ULTRASTRUCTURAL OBSERVATIONS OF PARENCHYMA AND SCLEREIDS IN DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII* (MIRB.) FRANCO) BARK[1]

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(Received December 1982)

ABSTRACT

Pitting that occurs in sclereids and parenchyma cells of Douglas-fir bark was examined, along with differences in structure of cell walls in parenchyma from inner and outer bark. Axial parenchyma is connected by pit areas that appear as a slightly thinner region in the adjacent walls where plasmodesmata are found. Sclereid-to-parenchyma pitting consists of a long pit canal through the sclereid wall about 1 μm in diameter with little or no evidence of a pit chamber. The pit membrane is the original double cell wall of the parenchyma as found in parenchyma-to-parenchyma pitting. Pitting between two sclereids exhibits similar long pit canals through the sclereid walls, but a pit chamber is present with a thick membrane likewise consisting of the double cell wall of the parenchyma. During sclereid formation the secondary wall of the sclereid appears to be deposited by apposition on the original parenchyma cell wall. Parenchyma cells in inner bark have small wrinkles and folds in the cell wall, whereas enlarged parenchyma in outer bark have very smooth walls.

Keywords: Bark, Douglas-fir, anatomy, ultrastructure.

INTRODUCTION

Existing anatomical research on the woody or xylem portion of the tree stem has led to increased utilization of this raw material. If wood technologists are to utilize bark fully, further studies of its structure become more important. Therefore, the objective of this paper is to extend the basic knowledge of Douglas-fir bark by describing and illustrating: (1) ultrastructural characteristics of parenchyma cells present in inner bark, (2) changes that occur in cells of parenchyma of outer bark, and (3) pitting between sclereids and adjacent cells.

Although the study of bark is not so complete as that of wood, many morphological and some ultrastructural characteristics of Douglas-fir bark have been

[1] This is paper 1725 of the Forest Research Laboratory, College of Forestry, Oregon State University, Corvallis, OR 97331. The research was conducted while the senior author was a Graduate Research Assistant at Oregon State University and is part of his Ph.D. dissertation. It was presented at the Biology Technical Session of the 35th annual meeting of the Forest Products Research Society, St. Paul, MN, June 1981. Support for this research was provided through the Forest Research Laboratory and a doctoral fellowship sponsored by Weyerhaeuser Company.

reported (Chang 1954; Grillos and Smith 1959; Den Outer 1967; Ross and Krahmer 1971; Patel 1975; Yie-Hsiang 1975; Litvay and Krahmer 1977; Dougal 1981). Bark is a major component of Douglas-fir trees, ranging in thickness from 1 to 2 inches in thin-barked trees to 1 to 2 feet in older trees (Hall 1971).

The inner bark of Douglas-fir includes sieve cells, axial parenchyma cells, ray parenchyma cells and sclereids (Chang 1954; Grillos and Smith 1959; Ross and Krahmer 1971). Axial parenchyma cells are about 0.1 mm in length and are responsible for food storage. In the outer part of the inner bark, the axial parenchyma cells may act in one of three ways: (1) they may remain inactive; (2) they may begin to enlarge; or (3) they may differentiate into sclereids (Grillos and Smith 1959; Yie-Hsiang 1975). In the outer bark or rhytidome, parenchyma cells become enlarged and the resulting expansion crushes the sieve cells. This occurrence of enlarged phloem parenchyma accompanied by an increased accumulation of tannins with outer bark formation has been observed in many species. Patel (1975) added that lignification of sieve cells and axial parenchyma (those without crystals) occurs in the outer bark.

In Douglas-fir some axial parenchyma in the inner bark will differentiate into sclereids, generally about a year after the parenchyma is formed from the vascular cambium. Douglas-fir sclereids are about 1 mm in length and 50 μm in diameter (Ross and Krahmer 1971; Yie-Hsiang 1975). Sclereids have thick cell walls with distinct lamellae (Mia 1969; Nanko et al. 1978; Nanko and Côté 1980) and have been isolated for use in bark composition boards (Wellons and Krahmer 1973) and as a reinforcing agent in plastics (Miller et al. 1974).

PROCEDURES

A Philips Model EM-300 transmission electron microscope (TEM) and an AMR 1000A scanning electron microscope were used for ultrastructural observations.

In order to embed bark successfully for TEM, it was necessary to extract 1-cm^2 sections 40–100 μm in thickness with three different solvents. This process removed bark extractives that would interfere with the polymerization of the embedding medium. The first five extraction steps in Fig. 1 represent the sequence used on the sections.

Following solvent extraction of these large microtome sections, approximately 1-mm^2 areas were selected and excised. These were stained for 6 hours in a 0.5% solution of potassium permanganate, rinsed in distilled water, and then boiled in water for 6 hours as outlined in Fig. 1. Stained pieces were dehydrated and placed in Spurr's (1969) low-viscosity embedding medium for ultramicrotomy.

