INTERVASCULAR PITTING ACROSS THE ANNUAL RING BOUNDARY IN *BETULA PLATYPHYLLA* VAR. *JAPONICA* AND *FRAXINUS MANDSHURICA* VAR. *JAPONICA*

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SUMMARY

The structure of intervascular pits, located at the boundary between the outermost and the second youngest annual rings in *Betula platyphylla* var. *japonica* and *Fraxinus mandshurica* var. *japonica* was examined by field-emission scanning electron microscopy. Unilaterally compound pits were present in the intervascular common wall at the annual ring boundary in both species. On the outer annual ring side of the unilaterally compound pits, outlines of pit membranes were curved or trifoliate, and each pit aperture was often elongated and curved. The porosity of the intervascular pit membranes differed between the two species. In *B. platyphylla* var. *japonica*, microfibrils were loosely packed in the peripheral region of each pit membrane, and openings of up to 300 nm in width were observed. By contrast, microfibrils were densely packed throughout the entire pit membranes in *F. mandshurica* var. *japonica*, and no openings perforating the pit membranes entirely were found. In addition, each species exhibited some unique features. In *B. platyphylla* var. *japonica*, extensive ethanol-soluble material was detected not only in the intervascular pits but also on scalariform perforation plates. In *F. mandshurica* var. *japonica*, we observed fine curly fibrils of unkown chemical composition in the intervascular pit membranes.

Key words: Betula platyphylla var. *japonica*; *Fraxinus mandshurica* var. *japonica*; intervascular pits; unilaterally compound pits; field-emission scanning electron microscopy.

INTRODUCTION

In wood, pits provide one of the main pathways for the flow of liquid between cells. Their structure and distribution affect the movement of sap in living trees and the penetration of liquid in timber. Thus, intensive efforts have been made to clarify the structure and distribution of pits and to evaluate their effects on permeability to liquids.

The structure and distribution of pits in softwoods have been documented in considerable detail, and the structure and function of bordered pits between tracheids are fairly well understood. By contrast, the structure of pits in hardwoods has generally received less attention. The presence or absence and the morphology of vestured pits have been studied systematically because these features have diagnostic value (e.g., Ohtani

1983; Jansen et al. 1998), and the fine structure and development of some types of pit membrane in a few species have been reported (e.g., Dute & Rushing 1988; Dute et al. 1992). However, examinations of the fine structure of hardwood pits have been focused on certain particular types of pit in a limited number of species, in spite of the fact that there are many pit types and numerous hardwood species. Structural variations and the functions of hardwood pits remain to be clarified.

This report describes field-emission scanning electron microscopic studies of the fine structure of intervascular pits located at the annual ring boundary in two hardwood species, *Betula platyphylla* var. *japonica* and *Fraxinus mandshurica* var. *japonica*.

MATERIALS AND METHODS

Materials

Samples of outer sapwood (longitudinal, radial and tangential directions, 10, 2 and 5 cm) were excised at breast height with a chisel from *Betula platyphylla* var. *japonica* and *Fraxinus mandshurica* var. *japonica* (Table 1). Two to four samples were collected from each tree. Half of the samples were put into 30% ethanol and stored at room temperature. The remainder was immersed in liquid nitrogen, brought to the laboratory and stored at -80 °C.

Table 1. Sources of wood samples.

Sample trees		Height (m)	DBH (cm)	Site[1]	Collection[2]
Betula platyphylla	A	18	19	1	1
var. *japonica*	B	20	30	1	1
Fraxinus mandshurica	A	20	45	2	2
var. *japonica*	B	9	15	2	2

[1]) 1: Tomakomai Experimental Forest of Hokkaido University; 2: Sapporo Experimental Nursery of Hokkaido University.

[2]) Season of sample collection: 1: late spring when leaf expansion was progressing; 2: early autum soon after formation of current-year xylem had ceased.

Field-emission scanning electron microscopy

The specimens that had been stored in 30% ethanol were used for the examination of sections. Surfaces of samples were exposed by the method devised by Yumoto et al. (1982). In brief, small cubes of wood ($2 \times 2 \times 2$ mm^3) were cut and embedded in methacrylate resin (a mixture of n-butyl methacrylate and methyl methacrylate, 1 : 2, v/v). Transverse surfaces were planed on an ultramicrotome with a glass knife. Then the methacrylate resin was removed in acetone. The cubes were subsequently air-dried and affixed with electron-conductive carbon paste to aluminium stubs. After coating with carbon and gold plus palladium by vacuum evaporation or with osmium by plasmapolymerization (Sano et al. 1999), the samples were examined with a field-emission scanning electron microscope (FE-SEM; JSM-6301F, Jeol) at an accelerating voltage of 2.5 kV.

