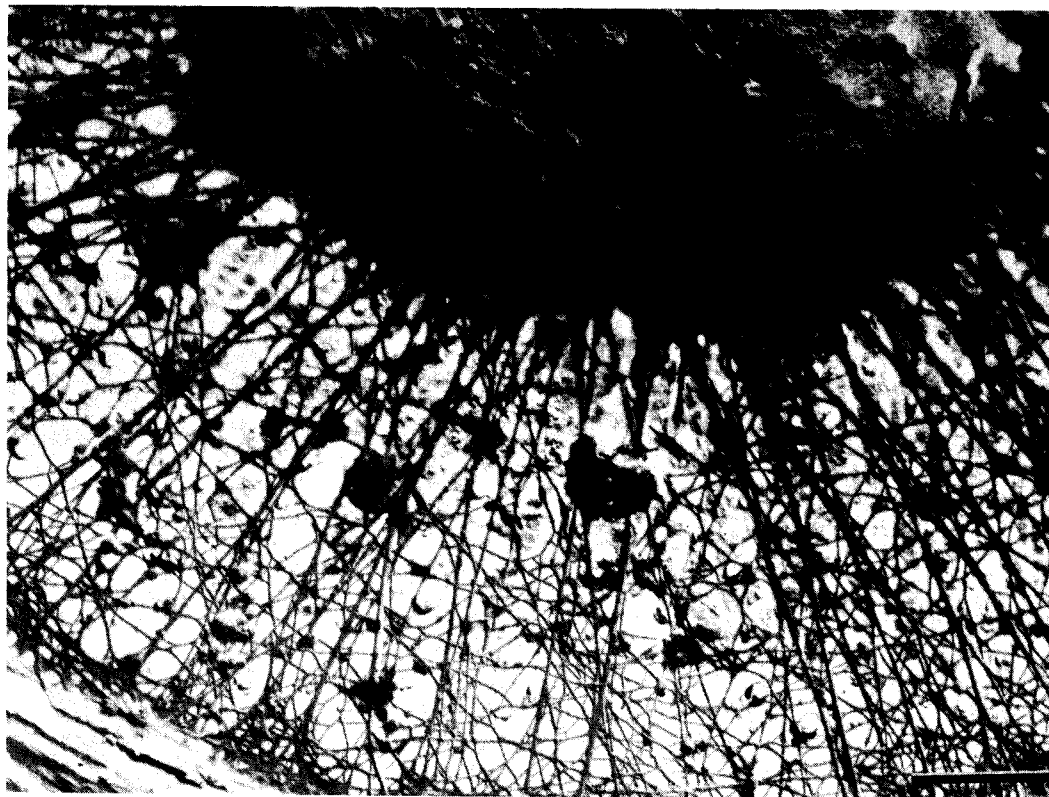
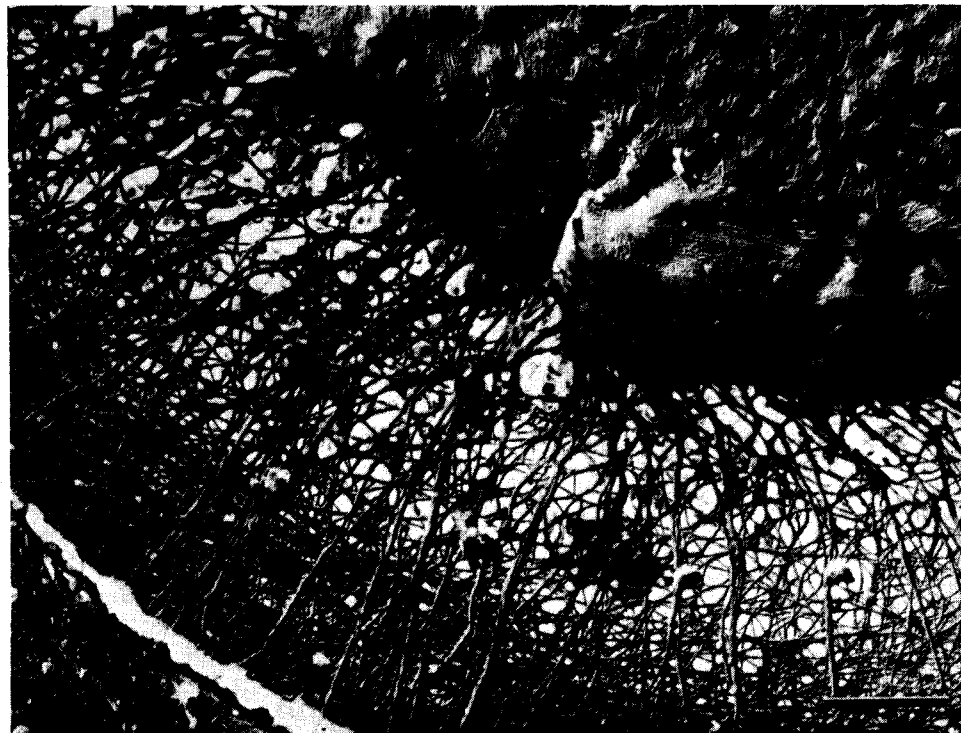
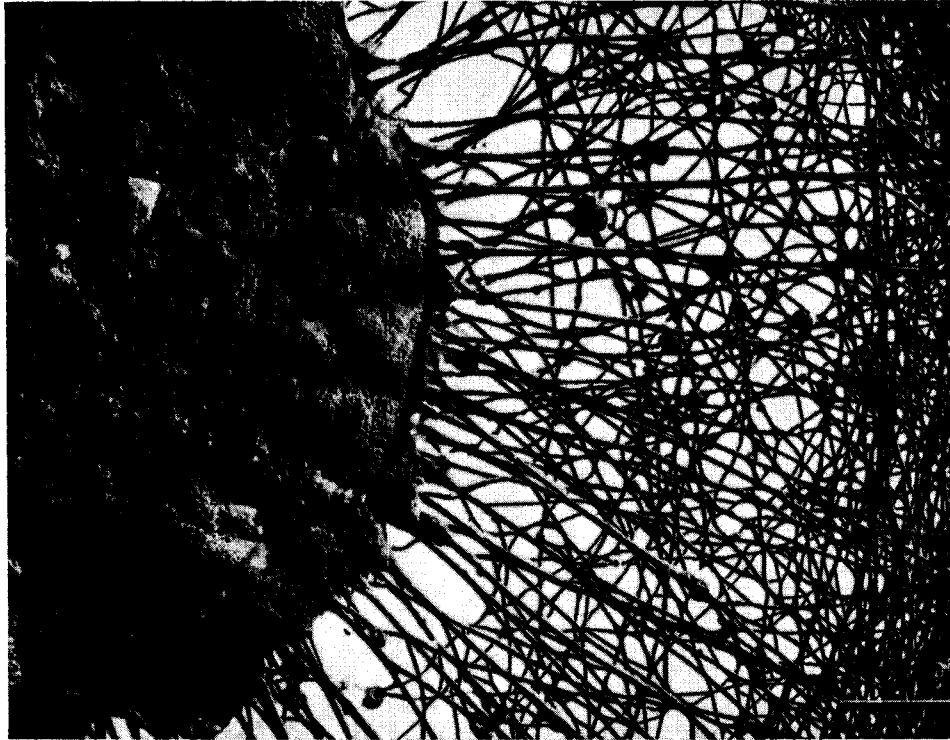


