MICROFIBRILLAR WEBS ACROSS VESSEL PIT APERTURES

B. G. Butterfield[1] and *B. A. Meylan*
Physics and Engineering Laboratory, Department of Scientific and Industrial Research, Lower Hutt, New Zealand

(Received 22 January 1973)

ABSTRACT

Microfibrillar webs traversing the pit apertures on the lumen side of vessel member walls were observed in *Coriaria arborea* Lindsay, *Corynocarpus laevigatus* J. R. et G. Forst., and *Knightia excelsa* R. Br. These webs originate from a layer of wall material occurring on top of the normal secondary wall.

Additional keywords: Coriaria arborea, Corynocarpus laevigatus, Knightia excelsa, ultrastructure, cell wall.

INTRODUCTION

The secondary walls of vessel members in angiosperm secondary xylem generally show a three-layered structure (Preston 1952; Wardrop 1964; Harada 1962; Mia 1969). The S_3 layer is sometimes overlaid by a warty layer. The microfibrils of the S_1 and S_3 layers tend to lie at right angles to the long axis of the cell, the direction of the slit openings of the bordered pits commonly being parallel to the microfibrillar orientation of these layers (Harada 1965). The thicker S_2 layer has its microfibrils aligned at about 70–80° to the long axis of the cell, though considerable variation occurs.

Evidence for the existence of an additional S_4 wall layer on top of the secondary wall in parenchyma cells was presented by Harada (1962), but doubt on its existence was later cast by the same author (Harada 1965). A wall layer termed the protective layer by Schmid (1965) has also been observed in parenchyma cells (Meyer and Côté 1968; Yata et al. 1970). This layer is similar to the secondary wall in appearance and may be the same structure as Harada's S_4 layer. No such corresponding layer has been reported to occur in vessel members.

During a recent study of the structure of more than fifty New Zealand woods, portions of a wall layer overlying the normal secondary wall were seen in the vessel members of *Coriaria arborea* Lindsay, *Corynocarpus laevigatus* J.R. et G. Forst., and *Knightia excelsa* R.Br. Where this layer passes over the pit apertures, it is drawn into microfibrillar webs similar in appearance to those reported recently occurring across the openings of the scalariform perforation plates in some woods (Meyer and Muhammed 1972; Butterfield and Meylan 1972a; Meylan and Butterfield 1973).

The object of this paper is to record the existence of this layer and its webs, although its nature remains largely unknown.

MATERIALS AND METHODS

Wood samples were collected from mature trees including *Coriaria arborea* (tree tutu, 10 cm diameter), *Corynocarpus laevigatus* (karaka, 40 cm diameter), and *Knightia excelsa* (rewarewa, 60 cm diameter) growing in a number of different localities. The samples were removed from the outside of the main trunks using a battery-operated one-inch-diameter hole cutter and transferred immediately to tubes of formalin aceto-alcohol.

Cubes of wood about 4 mm per side were cut from fully differentiated sapwood about 1 cm back from the cambium. Other cubes were prepared from wood adjacent

[1] On leave from Botany Department, University of Canterbury, Christchurch, New Zealand. The authors are indebted to Mr. R. R. Exley for technical assistance in the preparation of the wood specimens.