For examination of the fine surface structure of pit membranes, both samples stored at -80 °C and samples stored in 30% ethanol were used. Samples of both types were cut into small cubes ($5 \times 5 \times 5$ mm^3) that included the outermost annual ring boundary. The blocks cut from frozen samples were freeze-dried in a freeze-drying apparatus without immersion in organic solvent prior to drying. Samples that had been stored in 30% ethanol were air-dried after dehydration in absolute ethanol. All the dried blocks were split along a tangential plane at approximately the boundary between the outermost and the second annual rings and then specimens were affixed to aluminium stubs with electron-conductive carbon paste. They were coated with platinum by vacuum evaporation and examined with the FE-SEM as described above.

RESULTS

Examination of split faces

In both *Betula platyphylla* var. *japonica* and *Fraxinus mandshurica* var. *japonica*, separation within intervascular common walls that were located at an annual ring boundary almost always occurred on the outer annual ring side of the boundary. Therefore, intervascular pits that were located at the beginning of the annual ring were easily observed, while those that were located at the end of the previous annual ring were observed only rarely. The intervascular pits at the beginning of an annual ring exhibited characteristic morphology (Fig. 1–4). The pit aperture varied in length, and the long aperture was often curved (Fig. 1 & 2). The size and the outline of pit membranes also varied, often being elongated and curved (Fig. 3 & 4), or trifoliate (Fig. 4). By contrast, no similarly shaped pits were noted among the intervascular pits at the end of an annual ring, at least within the limited areas that were only rarely evident (Fig. 5 & 6). These observations implied that the curved and trifoliate intervascular pits at the beginning of the annual ring formed unilaterally compound pits with several smaller intervascular pits at the end of each annual ring. Diameters of pits at the end of the previous annual ring were 1.5 to 2.5 μm in *B. platyphylla* var. *japonica* and 3.5 to 4 μm in *F. mandshurica* var. *japonica*.

The porosity of pit membranes differed between the two species examined. In *B. platyphylla* var. *japonica*, microfibrils were loosely packed near the periphery of the pit membranes (Fig. 7), and openings of up to 300 nm in width were also found within the central region of densely packed microfibrils in some pit membranes (Fig. 3 & 7, arrows). By contrast, in *F. mandshurica* var. *japonica*, microfibrils were densely packed throughout the entire region of the pit membranes and there were no visible openings completely perforating the pit membranes (Fig. 8).

