RAY PARENCHYMA CELL-WALL ULTRASTRUCTURE AND FORMATION IN *PINUS BANKSIANA*

P. T. Mann

State University College of Environmental Science and Forestry, Syracuse, N. Y. 13210[1,2]

(Received 15 June 1973)

ABSTRACT

Ray parenchyma cells in seedlings of *Pinus banksiana* formed an unlignified multilayered primary cell wall during cell expansion. Some cells expanded radially four or five times the size of the ray initial. Evidence indicated that cell-wall extension occurred along the entire cell wall. After cell expansion, a secondary cell wall was rarely formed. Consequently, the ray crossing pits were considered to be blind pits.

Additional keywords: wood anatomy, ontogeny, electron microscopy.

INTRODUCTION

In the literature there are conflicting views about the nature of ray parenchyma cell walls in the wood of hard pines. Penhallow (1907) stated that 95% of the genera of North American conifers had ray parenchyma cell walls with secondary thickenings. However, ray parenchyma cells of the genus *Pinus* was an exception to which the terms "thick-walled" and "thin-walled" were applied. Bailey (1909), in describing the wood structure of Pineae, did not mention any variation in the ray parenchyma cell walls of hard pines. Bannan (1934) discussed variations of ray parenchyma cell-wall structure of *Pinus strobus*; however, he considered the rays of hard pines as having either typically tracheary or parenchymatous cells.

Bailey and Faull (1934) described the ray parenchyma cells of the wood of Taxodiaceae, Araucariaceae, Taxaceae, Podocarpaceae, Cupressaceae, and Cephalotaxaceae as having primary cell walls. They stated that only ray parenchyma cells of the subfamily Abietoideae of Pinaceae possessed secondary cell walls. Unfortunately, the structure of ray parenchyma cells of the subfamily Pinoideae of Pinaceae, which contains the genus *Pinus*, was not discussed.

Harada (1964) disagreed with Bailey and Faull that the ray parenchyma cells of Taxodiaceae have thickened primary cell walls. He described the ray parenchyma cell wall of *Cryptomeria japonica* as secondary from information obtained from ultrathin sections observed with an electron microscope. The ray parenchyma cell portion of ray crossing pits of *C. japonica* was blind. This "blind pitting" in ray parenchyma cells was also observed by Krahmer and Côté (1963) in the sapwood of *Thuja plicata* of Cupressaceae.

Balatinecz and Kennedy (1967) observed a delay in the formation of ray parenchyma cell walls of hard pines (Pinaster-Lariciones section). They examined disks from the cambium to the heartwood that had been collected during the winter from plantation-grown trees. Ray parenchyma cells near the cambium were thin-walled and were not lignified. However, near the heartwood-sapwood boundary, an abrupt increase occurred in the number of lignified ray parenchyma cells. These cells had smooth to "knoblike" cell-wall thickenings, which complemented the dentations of the ray

[1] Present Address: J. R. Simplot Company, Research Department, P. O. Box 1059, Caldwell, Idaho 83605

[2] This study is part of a Ph.D. dissertation supported by the Wood Products Engineering Department, for which I am grateful. I am indebted to Dr. W. A. Côté, Jr., for his guidance and review during this investigation. I wish to thank Dr. D. I. A. Goring, Research Director of the Pulp and Paper Institute of Canada, for permission to use his quartz microscope and Mr. A. C. Day, Mr. J. McKeon, and Mr. Y. Musha for their technical assistance. Also, I wish to thank Dr. R. A. Parham for taking the scanning electron micrographs.

tracheids and were devoid of a protoplast. Panshin et al. (1964) described the ray parenchyma cells of hard pines as thin-walled. Howard and Manwiller (1969) described ray parenchyma cells in the wood of southern pines as mostly thin-walled and unpitted. Pitted thick-walled cells apeared to be lignified and were distributed in a pattern reported by Balatinecz and Kennedy (1967). Mirov (1967) cited the variation in ray parenchyma cell-wall pitting of *Pinus* as a reflection of the "various stages of disappearance of the secondary walls in the ray parenchyma cells."