Surfaces of parenchyma cell walls were examined for ultrastructural detail by using both the direct carbon replica technique reported by Côté et al. (1964) for TEM and direct observation by SEM. Solvent-extracted sections, as described above for embedding, were freeze-dried and then replicated or mounted on stubs and coated with a 60/40 gold/platinum metal alloy for observation with SEM.

A detailed procedure was used to isolate, characterize and illustrate the structure of pitting in Douglas-fir sclereids with SEM. This pitting occurs only in the central portion of the sclereid where it differentiates from a parenchyma cell, and pits are not oriented with reference to any particular surface, i.e., radial or tangential.

To find sclereids with pits in proper orientation, sections of inner bark, 20–60 μm in thickness, were cut on a sliding microtome, solvent-extracted according to

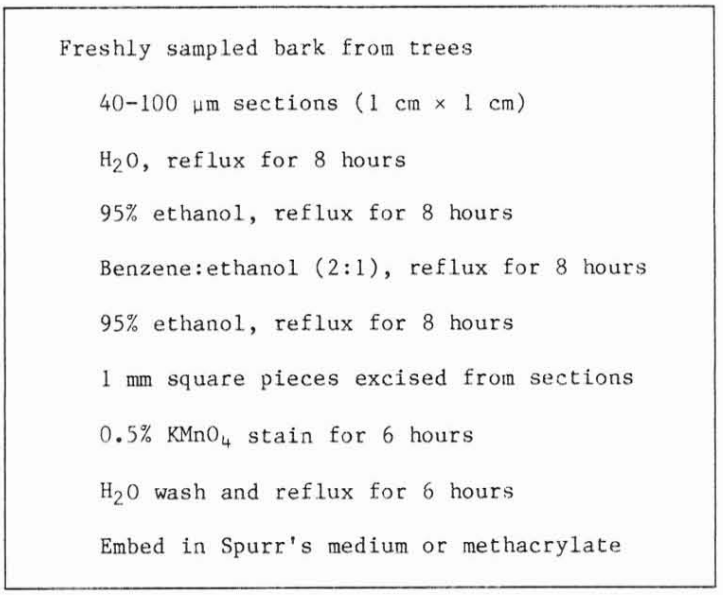

FIG. 1. Solvent extraction and staining sequence for microtomy of Douglas-fir bark.

the sequence in Fig. 1, and stained with phloroglucinol-HCl. The sclereid cell wall stained a pink-to-reddish color because of the presence of lignin. The contrast provided by this staining allowed many sections to be examined efficiently for properly oriented sclereids. Relatively few sclereids in microtomed sections had both the area representing the original parenchyma cell and the pit canals in proper orientation for further examination. One of these sclereids was found for about every five hundred examined. Figure 2 is a light micrograph of one of these tangential-longitudinal sections of inner bark. Pit canals extend from the sclereid lumen out to the adjacent parenchyma cell. Because these pit canals may be located within the section, and not at the surface for observation with SEM, the following procedure was used to locate the pit canals on the surface.

Thick sections were stained with acridine orange to enhance the fluorescence of the tissue surfaces for observation later with incident fluorescence microscopy. Then a 1-mm^2 area, which included the single sclereid with pitting and a few surrounding cells, was excised from the thick section. This small piece was dehydrated and embedded in methacrylate using standard procedures (Pease 1960; Berlyn 1963). Throughout the embedding process, care was taken to maintain the integrity and proper orientation of the sclereid.

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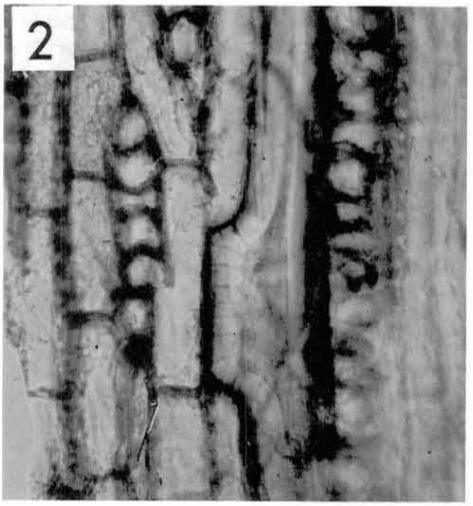
FIG. 2. Sclereid-to-parenchyma pitting in Douglas-fir bark. The pits occur only in the area of the original axial parenchyma cell from which the sclereid differentiated. Phase contrast: 200×.

FIG. 3. Plasmodesmata canals in a pit field of two adjacent axial parenchyma cells. TEM: 10,300×.

FIGS. 4 and 5. Pitting, appearing as thin areas in the wall between parenchyma cells in Douglas-fir bark. TEM: 400× and 11,800×.

FIG. 6. Direct carbon replica of a pit area in axial parenchyma. TEM: 3,300×.