In *F. mandshurica* var. *japonica*, there were fine curly fibrils in the intervascular pit membranes at the annual ring boundary (Fig. 4 & 8). Such fibrils were also found in all of the intervascular pit membranes between large earlywood vessels of the current-year xylem (Fig. 9), but there were none at all in the intervascular pit membranes between the small latewood vessels. In *B. platyphylla* var. *japonica*, no such fine curly fibrils were found in any of the intervascular pit membranes, irrespective of their location in an annual ring (Fig. 3 & 7).
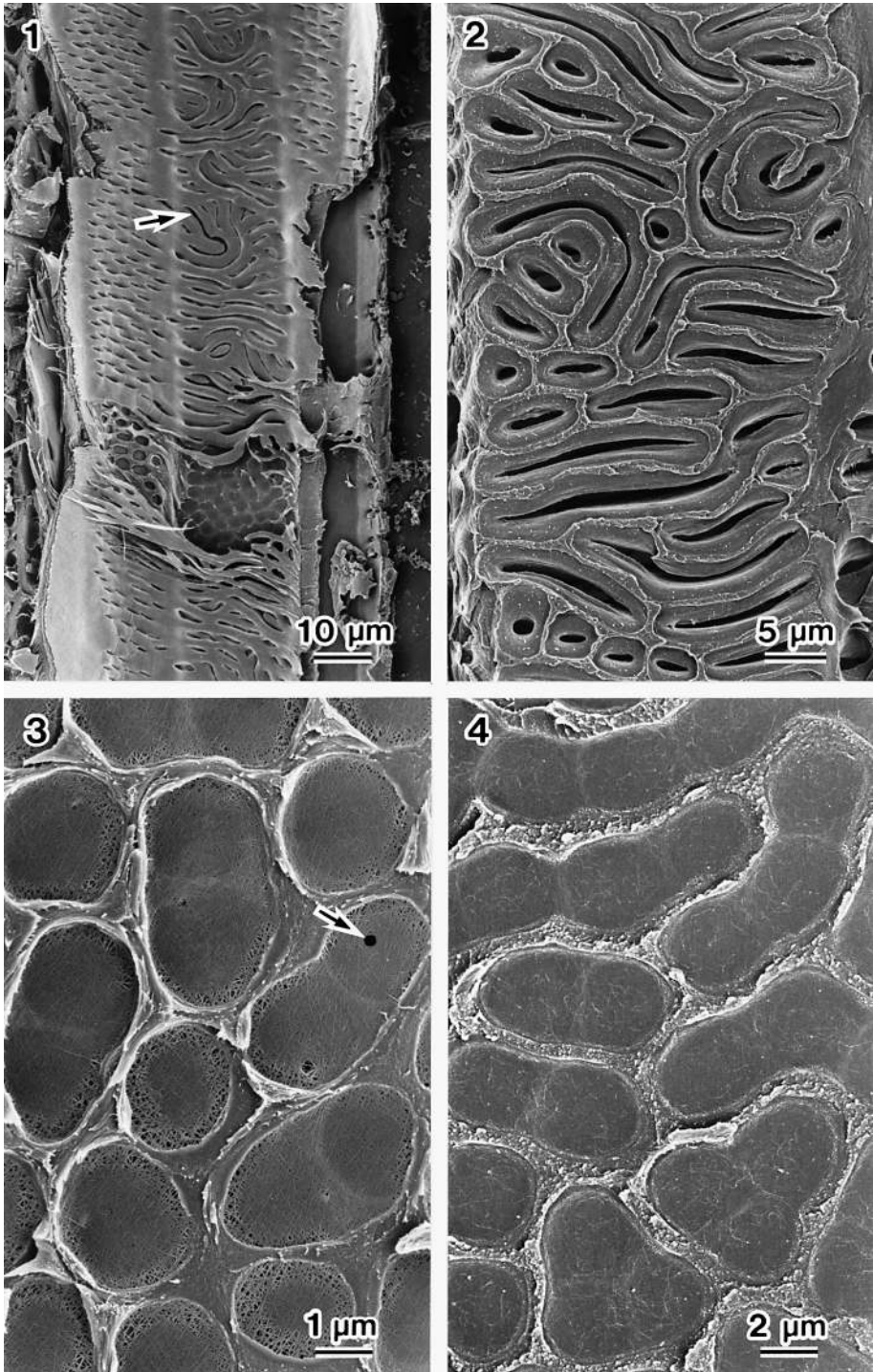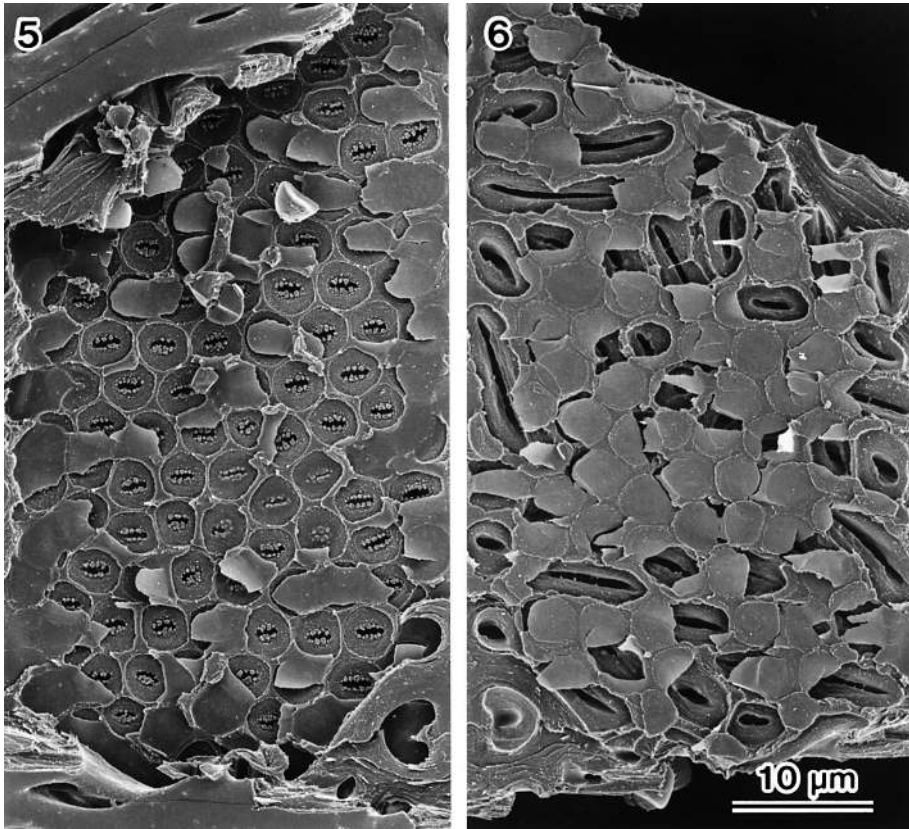
Fig. 5 & 6. A complimentary pair of fractured faces of intervascular pits across an annual ring boundary of *Fraxinus mandshurica* var. *japonica*. These images provide an example of the inner annual ring side of intervascular pits, which was rarely exposed by splitting.