In an extensive study of the ultrastructure of southern yellow pines, Côté and Day (1969) described ray parenchyma cells as having both primary and secondary cell walls. Primary cell walls possessed randomly oriented microfibrils and secondary cell walls were composed of lamellations. Also at the ultrastructural level, Thomas and Nicholas (1968) studied the pinoid pitting of four southern yellow pines: *P. taeda*, *P. echinata*, *P. serotina*, and *P. palustris*. They used random microfibril orientation and continuity of the parenchyma cell wall over the ray crossing pits as criteria for classifying these ray parenchyma cell walls as primary. Furthermore, they classified cell walls of thick-walled ray parenchyma cells as primary because the ray crossing pit membranes reflected the increase in the cell-wall thickness, in addition to possessing randomly oriented microfibrils.

As noted in the literature, several descriptive terms are used without being defined to describe the ray parenchyma cell wall in the wood of hard pines at the light microscope level. At the ultrastructural level, microfibril orientation, which is the criterion used to determine the nature of tracheid cell walls, is used to ascertain the nature of ray parenchyma cell walls without relating orientation to cell-wall formation. Therefore, the objective of this investigation was to ascertain whether ray parenchyma cell walls in the wood of a hard pine (*Pinus banksiana*) are primary or secondary by relating cell-wall structure to cell-wall formation.

MATERIALS AND METHODS

Pinus banksiana Lamb., a hard pine investigated extensively at the light microscope level by Balatinecz and Kennedy (1967), was selected for this research to complement their study at the ultrastructural level. A one-year-old seedling was collected each week from 18 April 1971 until 30 September 1971, from a greenhouse in which natural daylight was supplemented with artificial light from fluorescent tubes producing 400 ergs/cm^2-sec at the level of the seedlings so that the day length was 16 h. Also, one seedling was collected each week from seedbed 20-C at the College of Forestry Experiment Station from 23 July 1971 until 30 September 1971. The seed source for all seedlings was the Adirondack area and the seedlot number was S.U.N.Y. Silviculture Department Number 52-1.

Seedlings were dissected with a thin, double-edged stainless steel safety razor blade. Specimens were fixed in a solution of 3% glutaraldehyde, 2% formaldehyde, 2% acrolein, and 1% glucose in sodium cacodylate buffer at pH 7.2 for 3 h at 20 C. Tissue blocks were postfixed in a 1% solution of potassium permanganate in sodium cacodylate buffer at pH 7.2 for 3 h at 20 C to demonstrate the incorporation of lignin in the ray parenchyma cell walls and in uranyl acetate for 3 h at 20 C to increase contrast of cytoplasm. Potassium permanganate reacts with lignin to precipitate manganese dioxide (Crocker 1921), which is electron-dense. These fixation procedures are a modification of those published by Luft (1956), Hayat (1970), and Mollenhauer and Totten (1971). After postfixation, tissue blocks were dehydrated in ethanol and embedded in Spurr's "hard" low viscosity resin (Spurr 1969). Specimen blocks were prepared for ultramicrotomy by the method of Mann (1971).

Radial sections 100 μm thick from the stems of four-year-old seedlings were replicated to observe microfibril orientation in the radial cell walls of ray parenchyma cells (Côté et al. 1964). Specimens were treated with sodium chlorite to remove cytoplasmic debris and encrusting material from

the lumen surface (Koran 1964). Ultrathin sections and replicas were examined with an RCA EMU-4 electron microscope at 100 kV. Also, wood of four-year-old seedlings was examined with a JEOL JSM-U3 scanning electron microscope. Sections were examined with a Leitz ultraviolet microscope by the method of Scott et al. (1969) to verify observations of the lignin distribution obtained with the transmission electron microscope.

OBSERVATIONS AND DISCUSSION

Growth of ray parenchyma cells is different from the growth described for longitudinal tracheids by Wardrop (1957). Initiation of a tracheid starts an ontogenetic sequence that terminates with the senescence of the cell protoplast. Such an ontogenetic sequence was not observed in ray parenchyma cells of *P. banksiana* seedlings. In Fig. 1, the third ray parenchyma cell from the cambium is adjacent to longitudinal tracheids that have fully developed lignified cell walls and are devoid of protoplasts; the ray parenchyma cell does not exhibit similar characteristics of cell maturity. Some ray parenchyma cells expand four to five times the size of the ray initial centrifugally along a ray (Fig. 1). They are prevented from growing vertically by ray tracheids and other ray parenchyma cells. In transverse view, ray parenchyma cells in mature xylem are rectangular in

←

KEY TO ABBREVIATIONS

Cm—Cambial zone
Er—Endoplasmic reticulum
M—Mitochrondia
N—Nucleus
P—Plasmodesmata
Ri—Ray initial
St—Starch granule
V—Vacuole

FIG. 1. Ray parenchyma cells in different stages of cell enlargement. Compare the thickness of the primary cell wall of the ray parenchyma and that of a longitudinal tracheid (arrow). Also note the large number of plasmodesmata in the tangential cell walls. Plate Nos. 6677–6680. 1900×.