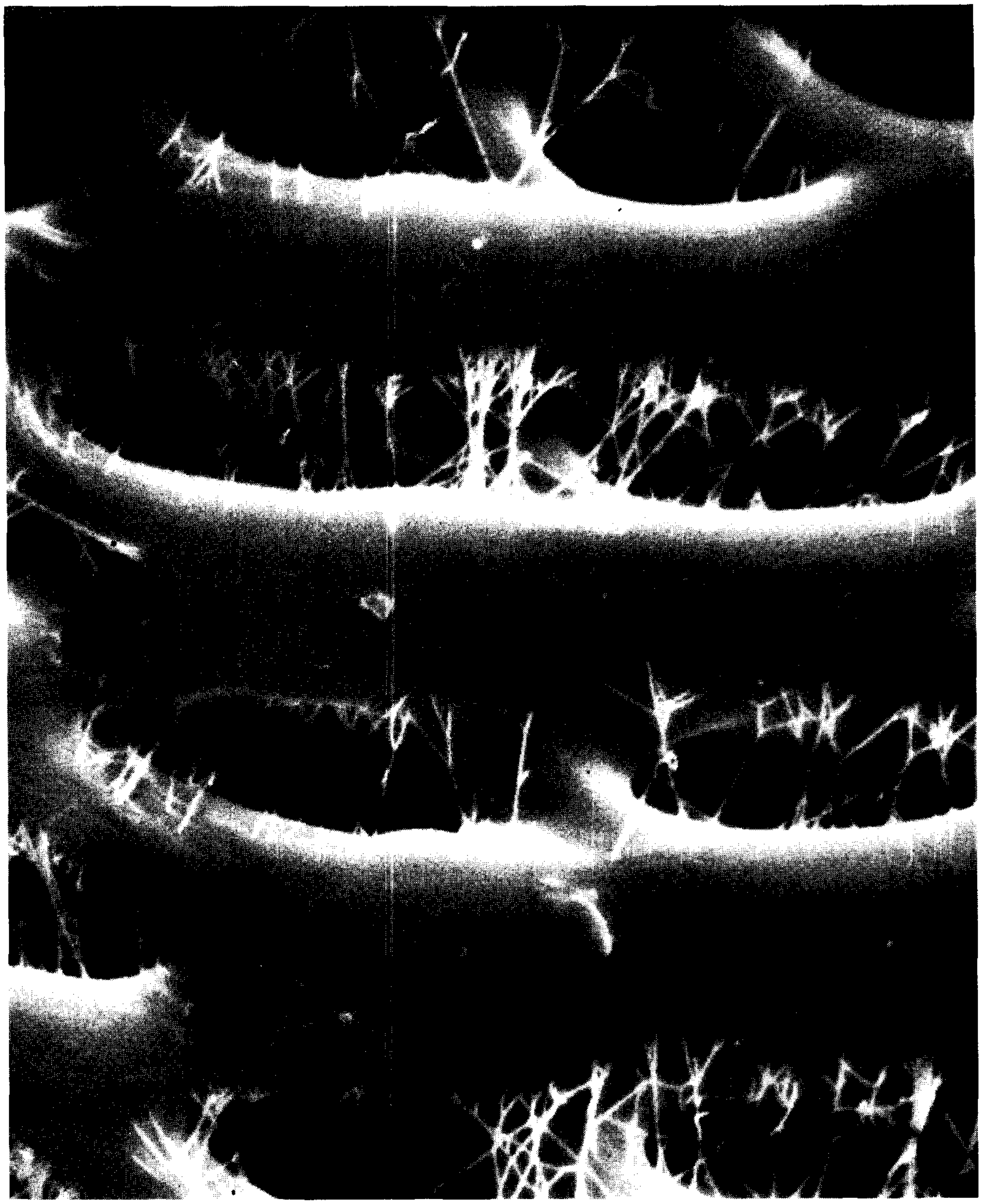

FIG. 1. Microfibrillar webs across the vessel pit apertures in *Coriaria arborea*. The webs are continuous with a layer of material lying on the top of the normal secondary wall (air-dried, hypochlorite-treated). × 10,500.

to the cambium. Some specimens were air-dried while others were dehydrated by a methanol-acetone solvent-exchange process. Additional specimens of *Coriaria arborea* were soaked in 100 mol m^{-3} HCl for 1, 8 and 48 hr; a 15% solution of sodium hypochlorite for 1 hr, or 500 mol m^{-3} KOH for 1, 4 and 17 hr. All specimens were then