Legends to Figures

Note: Among figures showing split faces, Fig. 1, 3, 7 and 16 are micrographs of air-dried samples, which were immersed in ethanol prior to drying, whereas the remainder are micrographs of freeze-dried samples, which were never soaked in ethanol.

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Fig. 1. Intervascular pits at an annual ring boundary in *Betula platyphylla* var. *japonica*, viewed from the outer annual ring side. The arrow indicates a long curved pit aperture. — Fig. 2. Pit border and outer apertures of intervascular pits located at the beginning of an annual ring in *Fraxinus mandshurica* var. *japonica*, viewed from the pit chamber side. — Fig. 3 & 4. Outer annual ring side of intervascular pit membranes located at an annual ring boundary in *Betula platyphylla* var. *japonica* (3) and *Fraxinus mandshurica* var. *japonica* (4). The arrow in Fig. 3 indicates the opening of a pit membrane.
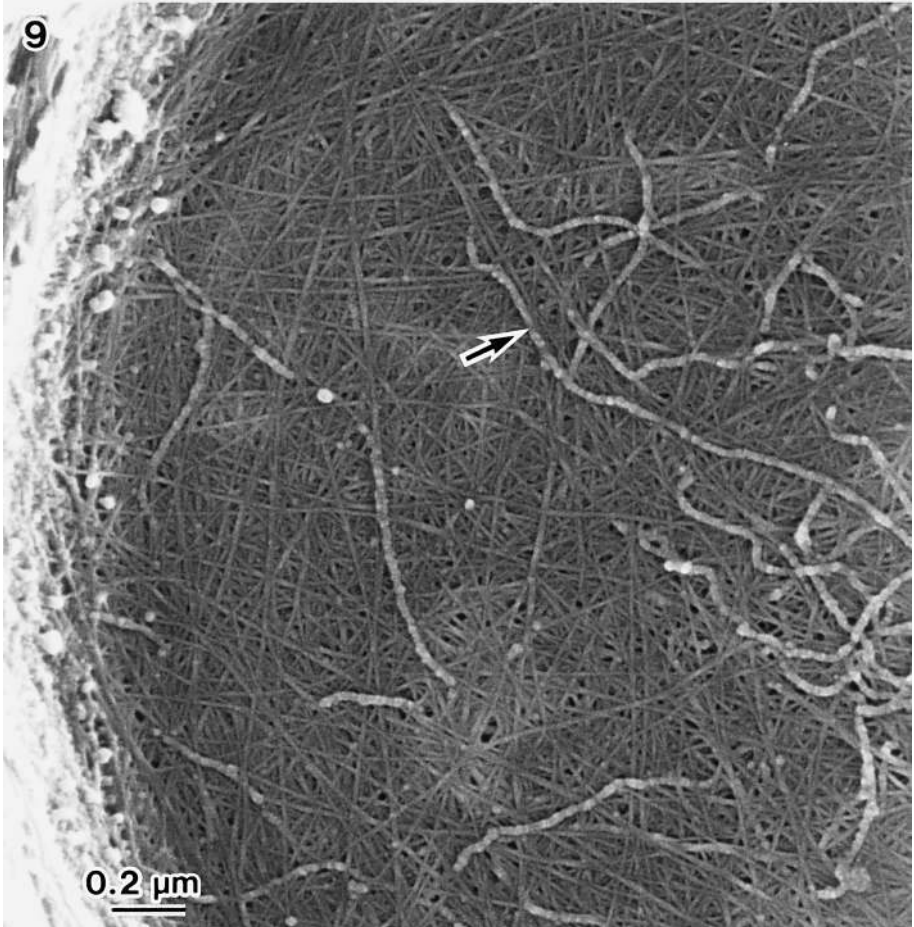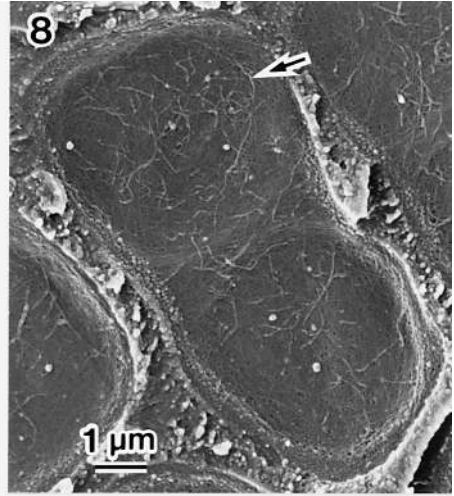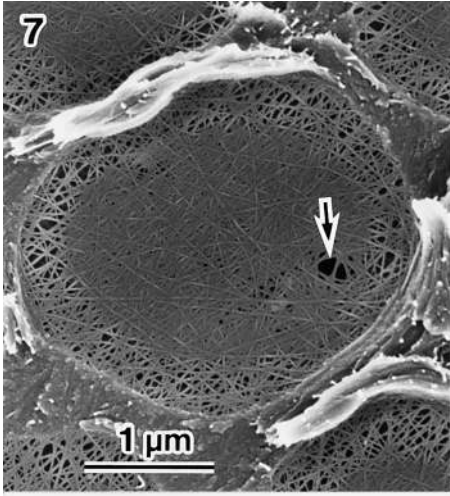
Examination of planed transverse faces