FIG. 7. Scanning electron micrograph of three pit areas in the wall of a parenchyma cell. 4,500×.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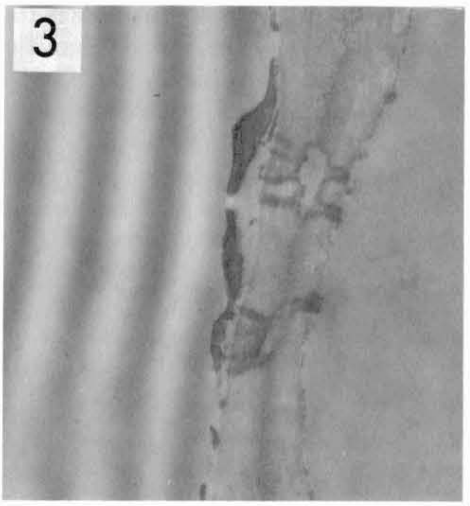
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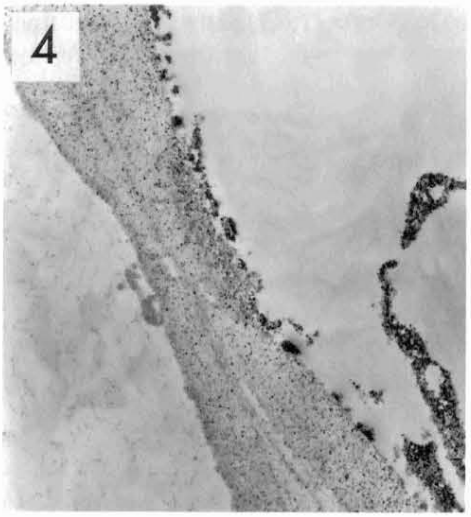
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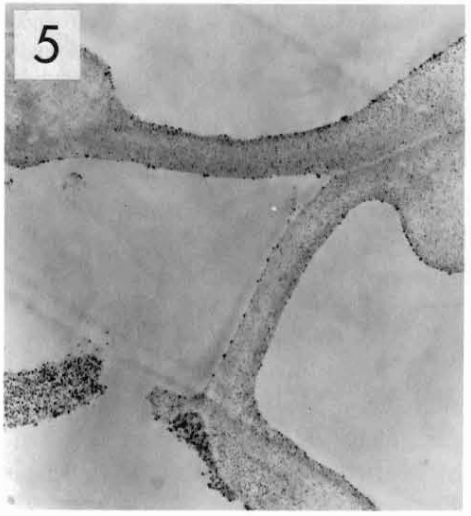
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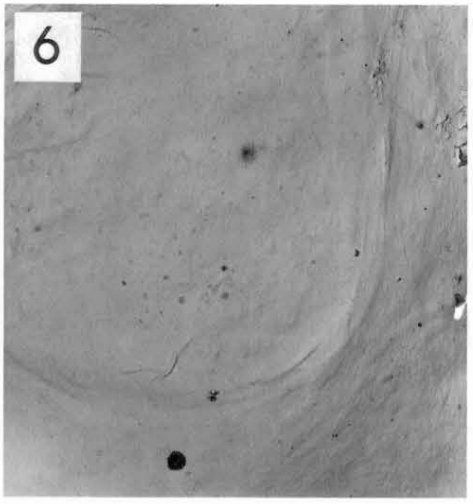
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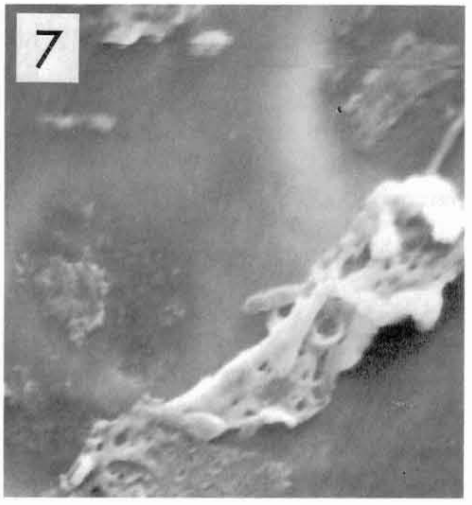
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Ultrathin sections approximately 0.05 μm thick were cut from the embedded bark. After about 20 sections were cut, the entire block was removed from the ultramicrotome and observed with an incident fluorescence microscope to determine if the pit canals appeared at the surface. If not, the block was returned to the microtome and the procedure was repeated until pit canals were exposed on the surface. Where possible, some ultrathin sections were picked up on grids for observation with TEM. Unfortunately, these thin sections of thick-walled sclereids were usually badly torn, wrinkled, and otherwise damaged.

Once the sclereid pit canals appeared to be exposed on the microtomed surface of the methacrylate-embedded bark, the tip of the block was cut off with a razor blade and placed in a small beaker of chloroform to dissolve the methacrylate from the bark. This small piece of bark was carefully oriented on a SEM stub, air-dried, and then coated with gold/platinum for observation.