FIG. 3. A portion of a non-aspirated, springwood bordered pit membrane from freeze-dried outer sapwood of pond pine.

differences in ultrastructure, and that the following characterization of the mature bordered pit membrane is applicable to all three species. Thus the conclusions of a study by Thomas (1967a) on the longleaf pine bordered pit membrane are applicable to this study. The inclusion of two additional species and the use of additional specialized drying techniques augment the earlier findings and introduce additional information.

Specialized drying techniques which prevented pit aspiration throughout the outer sapwood region (outermost five rings) revealed a margo with considerable variation in density. Figs. 1 through 7 represent the various pit membranes encountered throughout the outer sapwood of the three species examined. Note that in many cases the part of the margo near the torus is considerably more open than the outer

portion (Figs. 3 through 6). Pit membranes with extremely dense margo regions similar to those depicted in Figs. 6 and 7 were detected only rarely and in most cases were found in the outermost growth ring. The examination of many micrographs has indicated that the variation of margo density within an outer sapwood growth ring is as large as between rings. Furthermore, the variation within a tracheid often appeared to be as large as between tracheids. In the majority of cases, the variation appeared to be controlled by the number of the smaller, randomly-oriented microfibrils, rather than by any change in the number of the larger radially-oriented microfibrils. A comparison of non-aspirated pit membranes to aspirated pit membranes reveals essentially no difference insofar as the larger radiating margo microfibrils are concerned. However, since more of the