In both species examined, there were unilaterally compound pits in intervascular common walls at the annual ring boundary (Fig. 10–13). There were no unilaterally compound pits in common walls between large earlywood vessel elements or between small latewood vessel elements. The unilaterally compound pits across the annual ring boundary always consisted of one larger pit of the outer annual ring and several smaller pits of the inner annual ring (Fig. 11, 13).

Ethanol-extractable material in Betula platyphylla var. japonica

In *B. platyphylla* var. *japonica*, the intervascular pit membranes and the split faces of vessel walls around them were covered by a coating in freeze-dried specimens that had not been soaked in organic solvents during the preparation of samples (Fig. 14). This type of coating was not apparent in air-dried specimens that had been immersed in ethanol prior to drying (Fig. 1, 3, 7, 10, 11). The ethanol-soluble coating was also apparent in pit canals and pit apertures (Fig. 15 & 16) and similar ethanol-soluble material was noted on the bars of scalariform perforation plates (Fig. 17) and ray parenchyma. Thus, the aforementioned results on the fine surface structure of pits of *B. platyphylla* var. *japonica* (Fig. 1, 3, 7) were based on examinations of air-dried specimens after immersion in ethanol.

No similar differences, in terms of the texture of split faces, were detectable between the various samples of *F. mandshurica* var. *japonica*, indicating that the ethanol-soluble coating observed in *B. platyphylla* var. *japonica* was absent.

Occurrence of vestures in Fraxinus mandshurica var. japonica

The intervascular pits at the end of an annual ring in *F. mandshurica* var. *japonica* were vestured while those at the beginning of the next annual ring were not (Fig. 2, 5, 6, 12, 13). Vestures were observed at the outer apertures of the pits (Fig. 5, 13). Intervascular pits between small latewood vessels were also vestured in a similar manner (Fig. 12). By contrast, no distinct vesturing was noted in the intervascular pits between large earlywood vessel elements.

DISCUSSION

Unilaterally compound pits have been noted in the vessel-to-ray pits of hardwood species (e.g., Baas et al. 1988; Ohtani 2000), and the occurrence of vessel-to-ray unilaterally compound pitting is a feature that has been used for the identification of some hardwoods (IAWA Committee 1989). Unilaterally compound pits have also been found in the fiber-to-parenchyma pits in *Betula alleghaniensis* (Yang 1978). By contrast, this