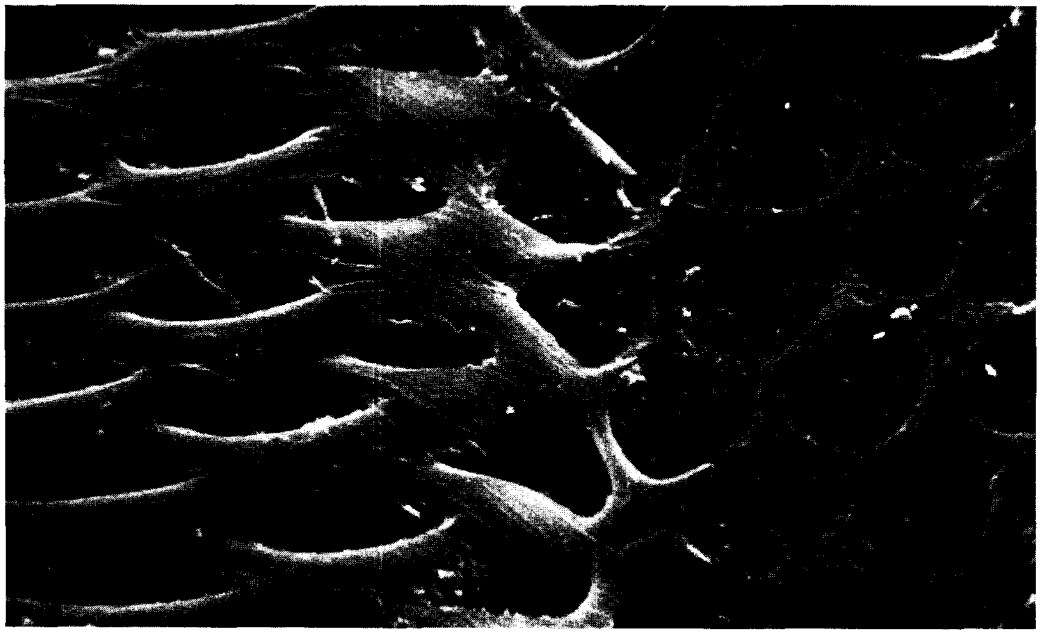

FIG. 2. An oblique cut through the cell wall of a vessel member of *Coriaria arborea* showing webs traversing the pit apertures to the left, partly removed secondary wall to the middle, and exposed circular pit membranes to the right (solvent-exchanged, otherwise untreated). ×4,600.

coated with about 20 nm of carbon and a similar amount of gold-palladium while being rotated, and examined in the vacuum-dry state in the column of a Cambridge Series IIA scanning electron microscope.

RESULTS

An obvious feature of the wood of *Coriaria arborea*, when viewed under the scanning electron microscope, is the presence of microfibrillar webs traversing the pit apertures on the lumen side of the secondary walls in some vessel members (Fig. 1). They could also be seen, with some difficulty, under the light microscope. The webs were observed to occur in wood close to the cambium and also in wood further removed from it in up to one-third of the vessels and were a feature of both the air-dried and the solvent-exchanged specimens. In *Coriaria arborea*, the inter-vessel pit pairs are separated by more or less circular pit membranes overarched on each side by the secondary walls of the adjoining vessel members (Figs. 2 and 3). The pit apertures are narrow slit-extended to oval in outline. When present, the microfibrillar webs traverse the apertures predominantly at right angles to the opening and adhere to the innermost layer of the secondary wall. In many of the vessels examined in *Coriaria*, the microfibrillar webs appeared to originate from an additional layer of wall material adhering to the lumen side of the normal secondary wall. The density of this layer varies considerably from cell to cell and even within any one vessel member. It can completely cover the normal cell wall including the pit apertures; as strips of fibrillar material passing over certain parts of the cell wall only (Fig. 4); or it can be so thinly dispersed that it is clearly visible only where it passes across the pit apertures. In the last case, in particular, the layer is drawn into fibrils across the pits. It can be easily distinguished from the normal secondary wall (Fig. 3).

Microfibrillar webs similar to those observed in *Coriaria arborea* were also seen

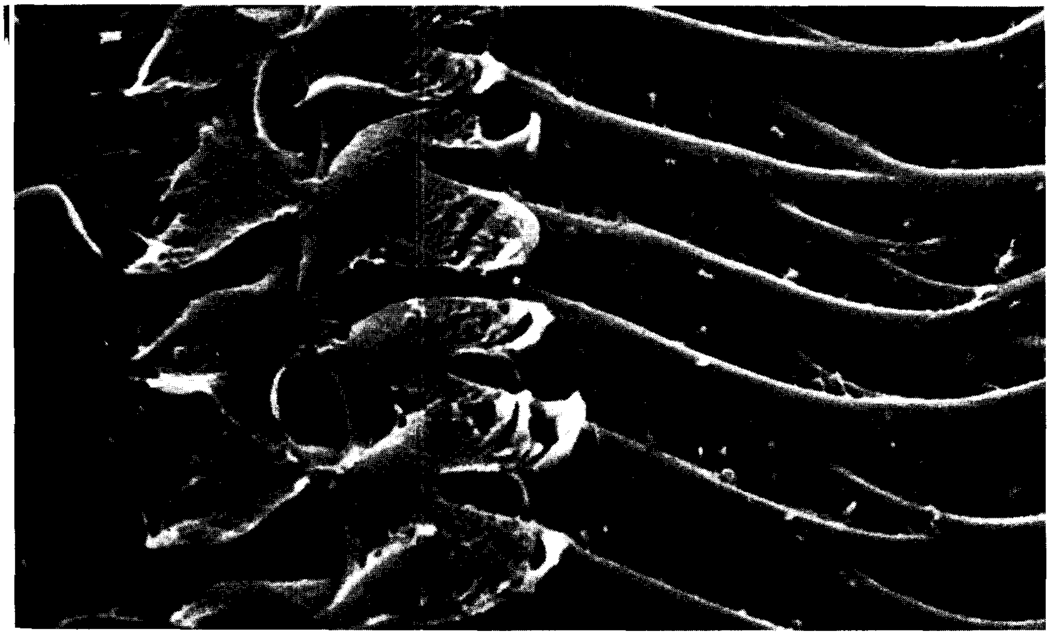

FIG. 3. A cross section through the walls of two adjacent vessel members in *Coriaria*. The additional wall layer in the cell to the left has separated from the rest of the vessel member wall. Microfibrillar webs can be seen traversing the pit apertures in the cell to the right (solvent-exchanged, otherwise untreated). ×3,750.