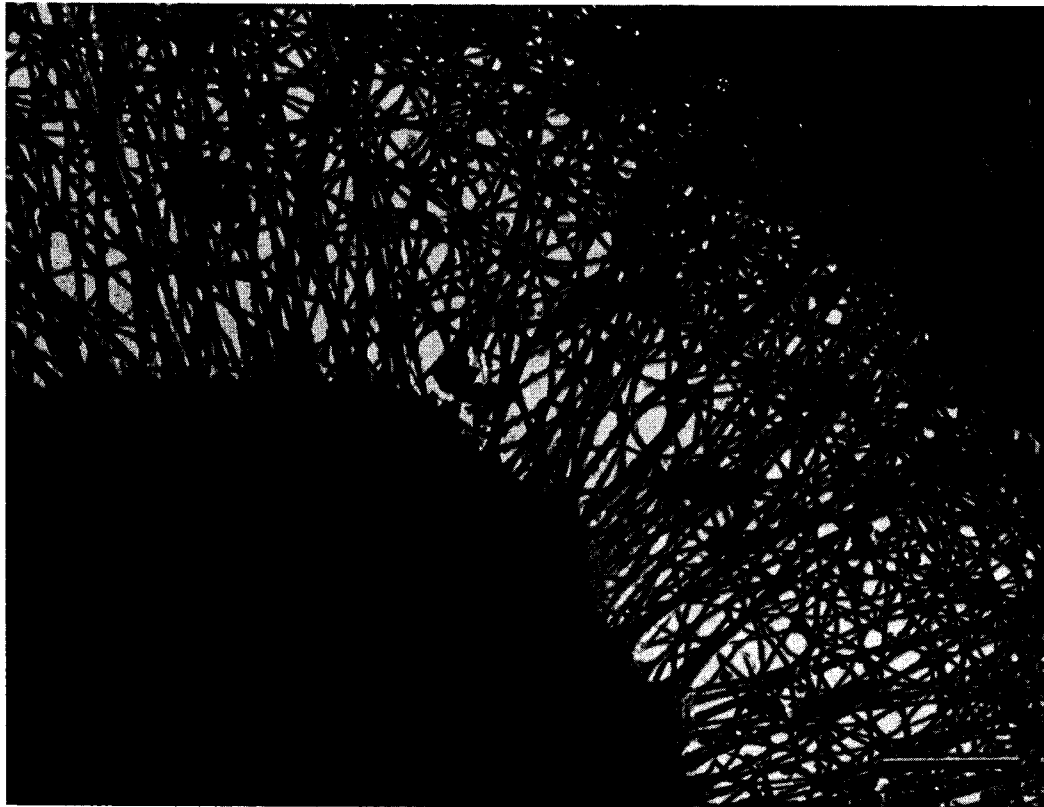


FIG. 6. A portion of a non-aspirated, springwood bordered pit membrane from solvent-dried outer sapwood of pond pine.

smaller, randomly-oriented microfibrils are revealed in the non-aspirated membrane, these membranes show a margo with smaller openings than previously depicted in the literature.

Hart and Thomas (1967) have speculated that the largest opening in the dry, non-aspirated margo, as depicted from pentane-dried specimens, may not reflect the largest opening in a green margo with any degree of accuracy. They state that when the first air hole appears in the drying margo, that hole may be considerably enlarged by the high tensile forces surround-

ing it, thus becoming larger in the dry state. The lower surface tension of pentane as compared to water (16 to 72.75 dynes per cm) should result in a smaller enlargement than water. Therefore, the use of drying techniques which further reduce the surface tension should reveal a margo with even smaller openings. Because the solvent drying technique utilized did not eliminate surface tension but only reduced it to 16 dynes per cm, an effort was made to determine if critical-point drying (a theoretical surface tension of zero) revealed a margo structure different from that observed on

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FIG. 4 (top). A portion of a non-aspirated, springwood bordered pit membrane from the outer sapwood of pond pine. The specimens were passed through the solvent-exchange liquids, back to water and freeze-dried.

FIG. 5 (bottom). A portion of a non-aspirated, springwood bordered pit membrane from solvent-dried outer sapwood of pond pine.

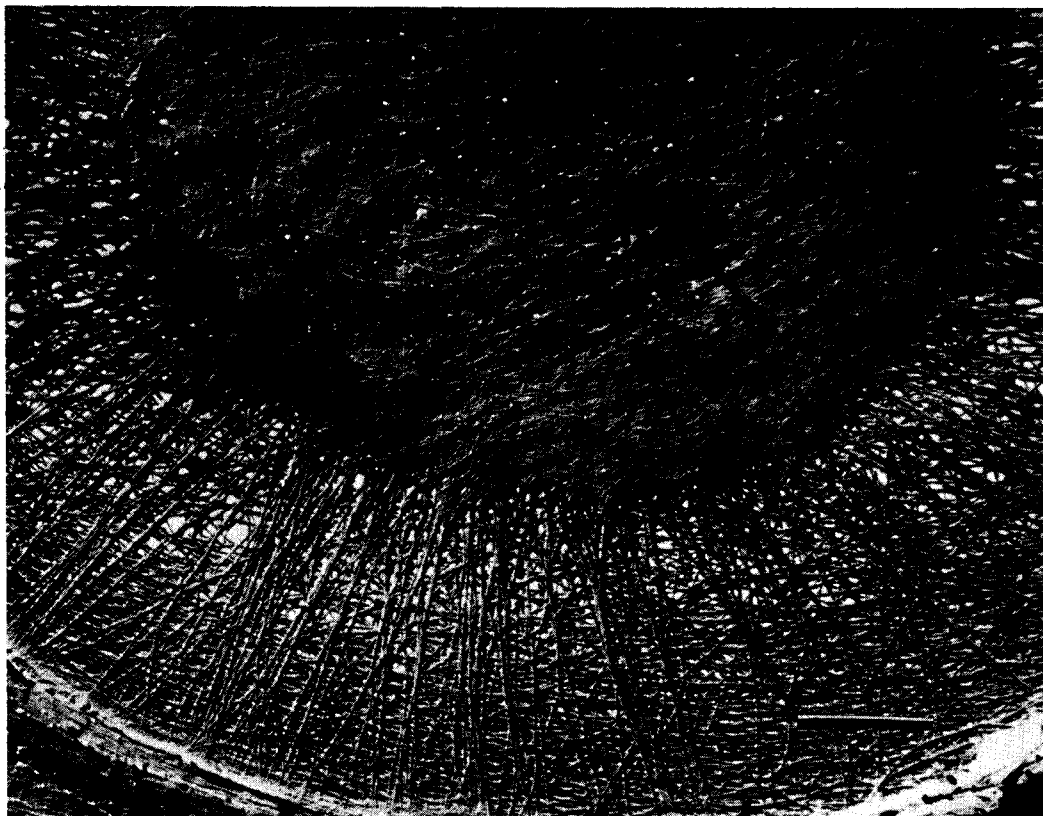


FIG. 7. A portion of a non-aspirated, springwood bordered pit membrane from solvent-dried outer sapwood of loblolly pine.

solvent-dried specimens. Except for the removal of additional incrustations by the amyl-acetate and/or liquid carbon dioxide from the torus surface (Fig. 2), no difference in pit membrane structure was noted.