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Fig. 7 & 8. Outer annual ring side of intervascular pit membranes located at an annual ring boundary of *Betula platyphylla* var. *japonica* (7) and *Fraxinus mandshurica* var. *japonica* (8). The arrow in Fig. 7 indicates an opening in the pit membrane. — Fig. 9. Part of the intervascular pit membrane between large earlywood vessel elements of *Fraxinus mandshurica* var. *japonica*. — The arrows in Fig. 8 and 9 indicate a fine curly fibril.
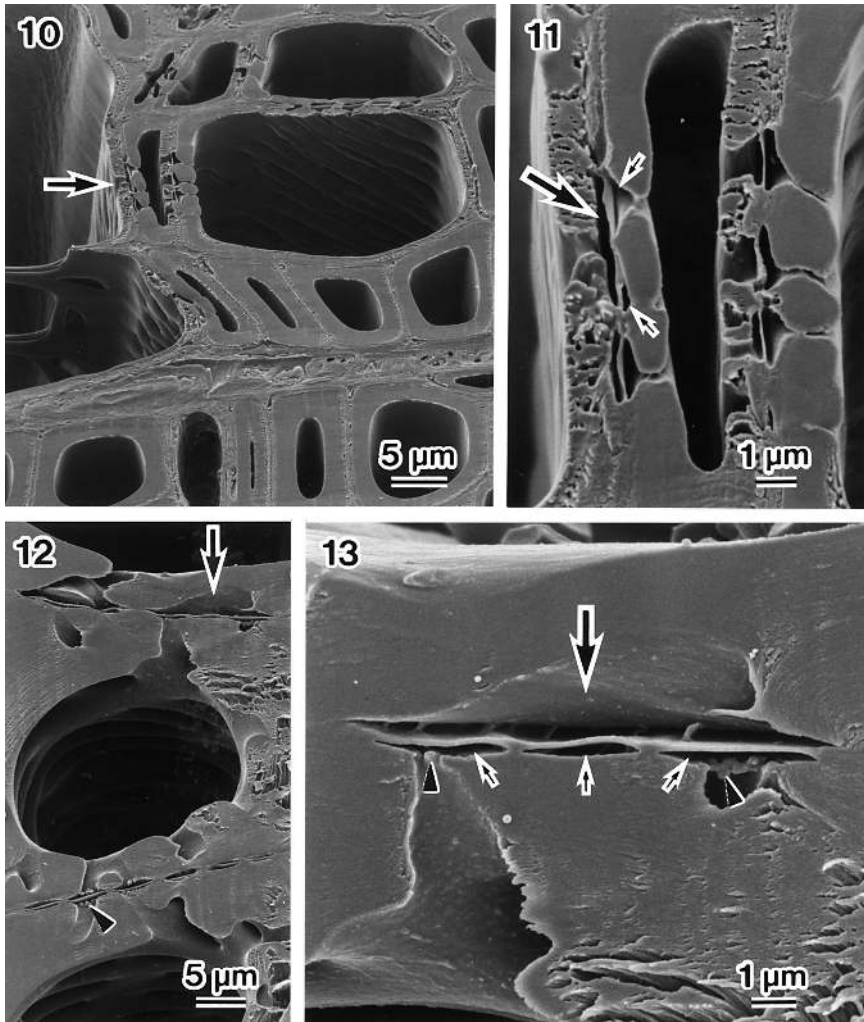
Fig. 10. Intervascular pits across an annual ring boundary of *Betula platyphylla* var. *japonica*. The arrow indicates the pit shown in Fig. 11. — Fig. 11. Detail of Fig. 10. Large and small arrows indicate, respectively, the pit chambers on the outer annual ring side and the inner annual ring side of the unilaterally compound pit. — Fig. 12. Intervascular pits across an annual ring boundary of *Fraxinus mandshurica* var. *japonica*. The arrow indicates the pit shown in Fig. 13. The arrowhead indicates vestures. — Fig. 13. Detail of Fig. 12. Large and small arrows indicate, respectively, pit chambers on the outer annual ring side and the inner annual ring side of an unilaterally compound pit. The arrowheads indicate vestures.