OBSERVATIONS AND DISCUSSION

Pitting in parenchyma cell walls

Detailed drawings of Douglas-fir bark by Den Outer (1967) show simple pitting between longitudinal parenchyma in phloem to be similar to pitting between ray parenchyma in xylem; however, ultrastructural detail was not illustrated. Ultrastructural observations of secondary phloem in *Pinus strobus* by Murmanis and Evert (1967) showed pitting between parenchyma as thin areas in the adjacent walls traversed by plasmodesmata.

Figure 3 is a transmission electron micrograph of an ultrathin section of the end wall between two longitudinal parenchyma cells located near the vascular cambium in the inner bark of Douglas-fir. Four plasmodesmata connections are visible crossing the walls common to the two cells. In young and developing plant cells, the plasmodesmata are the first form of communication between two cells (Esau 1969), forming the primary pit field. Where plasmodesmata cross thin pit areas, there appear to be large openings in the middle lamella region of the pit pair. These are similar to nodules on the plasmodesmata in white pine as reported by Murmanis and Evert (1967). Also, lying on the lumen surface of the parenchyma cell wall is a deposition of dark material, possibly unremoved extractive.

In longitudinal parenchyma cells located further from the cambium, the plasmodesmata are no longer visible. Only thin pit areas remain, as shown in Figs. 4 and 5.

Parenchyma cell walls were examined in surface view using direct carbon replicas to determine if thin pit areas were visible. In Fig. 6, the circular depression is interpreted to be a thin pit area viewed from the lumen side of one of the parenchyma cells. Encrustations mask any orientation of the cellulose microfibrils

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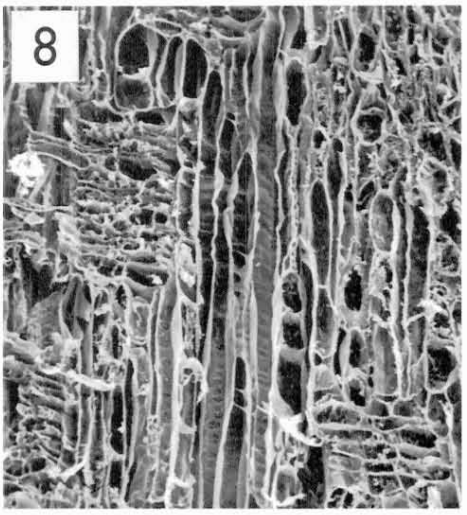
FIGS. 8 and 9. Radial surfaces of inner bark and outer bark, respectively. In the outer bark, the ray parenchyma and axial parenchyma are enlarged, crushing the sieve cells. SEM: 90×.

FIGS. 10 and 11. Tangential sections of inner bark and outer bark, respectively. Fusiform ray tissue and axial parenchyma are enlarged in the outer bark. 50×.