Earlier work (Thomas 1967) showed that freeze-drying wood prevented pit aspiration. The removal of water without an air-liquid interface prevents formation of the surface tension forces necessary for pit aspiration. Pit membranes from freeze-dried specimens revealed essentially the same margo structure as solvent-exchange and critical-point drying techniques (Figs. 3 and 4). Thus it appears that the surface tension of the evaporating liquid is important only with regard to pit aspiration and does not alter margo structure to any substantial degree.

Pit membrane incrustation, which has been described previously by various

workers for a number of species (Frey-Wyssling et al. 1956; Krahmer and Côté 1963; Koran 1964; Sebastian et al. 1965), was not prevalent on the springwood bordered pit membranes from solvent-exchange or critical-point dried specimens. Since the solvents may have removed various incrusting materials, freeze-drying which does not remove incrustations was utilized. Fig. 3 illustrates the typical pit membrane encountered from freeze-dried outer sapwood. Note that the margo is relatively free of incrusting materials. Fig. 4 depicts a membrane which was passed through the solvent-exchange system, back to water and freeze-dried, thus presenting a pit membrane with the solvent soluble incrustants removed. A comparison of Figs. 3 and 4 shows that the minor amount of incrustations present does not alter margo density sufficiently to interfere with liquid flow.

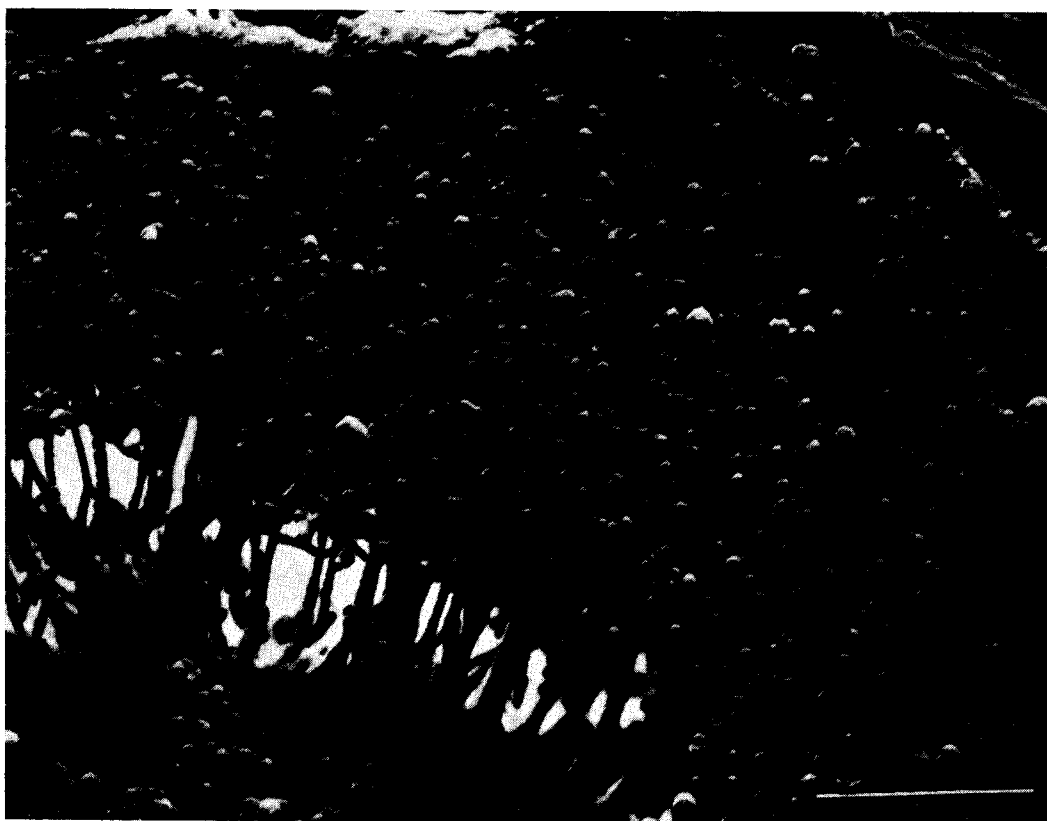


FIG. 8. A non-aspirated, incrustated, summerwood bordered pit membrane from freeze-dried outer sapwood of longleaf pine.

A study of freeze-dried bordered pit membranes from summerwood tracheids of all three species revealed, as reported for longleaf pine (Thomas 1967), a membrane with a very high degree of incrustations (Fig. 8). The incrustation is so complete that in many cases openings cannot be detected in the margo. Use of solvent-exchange drying systems resulted in almost complete removal of the incrustation from the pit membranes in the outer two growth rings and partial removal from the membranes throughout the inner growth rings. Incrustation removal showed that the summerwood pit membranes contain a torus and perforated margo. The number of margo microfibrils (and thus margo density) was consistently higher in the summerwood portion of the growth ring than in the springwood.

The summerwood bordered pit pairs were generally smaller and varied considerably more in size than the springwood bordered pit pairs. The summerwood pit membranes ranged from 8 to 15 microns in diameter while the springwood pit membranes ranged from 20 to 25 microns. The larger summerwood pit membranes are associated with the first formed summerwood tracheids. As the radial diameter of the tracheids decreases in the later-formed summerwood, the pit membrane diameter decreases.