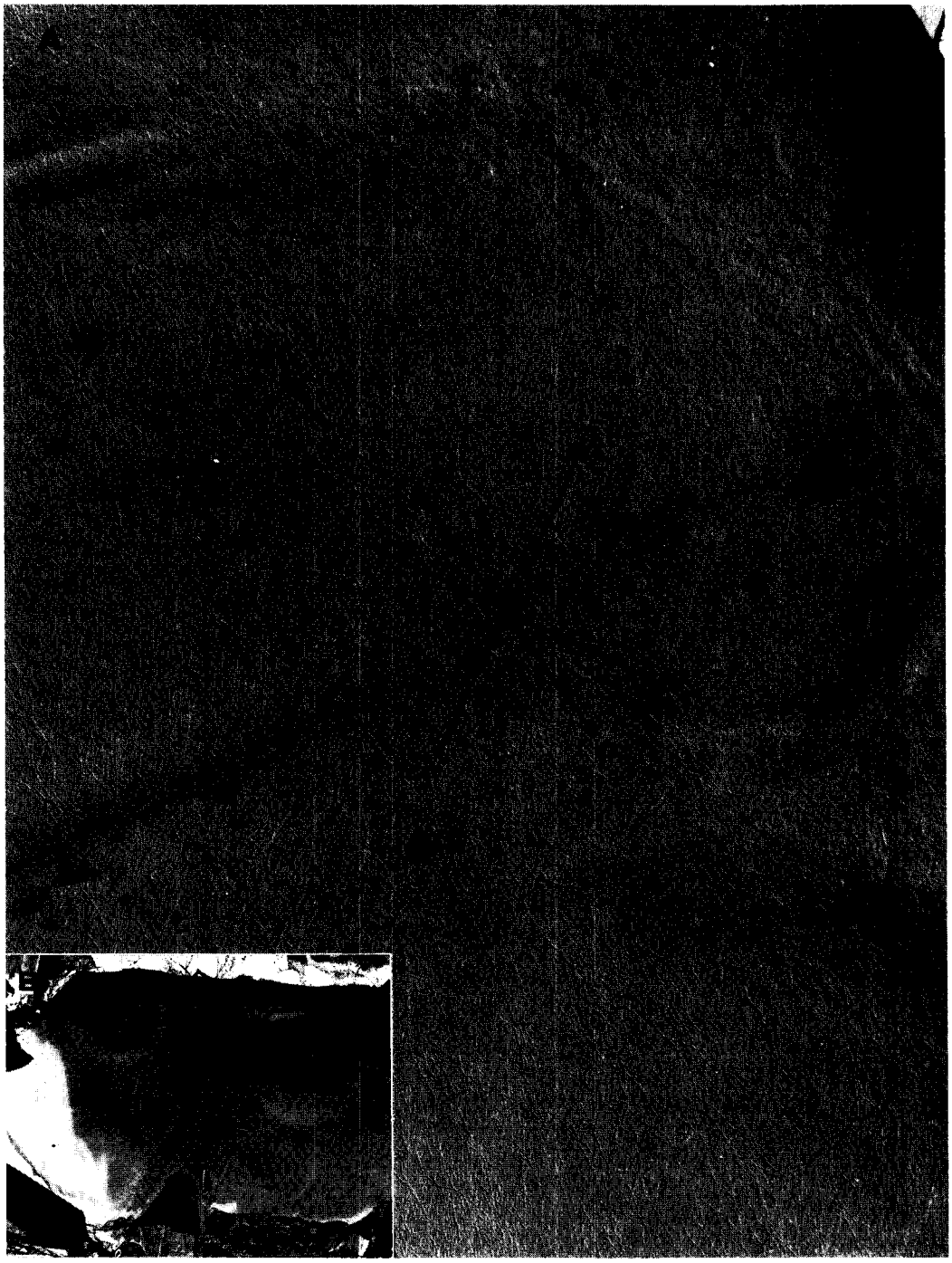


FIG. 2. A. A replica showing several layers within the ray parenchyma cell wall. A sparse network of microfibrils comprises the surface layer. Beneath this layer the microfibrils (a) are oriented 70° to the cell axis (c). A third layer of microfibrils (b) is visible through holes in the second layer having an orientation 25° to the cell axis (c). Plate No. 6921. 13,000×.

B. A lower magnification view indicating the relative position of A to the tangential cell wall of the contiguous longitudinal tracheid. Plate No. 6922. 1200×.

FIG. 3. Scanning electron micrograph of a ray parenchyma cell in tangential view showing the lack of secondary thickening in the ray parenchyma cell wall between pinoid pits (arrow). Cytoplasmic remains are seen inside the ray parenchyma cell. 4000×.

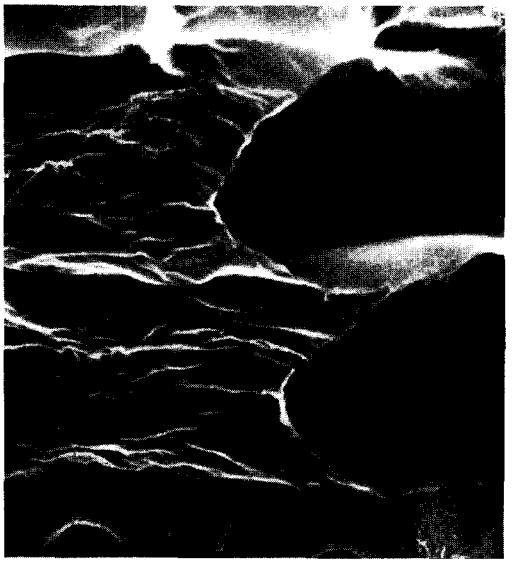


FIG. 4. Scanning electron micrograph of ray parenchyma cells in radial view showing the thin primary cell wall which collapsed without secondary cell wall thickening to give support. 1600×.

shape (Fig. 1). Intrusive tip growth by ray parenchyma cells was not observed; i.e., end walls of ray parenchyma cells were not tapered as if one ray parenchyma cell grew past another.