FIGS. 12 and 13. Scanning electron micrographs of the inner cell-wall surface of parenchyma cells from inner bark and outer bark, respectively. Small wrinkles and folds in the wall are present in the inner bark cells, but have disappeared in enlarged cells in the outer bark. 4,600× and 1,600×.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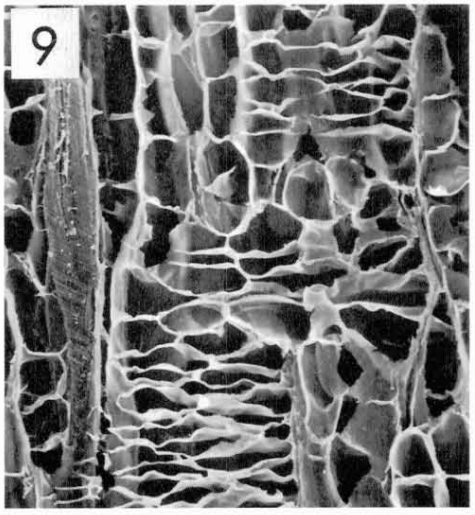
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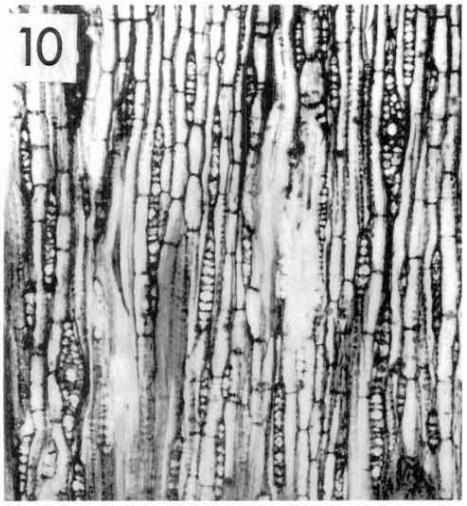
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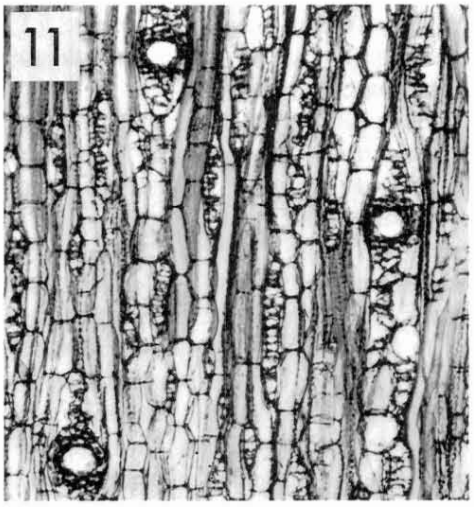
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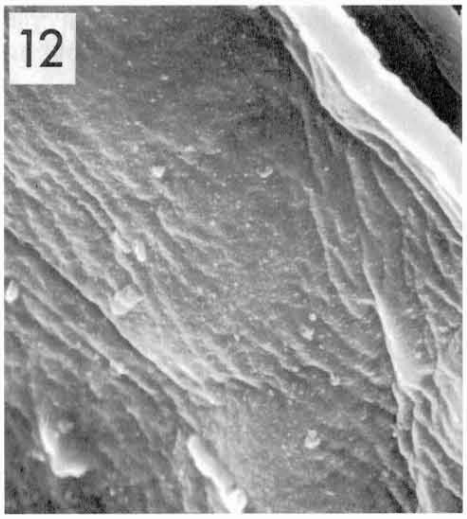
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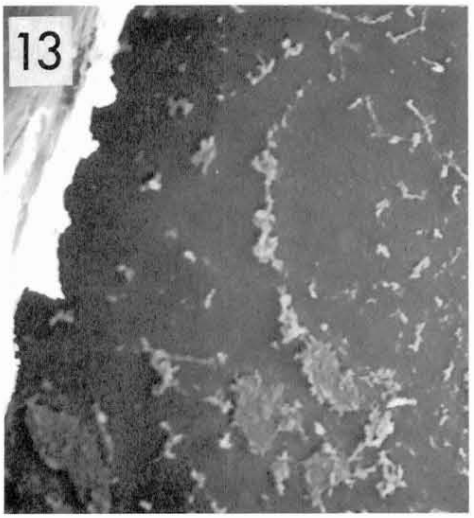
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in the membrane, but some orientation does appear in the cell wall adjacent to the membrane.

A scanning electron micrograph of a parenchyma cell wall is shown in Fig. 7. The three depressions are thin pit areas in the parenchyma cell wall. Because these depressions do not have well-defined boundaries, they are not easily found with SEM.

Enlarged phloem parenchyma

Douglas-fir longitudinal and ray parenchyma cells are enlarged in the outer bark. Some enlargement is observed frequently in the outer part of the inner bark. Figure 8 is a scanning electron micrograph of the inner bark adjacent to the last-formed cork layer. The appearance of ray tissue, sieve cells, and longitudinal parenchyma is typical for inner bark. Figure 9 is a scanning electron micrograph (at the same magnification) of outer bark tissue adjacent to the last-formed cork layer. The ray and longitudinal parenchyma have enlarged, crushing the sieve cells, which are no longer visible.

Enlargement was observed also in the fusiform rays. Figures 10 and 11 are light micrographs of tangential sections of inner and outer bark, respectively. In the outer bark, the fusiform rays have enlarged along with the surrounding longitudinal parenchyma.

Longitudinal and ray parenchyma cells were examined to determine what ultrastructural changes could be observed as a result of their enlargement in the outer bark. In inner bark, both longitudinal and ray parenchyma cell walls had rows of wrinkles oriented in the direction of the cell axis (Fig. 12). This wrinkled state was especially noticeable in the cell corners of parenchyma cells. After cell enlargement, the wrinkles, folds, and ridges had disappeared from the parenchyma walls, leaving a smooth inner surface (Fig. 13).

In a wide range of dry seeds observed with SEM by Webb and Arnott (1982), cell walls of internal tissues exhibited a unique collapsed structure ranging from a highly regular folding pattern in some species to random wrinkling in others. Their evidence suggests that a regular pattern of wall folding may result from a mechanism located in the cell wall and that this pattern is essential for preserving the structural integrity of the tissue during desiccation. We originally hypothesized that a stretching or shifting of cellulose microfibrils might accompany parenchyma enlargement in Douglas-fir and could be observed as a change in microfibril

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FIG. 14. Pit membranes of sclereid-to-parenchyma pitting, viewed from the parenchyma cell lumen. The membranes were probably ruptured in the electron beam of the microscope. SEM: 4,700×.