Because of the presence of heavy incrustations, considerable reduction of liquid flow between summerwood tracheids is to be expected. The studies of Kozłowski and co-workers (1966) on gymnosperm seedlings revealed that the last formed small-diameter tracheids often conducted no



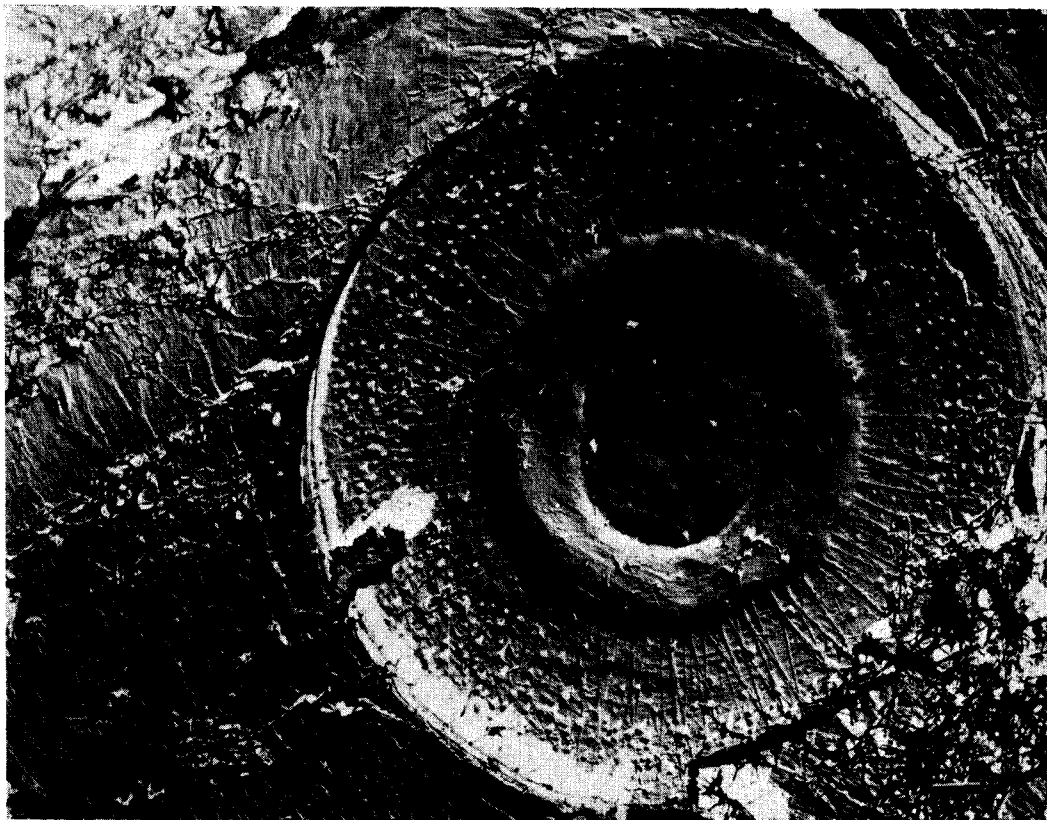


FIG. 9. An aspirated springwood bordered pit membrane from solvent-dried, inner heartwood of pond pine.

water upward in the tree. In addition, they found that tangential movement of water from rays into more than one row of latewood tracheids was limited by restricted lateral transport from one tracheid to another. Thus, the failure of the summerwood tracheids to translocate water appears to be directly related to the ultrastructure of the pit membrane.

The action of the various solvents on the torus surface was variable. In many cases the circular orientation of microfibrils around the periphery of torus was revealed (Figs. 1, 2, and 5) when incrusting materials were removed from the torus surface. Another interesting aspect which resulted from the solvent-exchange treatments was the detection in the torus of what appear to be small depressions or openings (Figs. 5, 7, 11, and 12). Although solvent drying

reveals these structures in most of the tori from ray tracheid to longitudinal tracheid bordered pit pairs (Thomas 1969), they were not detected as often in the tori of the longitudinal tracheid bordered pit pairs. A closer study of the structure (Fig. 13) reveals a circular depression with a raised central portion. Thus, they appear to meet the description of plasmodesmata (Cronshaw 1965) with the raised central portion corresponding to the central electron opaque core.

The aspiration of pit membranes throughout much of the heartwood zone in the living tree cancelled the major attribute of solvent-exchange drying. A study of heartwood specimens which were either air-dried, solvent-dried, or freeze-dried revealed only aspirated pits. Some variation in the degree of aspiration was encountered.