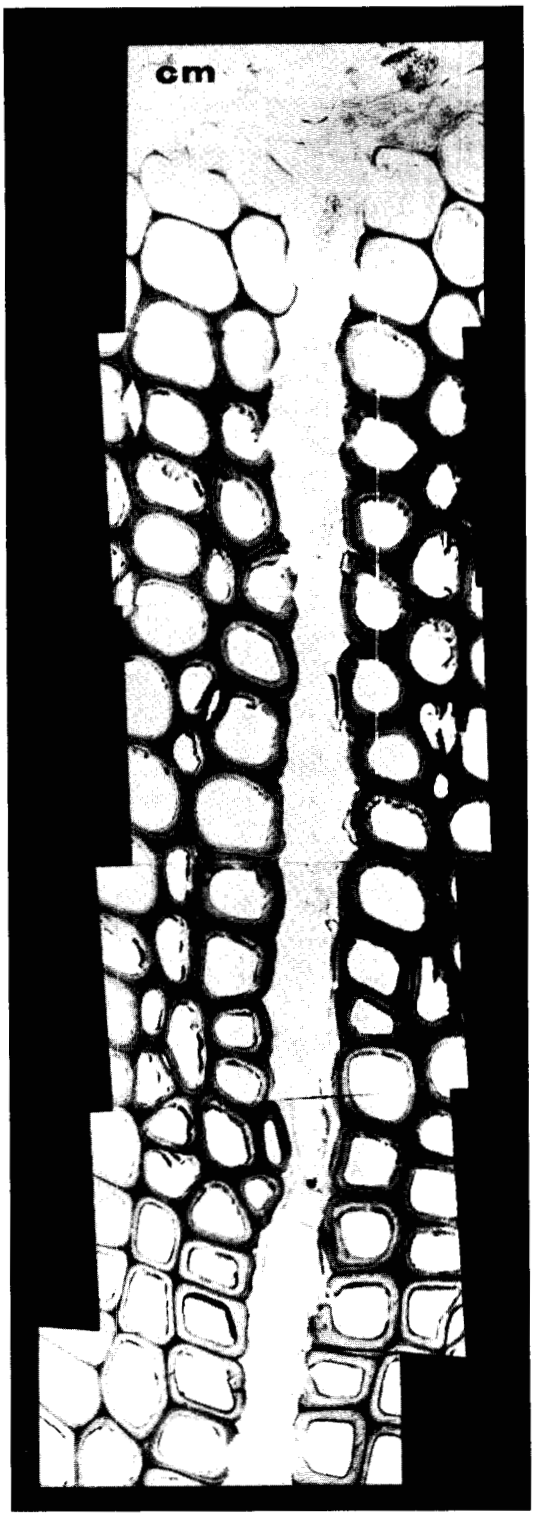
Ray parenchyma cell expansion is comparable to an elongating cylinder. Since intrusive tip growth was not evident, elongation occurred as an extension of the lateral cell walls. It is of interest whether expansion occurred uniformly along the lateral cell walls of a ray parenchyma cell or in localized areas. If expansion occurred uniformly along the lateral cell walls, there would be slippage between the radial cell wall of a ray parenchyma cell and the radial cell wall of a contiguous tracheid. This slippage would occur because the adjacent longitudinal tracheids would be in different stages of radial expansion and secondary cell-wall formation (Fig. 1) and uneven stresses would develop. In considering a group of tracheids adjacent to an expanding ray parenchyma cell at a given point in time, the tracheids nearest the center of the stem may have completed radial expansion, but those nearest the cambium may be expanding; thus, the amount of radial expansion by the expanding tracheids would be accommodated by the entire ray parenchyma cell wall.