but less frequently in vessel members of *Corynocarpus laevigatus* (Fig. 5) and were observed once in a vessel of *Knightia excelsa* but were not seen in any of the other species examined. They were not removed by any of the chemical treatments used. Sodium hypochlorite has previously been used successfully by the present authors to remove proteinaceous material that has been deposited from the cell cytoplasm. In *Coriaria*, however, as in the HCl and KOH treatments, it failed to have any effect on the webs, although it removed most of the cytoplasmic debris.

DISCUSSION

It was not possible to distinguish between the individual secondary wall layers in the vessel members of *Coriaria* and *Corynocarpus* during this scanning electron microscope investigation. Photographs published elsewhere (Butterfield and Meylan 1972b), however, indicate that the secondary wall layers in *Knightia* follow the expected pattern. A normal S_3 layer is clearly present in this species. The microfibrillar webs illustrated here appear to originate from an additional layer of material laid on top of the normal secondary wall in all three species and cross the pit apertures on the lumen side of the pit canals.

One interpretation of this layer of material is that it is of cytoplasmic origin, but chemical treatments normally used to remove such material have failed to affect it. It is also unlikely to be parts of a tylose wall since the layer does not always cover all the side walls of any one vessel member. In any case, a careful examination of the vessels in the wood of each species has failed to reveal any tyloses.

The appearance of the wall material when viewed in the scanning electron microscope (Fig. 4) is suggestive of its being cellulosic, and the results of the limited chemical tests do nothing to disprove this. The marked similarity in the form of the webs over the pit apertures to those found between the