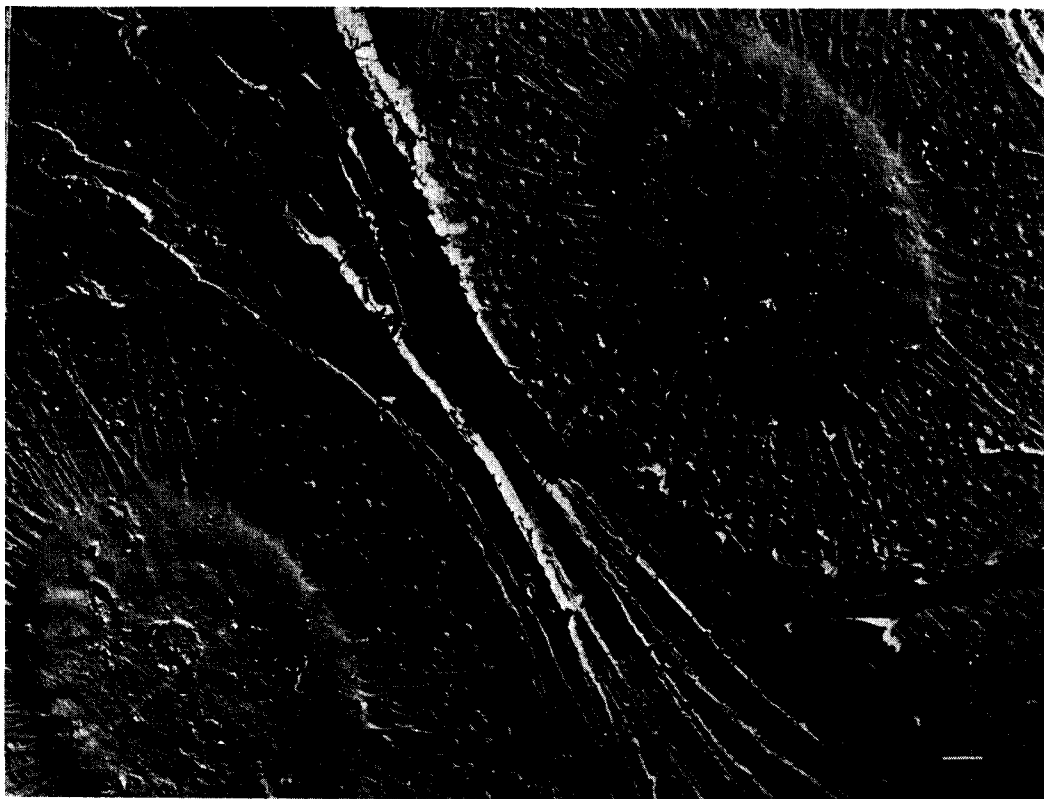


FIG. 10. Aspirated, springwood bordered pit membranes from solvent-dried, outer heartwood of longleaf pine.

For example, throughout the discolored heartwood zone extremely tight pit aspiration occurred, as indicated by the fact that the outline of the pit aperture can be detected through the torus (Fig. 9).

A transition zone of five to ten growth rings exists to the outside of the region of tight pit aspiration, within which not all pits are in the aspirated condition. Although some tracheids showed aspiration of every pit, other tracheids revealed all pits in the non-aspirated condition. In addition, aspirated and non-aspirated pits were observed within a single tracheid. Also, the pit membranes were not as tightly aspirated (Fig. 10) as the pits from the discolored heartwood region. The condition of loose aspiration, as opposed to the tight aspiration, was typical of the aspirated pits in the transition zone. It was also noted earlier in the study that the bordered pits of the

outer sapwood region aspirated in the same loose manner when air-dried from water.

The second most noticeable structural change of the pit membrane, as reflected by age, was the tendency for the number of margo microfibrils to decrease toward the center of the tree. Fig. 11 reveals the typical membrane encountered in the transition zone of longleaf and pond pine. Note the very definite decrease in the margo microfibril network. In order to ascertain whether or not margo density varies according to the age of the tree at the time of pit formation, a study of several trees from younger age classes (10 to 27 years) was undertaken. The results showed essentially the same margo density variation within the outermost solvent-dried ten to fifteen growth rings as existed in the counterpart outer sapwood zone of 60-year-old trees. Thus, the age of the tree at the time of pit

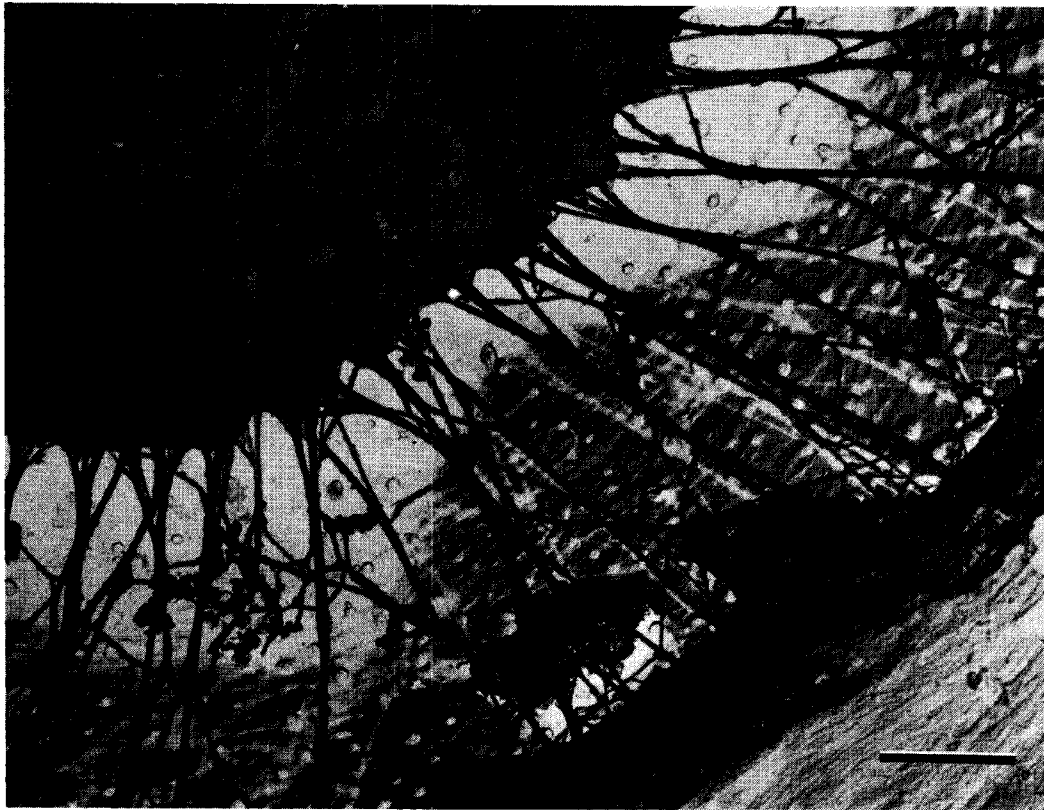


FIG. 11. A portion of a non-aspirated springwood bordered pit membrane from the 20th ring of a 60-year-old solvent-dried longleaf pine.