This hypothesis is supported by the lack of plasmodesmata in the radial cell walls of ray parenchyma cells which would have been have been disrupted during cell expansion and cell-wall movement. Plasmodesmata are evident in cell walls that were inactive in cell expansion (Fig. 1). Also in Fig. 1, the sharp separation between the primary cell wall of the longitudinal tracheids and the radial ray parenchyma cell wall might be the result of movement between the two cells. Generally, the primary cell walls cannot be distinguished from one another in the compound middle lamella.

If there were no relative movement between ray parenchyma cells and adjacent tracheids, ray parenchyma cell expansion might occur in localized areas contiguous to those tracheids that are expanding radially. Thus, those areas of the ray parenchyma cell wall adjacent to the tangential cell wall of a tracheid would not be subject to stress and the microfibrils would not be oriented or aligned differently than when they were formed. Figure 2, a replica of a ray parenchyma cell wall adjacent to the tangential cell wall of a longitudinal tracheid, shows several layers of microfibrils. The surface layer of randomly oriented microfibrils is sparse, permitting observation of microfibril orientations within the cell wall. Microfibrils of the first layer beneath the surface have an orientation 70 degrees to the cell axis, and the second layer of microfibrils has an orientation 25 degrees to the cell axis. A change in microfibril orientation was not observed as the microfibrils passed from a proposed area of cell-wall extension to a proposed area of no cell-wall extension.

The cell-wall structure observed may be explained by the multinet growth theory of Roelofsen and Houwink (1953). The outer layer, the layer nearest the middle lamella, has a longitudinal microfibril orientation, but the layer deposited on the inside has a more transverse microfibril orientation. Microfibrils of the outer layer, the oldest layer, were deposited with a transverse orientation and were reoriented during cell-wall extension to a longitudinal orientation. Microfibrils of the youngest layer were deposited at a time after which only a limited amount of cell-wall extension occurred, and thus the microfibrils are nearly transversely oriented.

Ray parenchyma cells did not form an additional cell wall immediately after cell expansion ceased, and the cell wall formed during cell expansion did not lignify. There is no evidence of cell-wall material depos-



→

FIG. 5. Photomicrograph of a 0.5-μm section taken with 280 nm light. Note the lack of lignin in the ray parenchyma cells. 750×.