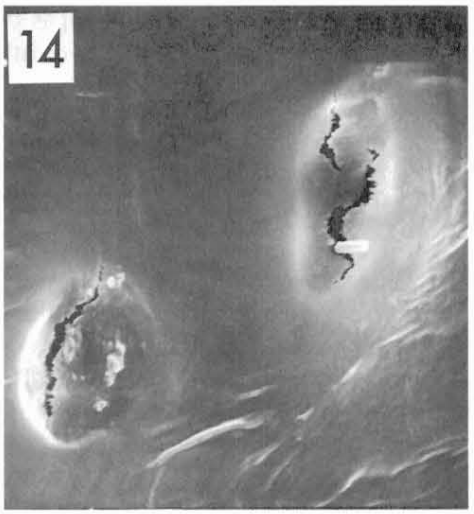
FIG. 15. Pit aperture in a Douglas-fir sclereid (arrow). SEM: 2,500×.

FIG. 16. Ultramicrotomed, tangential surface of bark, exposing sclereid-to-parenchyma pitting (arrow). Compare with Fig. 2. SEM: 670×.

FIG. 17. Enlargement of pit in Fig. 16, illustrating detail of the pit canal in the sclereid and the membrane region at the sclereid/parenchyma boundary. SEM: 9,000×.

FIG. 18. Ultrathin section of sclereid-to-parenchyma pitting, showing the pit membrane (arrow) as a thinning of the original adjacent parenchyma cell walls (P), on which secondary wall thickening of the sclereid (S) has formed a pit chamber occluded with extraneous material. TEM: 7,700×.

FIG. 19. Ultramicrotomed surface of sclereid-to-sclereid pitting in Douglas-fir. A pit canal, chamber and membrane are apparent (arrow). SEM: 600×.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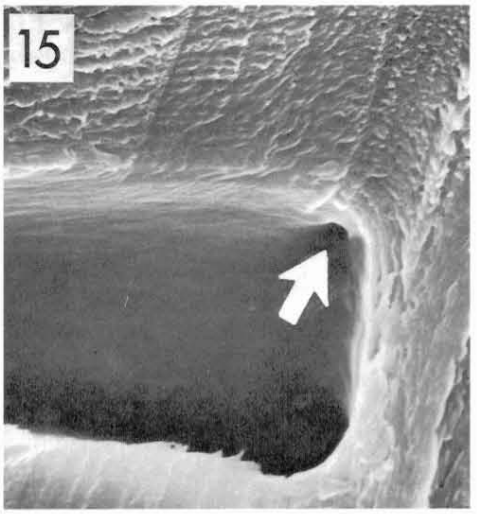
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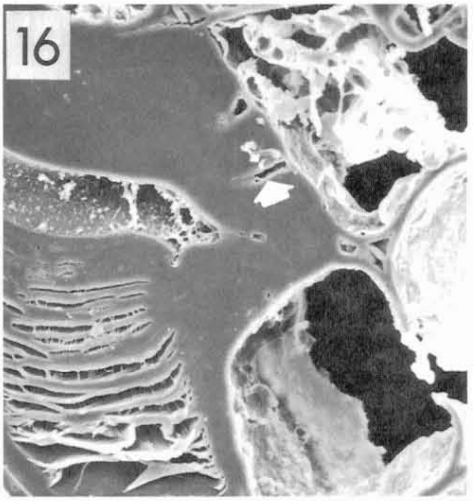
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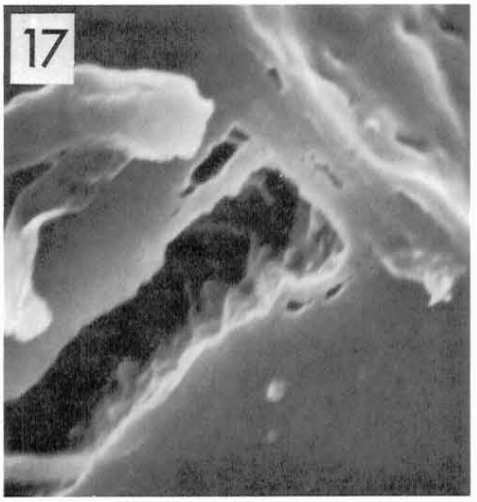
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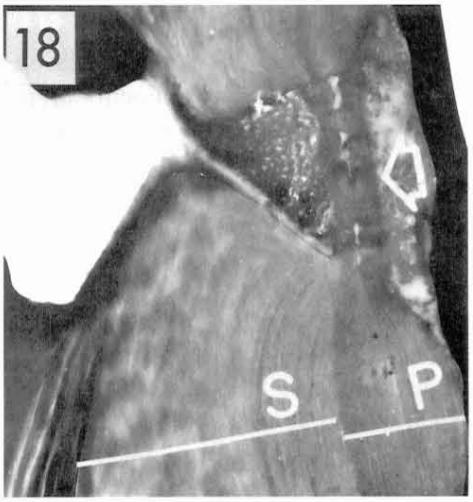
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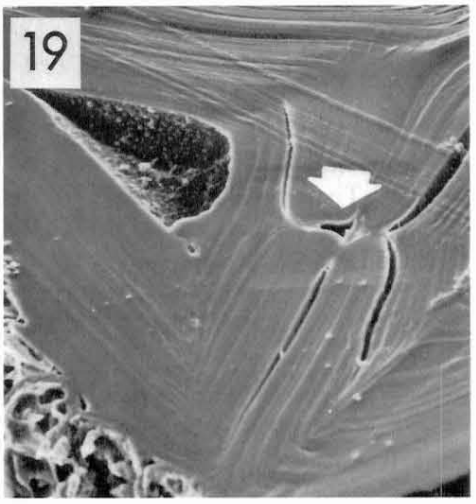
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orientation in the cell-wall layers. However, because sufficiently clean surfaces and appropriate fractures could not be obtained, changes in the orientation of wall components were not studied. A number of phenomena undoubtedly occur during the enlargement of these parenchyma cells.