formation cannot account for the change in the margo. Frey-Wyssling and co-workers (1959) have reported a qualitative decrease in the number of microfibril strands and increase in the thickness of the remaining fibrils with age because of aggregation of smaller microfibrils. Though the decrease in the number of randomly-oriented microfibrils was noted in this study, no detectable increase in the size of the large radially-oriented strands was found (compare Fig. 11 with Figs. 3 through 6). Consequently, a definite statement about the reason for the apparent decrease in margo microfibrils with age cannot be made. But, since the decrease does not appear to be due to aggregation of smaller microfibrils or the age of wood at the time of pit formation, it can be speculated that enzyme activity persists deep in the xylem region and may be responsible for the decrease in margo

microfibrils. Although a few pit membranes with a very high density (Fig. 12) were detected inside of the outer sapwood zone, most membranes of this type were located in the outermost ring, just inside of the differentiating zone (Fig. 7). Both Fengel (1966) and Thomas (1967, 1968) have shown that the differentiating membrane is imperforate with the membrane structure apparently formed within a matrix substance. Removal of the matrix substance reveals a membrane with a perforated margo of variable density. One can surmise that the mechanism responsible for the removal of the smaller, randomly-oriented microfibrils proceeds at varying rates in different pits, thus accounting for the margo density variation. The location of a high density margo in the innermost ring of a 10-year-old longleaf pine (Fig. 12) indicates a failure of the removal mech-

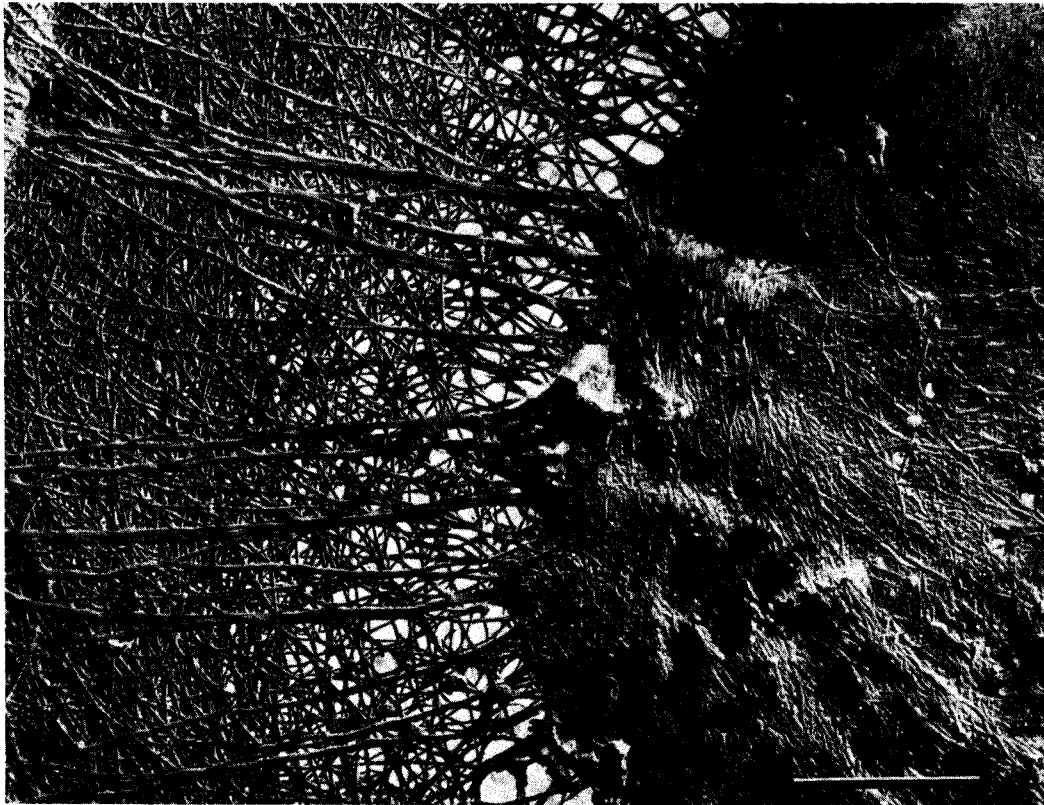


FIG. 12. A portion of a non-aspirated springwood bordered pit membrane from the innermost ring of a 10-year-old solvent-dried longleaf pine.

anism. Since adjacent pit membranes had the very low margo density characteristic of older wood, the failure was an isolated occurrence. In general, the transformation of the springwood pit membrane from a very high margo microfibril density (Figs. 6, 7, and 12) to a medium density with considerable variation (Figs. 1 through 5) appears to occur within one year, while the change to a low density margo microfibril network (Fig. 11) is a much slower process.

#### CONCLUSIONS

The following conclusions apply to the longitudinal tracheid bordered pit membranes of southern pine species:

1) The bordered pit membrane consists of a perforated margo with variable density and an imperforated, centrally located, thickened torus. The density variation ap-

pears to be controlled by the number of randomly-oriented, small diameter microfibrils present throughout the margo. A definite circular orientation of microfibrils around the periphery of the torus was noted.