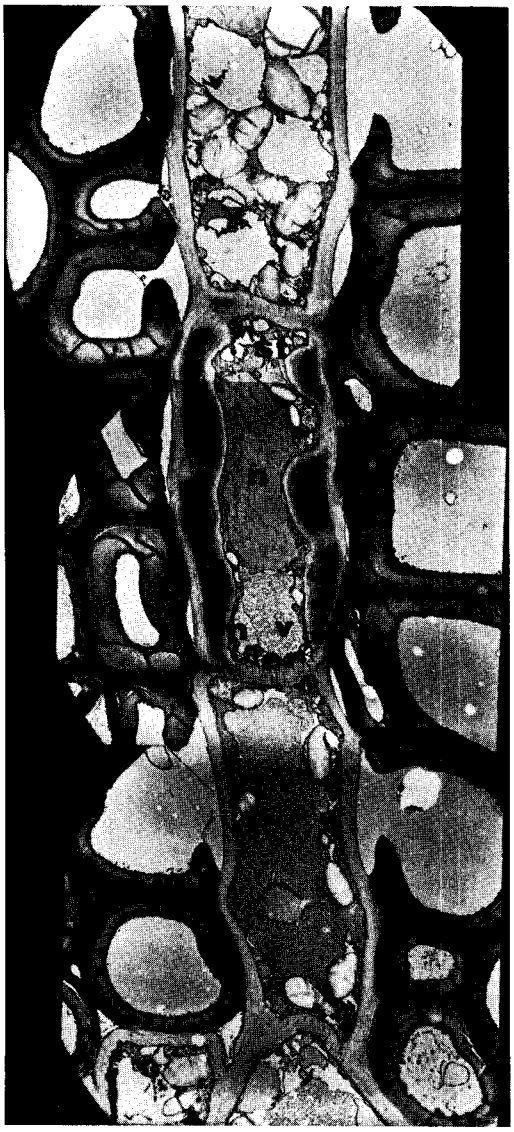


FIG. 6. An isolated ray parenchyma cell that developed a lignified secondary cell wall. Note the distinct cell contents. Plate Nos. 6619–6621. 1300×.

ited other than that deposited during cell expansion, i.e., there is no cell-wall thickening on the ray parenchyma cell wall in areas adjacent to pinoid pits (Figs. 3 and 4). The lack of lignin is seen both in photomicrographs taken with a quartz microscope (Fig. 5) and electron micrographs of specimens stained with potassium permanganate (Figs. 1 and 6). In the photomicrograph, the ray parenchyma cell walls did not absorb at the 280-nm wavelength, which is the wavelength of maximum absorbance by lignin, and are almost undetectable. In electron micrographs, ray parenchyma cell walls did not stain with potassium permanganate although they are distinct.

The cell walls of *P. banksiana* ray parenchyma cells examined in this investigation are considered to be primary in nature. The cell wall was formed during the expansion phase of growth, which is the criterion employed by many authorities (Committee of Nomenclature 1964; Esau 1953; Panshin et al. 1964). In addition, Esau (1960) states that a primary cell wall may have various microfibril orientations ranging from random to more or less parallel, which may change considerably during cell expansion.

Isolated ray parenchyma cells were observed with an additional cell wall which is considered secondary in nature (Fig. 6). Notice that the additional cell wall is lignified and that the cell has retained the protoplast with a distinct nucleus, mitochrondria, starch granules, endoplasmic reticulum, and vacuoles, which contrasts with the observations of Balatinecz and Kennedy (1967) pertaining to lignified ray parenchyma cells in hard pines. The knob-like thickenings observed in ray parenchyma cells of hard pines (Balatinecz and Kennedy 1967) and the pitted thick-walled cell walls observed in ray parenchyma of southern pines (Howard and Manwiller 1969) are considered to be secondary cell-wall thickenings.

Ray crossing pits in *P. banksiana* cannot be considered half-bordered pit pairs. A bordered pit is present in the longitudinal tracheid, but the complementary simple pit is not present because the secondary cell wall in the contiguous ray parenchyma cell is wanting. Should the ray parenchyma portion of the ray crossing pitting be considered a primary pit-field? It could be, in that one or more pits would probably form within its limits if a secondary cell wall formed (Esau 1960; Committee on Nomenclature 1964). However, a depression or depressions were

not observed in ultrathin sections of the radial ray parenchyma cell wall (Fig. 1), nor were plasmodesmata observed in the radial cell walls which are essential for intercellular communication (Esau 1953). Therefore, the ray crossing pits in *P. banksiana* seedlings are considered to be blind pitting.