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Fig. 14. Intervascular pits located in a single annual ring of *Betula platyphylla* var. *japonica*. Freeze-dried sample. — Fig. 15 & 16. Intervascular pits located in a single annual ring of *Betula platyphylla* var. *japonica*, viewed from the lumen side. A freeze-dried sample (15) and an air-dried sample (16) are shown. The arrow in Fig. 15 shows a pit aperture that is partially obstructed by an ethanol-soluble coating — Fig. 17. A scalariform perforation plate of a freeze-dried sample of *Betula platyphylla* var. *japonica*. The arrow shows material that was not found in air-dried samples.
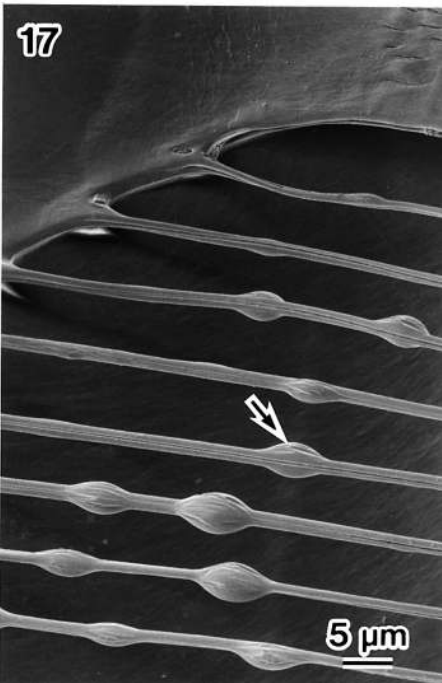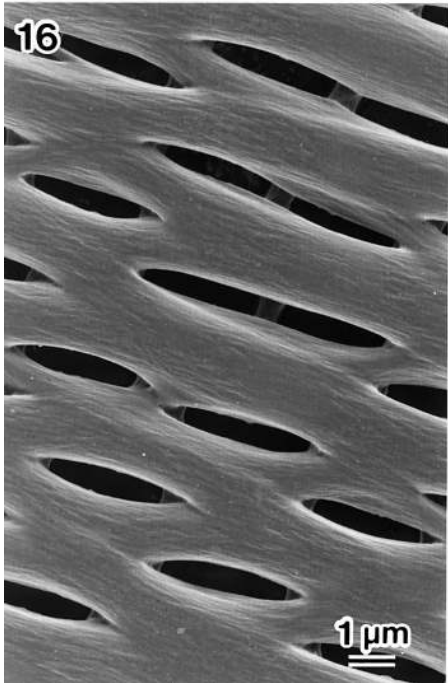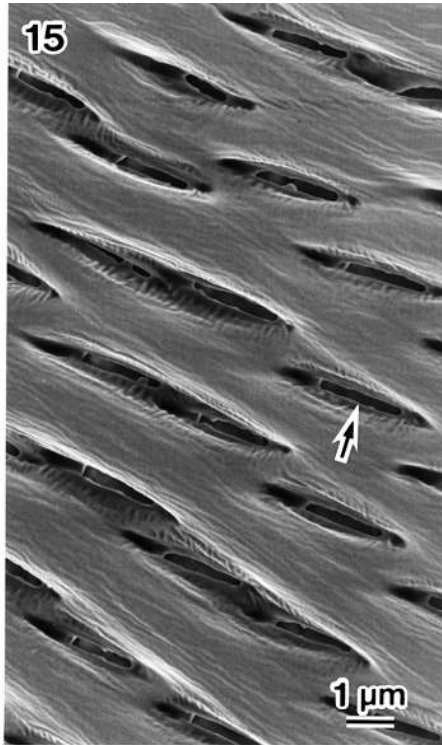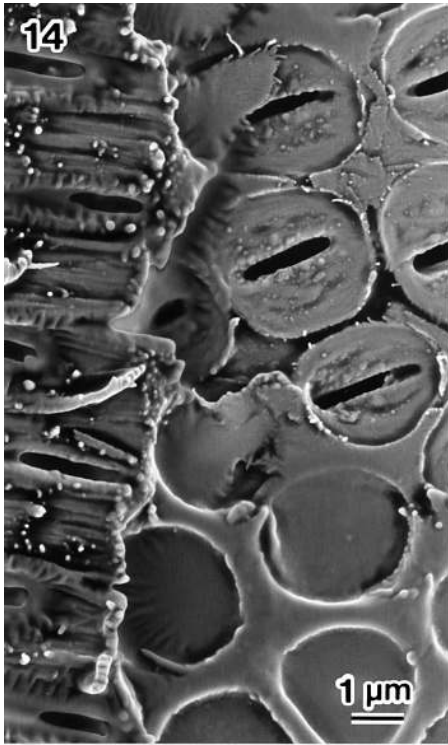
type of pit seems rare in common walls between the same kinds of element. There are no reports, to the author's knowledge, of this type of pitting in intervascular pits. However, it is not surprising that this type of pit should occur between vessel elements since such pits are similar to dimorphic perforation plates, with the exception that the perforation plates lack pit membranes.

Unilaterally compound pitting was confined to the intervascular pits across the annual ring boundary. Needless to say, elements at the end of one annual ring and those at the beginning of the next annual ring are formed at different times. Such differences in timing might be a condition for the formation of unilaterally compound pits. It seems likely that the later-forming cells cannot detect exactly the site at which the secondary wall has been deposited in adjacent mature cells.

The unilaterally compound pits observed in the present study might correspond to the 'anomalous pits' described by Carlquist (2001, fig. 3.14.3). The elongated and curved nature of the pit aperture of an anomalous pit in *Euphorbia anastomosans* (Onagraceae) resembles that of the outer annual ring side of the unilaterally compound pits found in the present study. In Carlquist's micrograph of 'anomalous pits', there are smaller, rounded to slightly polygonal compartments in each of the elongated and curved outlines of pits (Carlquist 2001, fig. 3.14.3). If the unilaterally compound pits found in the present study were to be viewed under a light microscope, the pit pairs might resemble these anomalous pits.