2) Incrustations capable of altering margo porosity were not found on springwood pit membranes throughout the sapwood region. Heavy incrustations were noted on summerwood pit membranes regardless of location.

3) Aspiration of the pit membranes in the heartwood region occurred in the standing tree. Extremely tight aspiration was noted throughout the discolored heartwood zone.

4) A definite decrease in the number of margo microfibrils with increasing age of the wood was detected. The study of trees from several age classes showed the dete-

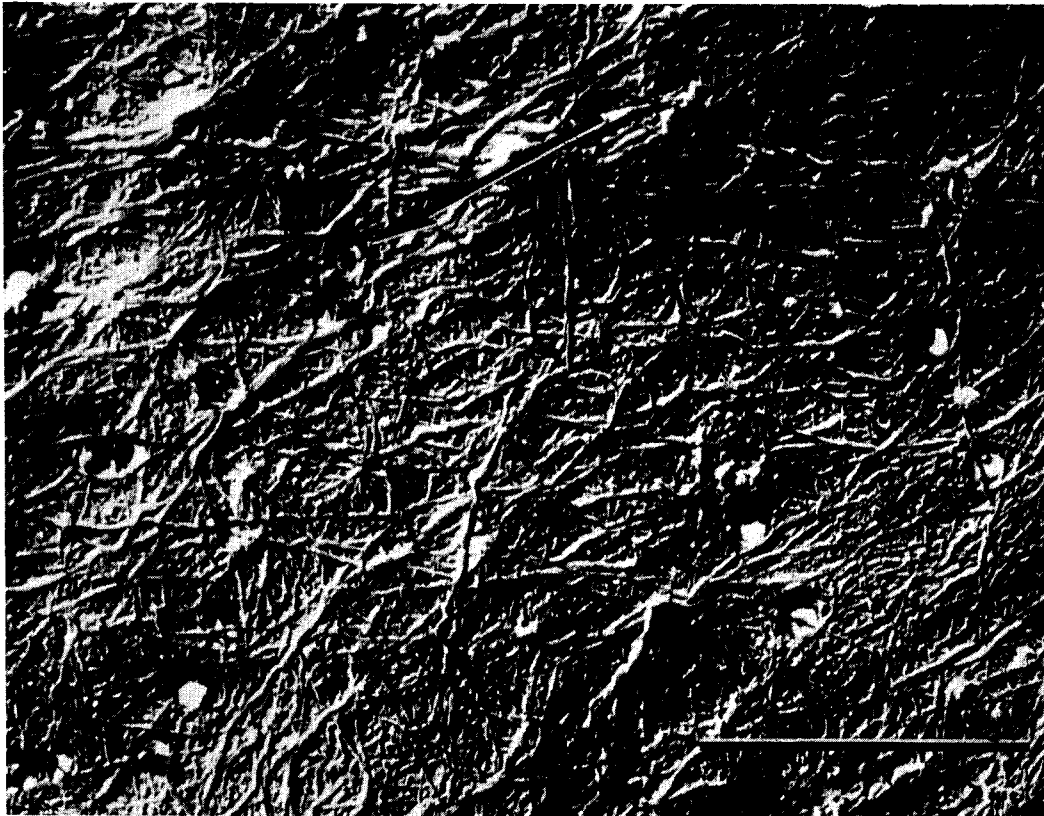


FIG. 13. A portion of a longleaf pine torus showing what appears to be the remains of plasmalesmata-like structures. Arrow indicates one such structure.

rioration of the margo to be related to the age of the wood rather than the time of wood formation.

5) The use of solvent-exchange, critical-point, and freeze-drying techniques indicated that the surface tension of the final liquid is important only with regard to pit aspiration and does not alter margo structure to any noticeable degree.

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KORECKÝ, A., and L. ČIŽEK. 1968. Construction of demountable buildings in the Research and Development Institute for Wood Industry (VVUD) in Prague. *Holztechnologie* 9(4): 219-220. Easily demountable wood buildings for various uses are under development. For economy, dimensions and construction of these buildings were standardized. Style of construction and materials can be varied. For poultry farms, glued truss girders proved very satisfactory. (RE)

KÜNZELMANN, E., and R. WAGENFÜHR. 1968. Observations of lacquer surfaces by means of light-optical and electron-microscope. *Holztechnologie* 9(4): 221-224. After various finishing-stages, the surface of a polyester lacquer was observed light-optical microscopically in the vertical bright field illumination and electron microscopically in the secondary electron emission microscope as well as by the transmission method. The testing methods used are described and the test results compared. (A)

KIRK, H. and G. SCHULTZE-DEWITZ. 1968. Elasticity and impact strength as well as their indicating values for the effectiveness of wood preservatives against ascomycetes, fungi imperfecti, and basidiomycetes. *Holztechnologie* 9(4): 249-254. In order to shorten the period of wood preservative tests, the influence of lower and higher wood-destroying fungi on elasticity and impact strength was examined and compared with other investigations. Impact strength has a higher index value than modulus of elasticity. A new determination of threshold values may be required. (A)

ZEPPENFELD, G. and H. DAMME. 1968. Characterizing wood fiber materials by dye-stuff absorption. *Holztechnologie* 9(4): 255-256. A dyeing method based on absorptive dyeing with Congo red in aqueous solution and made for the purpose of characterizing wood fiber materials for fiberboards is described. Details are given on the relation between swelling behavior of the finished board and dye absorption of the wood fiber. (A)