REFERENCES

BAILEY, I. W. 1909. The structure of the wood in the Pineae. Bot. Gaz. 48:47–55.

BAILEY, I. W., AND A. F. FAULL. 1934. The cambium and its derivitaive tissues. No. IX. Structural variability in the redwood, *Sequoia sempervirens*, and its significance in the identification of fossil woods. J. Arnold Arbor. 15:233–254.

BALATINECZ, J. J., AND R. W. KENNEDY. 1967. Maturation of ray parenchyma cells in pine. For. Prod. J. 17(10):57–64.

BANNAN, M. W. 1934. Origin and cellular character of xylem rays in the gymnosperms. Bot. Gaz. 96:260–281.

COMMITTEE ON NOMENCLATURE. 1964. Multilingual glossary of terms used in wood anatomy. International Association of Wood Anatomists. Zurich. 186 pp.

CÔTÉ, W. A., AND A. C. DAY. 1969. Wood ultrastructure of the southern yellow pines. Tech. Pub. No. 95. State University of New York College of Forestry. Syracuse. 70 pp.

CÔTÉ, W. A., Z. KORAN, AND A. C. DAY. 1964. Replica techniques for electron microscopy of wood and paper. Tappi 47(8):477–484.

CROCKER, E. C. 1921. Significance of "lignin" color reactions. Ind. Eng. Chem. 13:625–627.

ESAU, K. 1953. Plant anatomy. John Wiley and Sons, Inc. New York. 735 pp.

———. 1960. Anatomy of seed plants. John Wiley and Sons, Inc., New York. 376 pp.

HARADA, H. 1964. Further observations on the pit structure of wood. J. Jap. Wood Res. Soc. 10(6):221–225.

HAYAT, M. A. 1970. Principles and techniques of electron microscopy. v. 1: Biological applications. Van Nostrand Reinhold Co., New York. 412 pp.

HOWARD, E. T., AND F. G. MANWILLER. 1969. Anatomical characteristics of southern pine stemwood. Wood Sci. 2(2):77–86.

KORAN, Z. 1964. Ultrastructure of tyloses and a theory of their growth mechanism. Ph.D. Dissertation. New York State College of Forestry. Syracuse.

KRAHMER, R. L., AND W. A. CÔTÉ, JR. 1963. Changes in coniferous wood cells associated with heartwood formation. Tappi 46(1):42–49.

LUFT, J. H. 1956. Permanganate—A new fixative for electron microscopy. J. Biophys. Biochem. Cytol. 2:799–801.

MANN, P. T. 1971. Rapid method for rough-trimming specimen blocks for electron microscopy. Int. Assoc. Wood Anat. Bull. No. 2:10–12.

MIROV, N. T. 1967. The genus *Pinus*. Ronald Press Co., New York. 602 pp.

MOLLENHAUER, H. H., AND C. TOTTEN. 1971. Studies on seeds. I. Fixation of seeds. J. Cell Biol. 48:387–394.

PANSHIN, A. J., C. DE ZEEUW, AND H. P. BROWN. 1964. Textbook of wood technology. v. 1. McGraw-Hill Book Co., New York. 705 p.

PENHALLOW, D. P. 1907. North American gymnosperms. Ginn and Co., Boston. 374 pp.

ROELOFSEN, P. A., AND A. L. HOUWINK. 1953. Acta Bot. Neerl. 2:218–225. Original not seen. (Pages 69–149 *in* P. A. Roelofsen, 1965. Ultrastructure of the wall in growing cells. *In* R. D. Preston, ed. Advances in botanical research, v. 2. Academic Press, N.Y.)

SCOTT, J. A. N., A. R. PROCTER, B. J. FERGUS, AND D. A. I. GORING. 1969. The application of ultraviolet microscopy to the distribution of lignin in wood: Description and validity of the technique. Wood Sci. Technol. 3:73–92.

SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31–43.

THOMAS, R. J., AND D. D. NICHOLAS. 1968. The ultrastructure of the pinoid pit in southern yellow pine. Tappi 51(2):84–88.

WARDROP, A. B. 1957. The phase of lignification in the differentiation of wood fibers. Tappi 40:225–243.