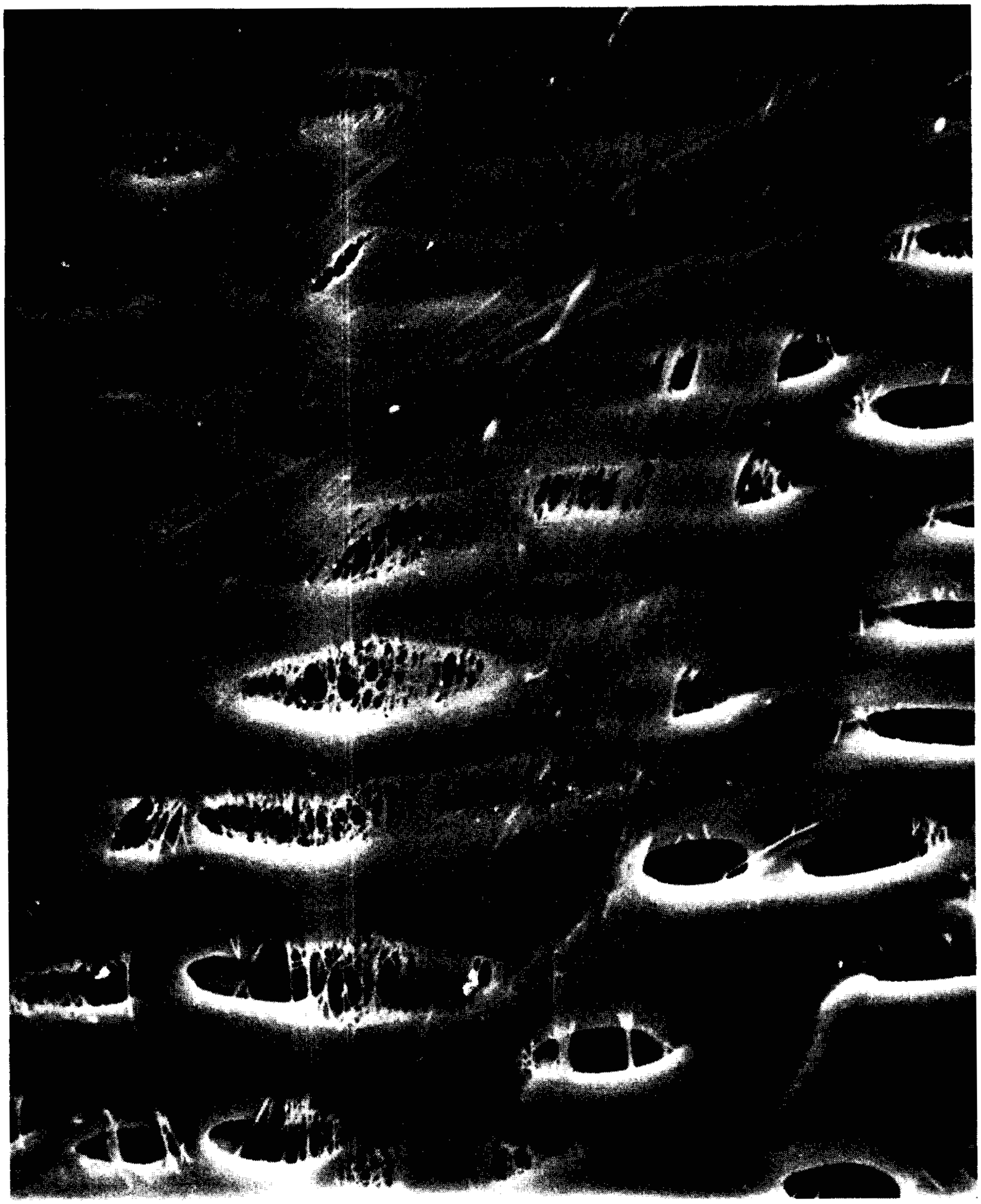

FIG. 4. A view of the inside surface of a vessel wall in *Coriaria arborea*. The microfibrillar webs appear to originate from fibrillar wall material laid down on top of the normal secondary wall. In some areas the layer completely covers the pit apertures (air-dried, hypochlorite-treated). × 4,200.

bars of scalariform perforation plates is also significant. The webs in scalariform perforation plates lie between the overarching secondary wall borders of the adjoining vessel members and originate from unlignified primary wall material left after the bulk of each perforation partition has been digested. Although the cell-wall layer

FIG. 5. Microfibrillar webs traversing the vessel pit apertures in *Corynocarpus laevigatus* (air-dried, hypochlorite-treated). × 4,500.

described in this paper clearly lies on top of the normal secondary wall, it is difficult to understand how cellulose wall material could be laid down in this position. It is clear that further studies are required in order to establish more definitely the origin and nature of this layer.

Whatever the origin, the behavior of this material when drawn over the pit apertures may be significant to the understanding of the development of other similar microfibrillar webs. In both air-dried and solvent-exchanged wood specimens, the webs illustrated here are similar to those found in the openings of scalariform perforation plates and are not unlike the microfibrils in the margo areas of conifer bordered pits although they overlie the secondary wall, rather than occurring within the compound middle lamella.

REFERENCES

BUTTERFIELD, B. G., AND B. A. MEYLAN. 1972a. Scalariform perforation plate development in *Laurelia novae-zelandiae* A. Cunn. A scanning electron microscope study. Aust. J. Bot. 20:252:9.

———. 1972b. Intervessel pit membranes in *Knightia excelsa* R. Br. Bull. Int. Assoc. Wood Anat., 1972/4:3–9.

HARADA, H. 1962. Electron microscopy of ultrathin sections of beech wood (*Fagus crenata* Blume). J. Jap. Wood Res. Soc., 8:252:258.

HARADA, H. 1965. Ultrastructure of angiosperm vessels and ray parenchyma. Pages 235–249 *in* W. A. Côté, ed., Cellular ultrastructure of woody plants. Syracuse University Press, New York.

MEYER, R. W., AND W. A. CÔTÉ. 1968. Formation of the protective layer and its role in tylosis development. Wood Sci. Technol. 2: 84–94.

MEYER, R. W., AND A. F. MUHAMMAD. 1972. Scalariform perforation plate fine structure. Wood Fiber 3:139–145.

MEYLAN, B. A., AND B. G. BUTTERFIELD. 1973. Scalariform perforation plates: Observations using scanning electron microscopy. Wood Fiber 4:225–233.

MIA, A. J. 1969. Study of cell walls in angiospermous plants using light and electron microscopes. Wood Sci. 2:1–10.

PRESTON, R. D. 1952. The molecular architecture of plant cell walls. Chapman and Hall, London.

SCHMID, R. 1965. The fine structure of pits in hardwoods. Pages 291–304 *in* W. A. Côté, ed., Cellular ultrastructure of woody plants. Syracuse University Press, New York.

WARDROP, A. B. 1964. The structure and formation of the cell wall in xylem. Pages 87–134 *in* M. H. Zimmerman, ed., The formation of wood in forest trees. Academic Press, New York.

YATA, S. T. ITOH, AND T. KISMA. 1970. Formation of perforation plates and bordered pits in differentiating vessel elements. Wood Res. 50:1–11.