Fit structure between sclereids and parenchyma

Sclereids grow intrusively in the longitudinal direction during cell elongation; in those parts of the sclereid that grow intrusively, no pitting has been reported. Only where the original parenchyma cell has existed, that is, where the original plasmodesmatal connections were found, would complete pits be expected to form.

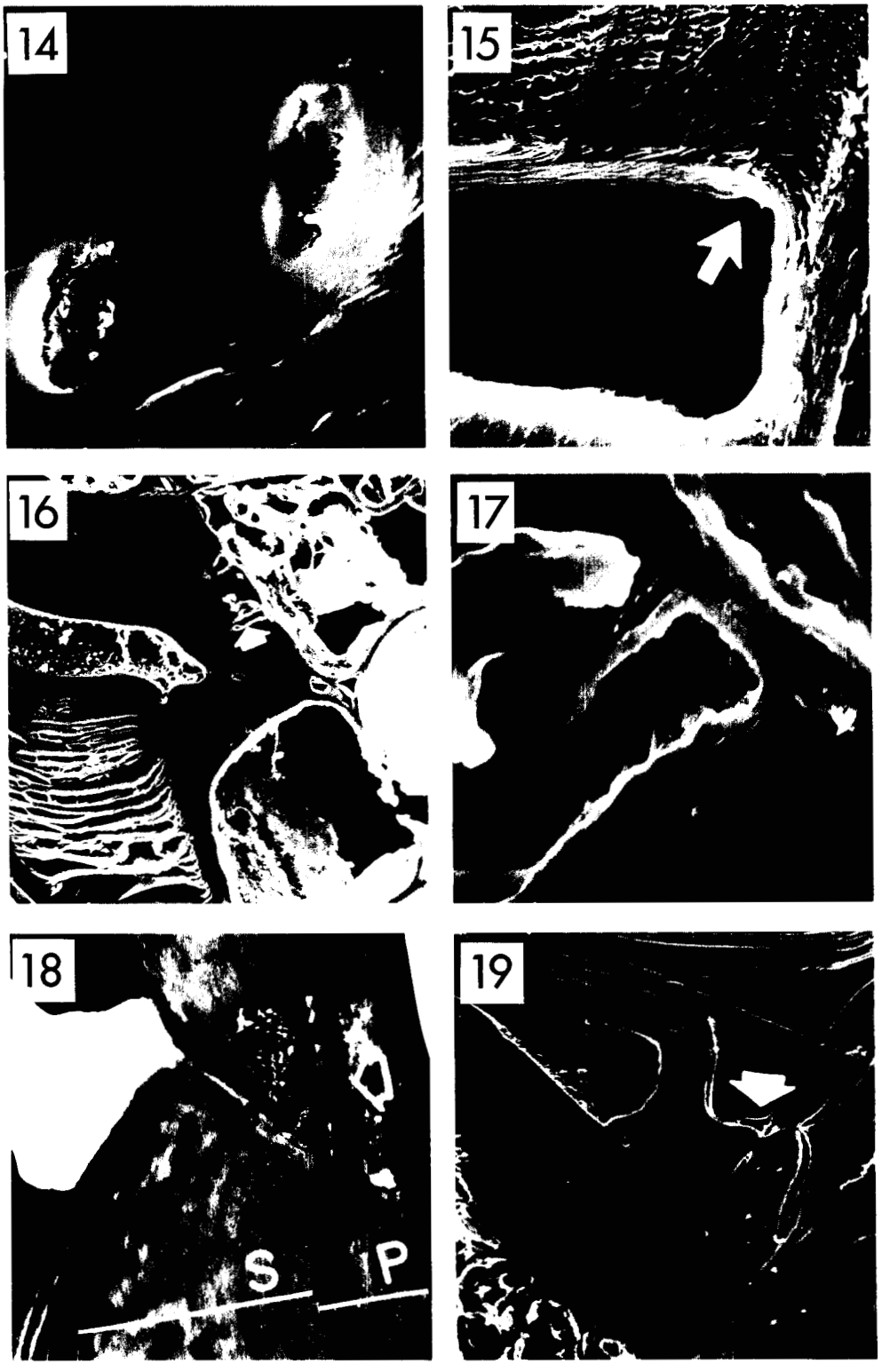
Lumen surfaces of parenchyma walls were examined with SEM, and sclereid-to-parenchyma pitting appeared as shown in Fig. 14. Pit membranes had diameters of 1 to 3 μm. At the lumen surface of the sclereid, the pit aperture leading to the pit canal appeared as a round hole about 1 μm in diameter (Fig. 15). Goldschmid and Folsom (1975) used SEM to show similar small holes, which they called simple pits, on the outer surface of hemlock sclereids. Bramhill and Kellogg (1979) also used SEM to show holes in hemlock sclereid walls; these holes were sometimes plugged with resinous material.