Early studies of the fine structure of hardwood pit membranes, notably those of Côté (1958), Harada et al. (1958), Schmid (1965) and Schmid & Machado (1968), consistently showed the absence of visible openings that could be resolved by transmission electron microscopy. However, openings have been detected in intervascular pit membranes of hardwoods in the genera *Liriodendron* (Bonner & Thomas 1972), *Pseudowintera* (Meylan & Butterfield 1982), *Acer* (Wheeler 1982; Sperry & Tyree 1988) and *Daphne* (Dute et al. 1992) both by transmission and scanning electron microscopy. Thus, previous research and the present study suggest that the porosity of intervascular pit membranes varies among species.

According to the 'air-seeding' model that was proposed by Zimmermann (1983) and supported by subsequent studies (e.g., Sperry & Tyree 1988; Tyree & Zimmermann 2002), the susceptibility to the progression, from one cavitated conduit to the adjacent water-filled conduit, of water stress-induced cavitation increases with increases in the maximum size of the pores in the pit membranes that are located between the two conduits. This relationship suggests that *Betula platyphylla* var. *japonica* is more susceptible to the progression of water stress-induced cavitation across annual ring boundaries than is *Fraxinus mandshurica* var. *japonica*. However, a mechanism exists in *B. platyphylla* var. *japonica* for refilling previously cavitated vessels in springtime before leaf expansion (Utsumi et al. 1998). By contrast, in *F. mandshurica* var. *japonica*, earlywood vessels are not refilled (Utsumi et al. 1996). Accordingly, it is possible that, in *B. platyphylla* var. *japonica*, the ability to refill cavitated vessels balances out the greater susceptibility to the progression of cavitation. Conversely, denser intervascular pit membranes might play a role in overcoming the absence of any ability to refill previously cavitated conduits in *F. mandshurica* var. *japonica*.

In *F. mandshurica* var. *japonica*, vestures were present at and around the outer apertures of intervascular pits of small latewood vessels but not of those of large earlywood vessels (Fig. 5, 6, 12, 13). Reports of the presence or absence of vestures in the genus *Fraxinus* are inconsistent (Jansen et al. 1998). Ohtani (1983) reported that exclusively non-vestured pits were present in all of four species of *Fraxinus* examined. By contrast, there are other reports that intervascular pits are distinctly or slightly vestured in some species of *Fraxinus*, with not all the intervascular pits being vestured (Wheeler 1981; Baas & Zhang 1986; Baas et al. 1988). Such inconsistencies might be due largely to differences in the locations of vessels in individual annual rings. Alternatively, cell size might be associated with the presence or absence of vestured pits in one species (e.g., Jansen et al. 2000).

The nature of the fine curly fibrils that were noted in the intervascular pit membranes of *Fraxinus mandshurica* var. *japonica* is unclear. In a previous study, using a conventional scanning electron microscope equipped with a thermoelectronic emission-type electron gun, Sano and Fukazawa (1993) failed to detect such fine fibrils in the intervessel pit membranes of this species. The failure was due, most probably, to a thicker coating of metal on samples and to a higher accelerating voltage. Wheeler (1981) examined the fine structure of intervascular pit membranes and the seasonal changes in their appearance in *Fraxinus americana* by transmission electron microscopy. Wheeler also did not mention the presence of any fine curly fibrils. Indeed, to the author's knowledge, no such fine fibrils have been recorded in any type of pit membrane of any other species. Further studies are required to determine the nature of these fine fibrils, as well as the extent of their occurrence.

The chemical components and the role of the ethanol-soluble material found in the intervascular pits, scalariform perforation plates and ray parenchyma of freeze-dried specimens of *Betula platyphylla* var. *japonica* remain to be determined. It seems likely that the coating is somewhat fluid since it covered not only the intrinsic surfaces of cell walls but also the faces exposed by splitting (Fig. 14). It is possible that the coating appears seasonally since it has been reported that, in *Fraxinus americana*, a thick layer of impervious material covers the intervascular pit membranes in winter (Wheeler 1981). However, the solubility in various solvents differs between the coating in *B. platyphylla* var. *japonica* and that in *Fraxinus americana*. Wheeler (1981) reported that in *Fraxinus americana* such coating was not extracted by organic solvents (methanol and acetone). Further studies are required to explore the nature of the coating and to evaluate its influence on the flow of water in those species that have such material in their water-conduction systems.

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