Nanko et al. (1978) studied the cell-wall structure of sclereids in the secondary phloem of *Populus euramericana* Guinier. They observed simple pits with canal-like cavities in the secondary wall of the sclereids and thought that microfibrils were oriented along the canal direction in the pit.

A clean, ultramicrotomed surface of Douglas-fir inner bark containing a sclereid is illustrated in Fig. 16. This scanning electron micrograph shows the portion of the thick-wall sclereid that was the original parenchyma cell from which it developed. The sclereid lumen is visible. Portions of several pit canals have been exposed. None of the pit canals extends the entire distance from the sclereid lumen to the parenchyma cell next to it because none was parallel to the cut surface of the sclereid.

Figure 17 shows one pit area from Fig. 16 at higher magnification. The pit canal is 1 or 2 μm in diameter and appears to broaden slightly at the outer surface of the sclereid. The membrane between the sclereid pit canal and the lumen of the adjacent parenchyma is not thin as are those normally associated with pitting in xylem. Instead, the parenchyma cell wall itself appears to be the so-called pit membrane. This structure is consistent with the pit structure of phloem parenchyma as discussed earlier. In the area of the plasmodesmata, which is the primary pit field, no apparent changes take place in the pit membrane with sclereid formation. The small holes in the center of the membrane region in Fig. 17 might, in fact, be the small openings resulting from plasmodesmata nodules in the middle lamella region as shown in Fig. 3.

Figure 18 is an ultrathin section showing the pitting from a sclereid to an adjacent parenchyma cell. The original parenchyma cell wall and a thin pit area with small openings in the middle lamella region are visible. Deposited onto this original parenchyma cell wall are the additional secondary wall layers of the sclereid, forming a pit chamber area that is occluded with extraneous material. The outermost layer of this sclereid cell wall appears to be the original parenchyma cell wall. Nanko et al. (1978) and Mia (1969), in describing the secondary wall structure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of fully differentiated sclereids in hardwoods, noted that the outermost layer of the secondary wall had a different structure from the inner layers of alternating bands of light and dark lamellae. This outermost layer that they described is probably the original parenchyma cell wall.

Pitting between two sclereids

If two adjacent parenchyma cells were to differentiate into sclereids simultaneously, intersclereid pitting might develop. This pit formation could follow a pattern similar to that of pitting in other thick-walled cells. In a review article on wood anatomy, Côté and deZeeuw (1962) showed an electron micrograph of adjacent thick-walled fibers in bamboo. The pit-pair connecting them has a long pit canal leading into a small chamber, which gives it the appearance of a bordered pit-pair. A relatively thick membrane separates the two pits.

Sclereids in Douglas-fir appear to differentiate in isolation from one another most of the time, and only one pair of adjacent sclereids having intersclereid pitting was found in this study (Fig. 19). Both sclereids were distorted in shape, possibly because of extreme restrictions to elongation, and, therefore, their structure is even more complex. A pit chamber and canal are exposed in one of the sclereids, and there is also evidence of a pit chamber in the adjacent sclereid, with what appears to be a membrane between the two pits.

CONCLUSIONS

1. Pitting between parenchyma in Douglas-fir creates shallow, circular depressions in the cell wall; these depressions occur in the vicinity of the plasmodesmata connections. A slightly thinner, double cell wall, therefore, appears as the pit membrane.

2. During differentiation of the sclereids from parenchyma cells in the inner bark, long pit canals are formed with secondary cell-wall thickening in areas of plasmodesmata (thin pit areas) in the original parenchyma wall. A sclereid pit, therefore, consists of a long pit canal extending through the secondary wall to a relatively thick membrane that appears to be the same as in the original parenchyma-to-parenchyma pitting. In parenchyma-to-sclereid pitting, distinct pit chambers are not evident in the sclereid, but in sclereid-to-sclereid pitting, a pit chamber does exist.

3. Cell walls of parenchyma in the inner bark have many small wrinkles and folds in them. In the outer bark where parenchyma cells are enlarged, cell walls appear smooth.

4. An outer layer of a sclereid cell wall in the pitted zone is the original wall of the parenchyma cell from which it differentiated.

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