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The effect of SEM preparation on pit membrane remnants in vessel element end-walls of primitive angiosperms

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ABSTRACT

Scanning electron microscope (SEM) is necessary to demonstrate presence or absence of pit membranes in possible perforations or the type of pit membrane remnants in perforations in vessel element end-walls of angiosperms, but it was unconfirmed and questionable whether pit membrane absence in pits was affected by the processing and handling before SEM observations. To solve this question, the secondary xylem of four woody species from primitive angiosperms, *Illicium henryi* Diels. (Illiciaceae), *Schisandra rubriflora* (Franch.) Rehd. et Wils. (Schisandraceae), *Tetracentron sinensis* Oliv. and *Trochodendron aralioides* Sieb. & Zucc. (Trochodendraceae) was chosen and the following techniques were used: (1) fresh materials were examined in low-vacuum with ESEM. (2) Air-dried materials were examined both in low- and high-vacuum with ESEM. (3) Fresh materials fixed in several different fixatives were observed in low-vacuum, respectively. (4) Smooth surface of the material by paraffin section methods was examined in high-vacuum. (5) Materials treated by Jeffrey's Fluid were observed in high-vacuum.

Pit membranes and remnants in perforations of fresh material were little different from that of treated materials. Absence of the pit membrane in perforations (pits) in the end-wall was not attributed to the processing and handling. Information of pit membranes and remnants in perforations in end-wall based on the SEM observation might be validly claimed.

Introduction

SEM (scanning electron microscope) has become a necessary tool to demonstrate the presence or absence of pit membrane in possible perforations or the variation of pit membrane remnants in perforations of vessel elements end-walls of angiosperms since the very beginnings of SEM use observing perforation plates (Butterfield and Meylan, 1972; Fagerlind and Dunbar, 1973; Meylan and Butterfield, 1978; Ohtani and Ishida, 1978). Pit membrane and remnants can be demonstrated much more clearly with SEM than with light microscope. Thus, vessel elements were discovered also in some angiosperms supposed to be vesselless, e.g., *Sarcandra* of Chloranthaceae (Carlquist, 1987; Takahashi, 1988), *Tetracentron* of Trochodendraceae (Ren et al., 2007), and some species of Nymphaeaceae (Schneider and Carlquist, 1995a,b). However, it was not unequivocal whether the absence of pit membranes in perforations (pits) in end-wall could have been affected by processing and handling before SEM observation. Absence of a pit membrane in the pit is a critical character for defining a vessel element as different from tracheids in the secondary xylem (Bailey, 1944; Frost, 1930) and the occurrence of membrane remnants may indicate an intermediate form between vessel elements and tracheids (Carlquist, 1992). The discoveries cited above have been credited as natural occurrences (Carlquist and Schneider, 2002a), but the debate still exists whether the absence of a pit membrane observed with SEM is an artifact of preparation or not. There were some studies testing preparation effects on pit membrane demonstration by SEM, but their focal points were the fine structures of pit membrane itself (Jansen et al., 2008, 2009; Shane et al., 2000) rather than type of pit membrane remnants or whether a pit membrane was present at all. Carlquist and Schneider (2002a) had tried to distinguish between damaged and natural remnants, but the processing and handling were not examined experimentally. So, the aim of the present study was to prove whether the absence of a pit membrane in possible perforations on end-walls of xylem elements is due to the processing and handling before SEM or evidence for missing intervessel pit membranes.

Four woody species from primitive angiosperms (basal angiosperms and basal eudicots) were involved in the present studies. *Illicium henryi* Diels of Illiciaceae and *Schisandra rubriflora* (Franch.) Rehd. et Wils. of Schisandraceae (basal angiosperms) are vessel-bearing. They were chosen because pit membrane remnants are commonly present in the perforation on the end-walls of ves-

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Table 1
Pit membranes and/or remnants in end walls of vessel elements observed in different treatments and species.

Treatment	*Illicium henryi*		*Schisandra rubriflora*		*Tetracentron sinense*		*Trochodendron aralioides*	
	Pit membrane remnant	Fig. 1	Pit membrane remnant	Fig. 2	Pit membrane (remnant)	Fig. 3	Pit membrane (remnant)	Fig. 4
Fresh, low-vacuum	Strip-like	A	Porous, thread-like	A	Intact, strip-like, debris	A, B	Intact, strip-like	A
Air-dried, low-vacuum	Strip-like, thread-like	B	Strip-like	B	Intact, strip-like	C	Intact, flake-like	B, C
Air-dried, high-vacuum	Strip-like, thread-like	C	Strip-, thread-, flake-like	C	Intact, strip-, thread-, flake-like	D, E	Intact, strip-like	D, E
70% alcohol fixed, low-vacuum	Strip-like	D	Strip-like	D	Intact, strip-like	F	–	–
FAA fixed, low-vacuum	Strip-, thread-like	E	Strip-like	E	Intact, thread-like	G	Intact, flake-like	F
Karnovsky's fixation, low-vacuum	Thread-like	F	Strip-, thread-like	F	Intact, thread-like	H	–	–
Technique of paraffin section, high-vacuum	Intact, strip-, thread-, flake-like	G, H	Thread-like	G	Intact, strip-, thread-, flake-like	I, J	Intact, strip-, thread-, flake-like	G
Jeffrey's Fluid treated, High-vacuum	Strip-like, porous	I, J	Thread-like	H	Intact, strip-, thread-, flake-like	K, L	Intact, thread-like	H, I

Note: –, not observed.

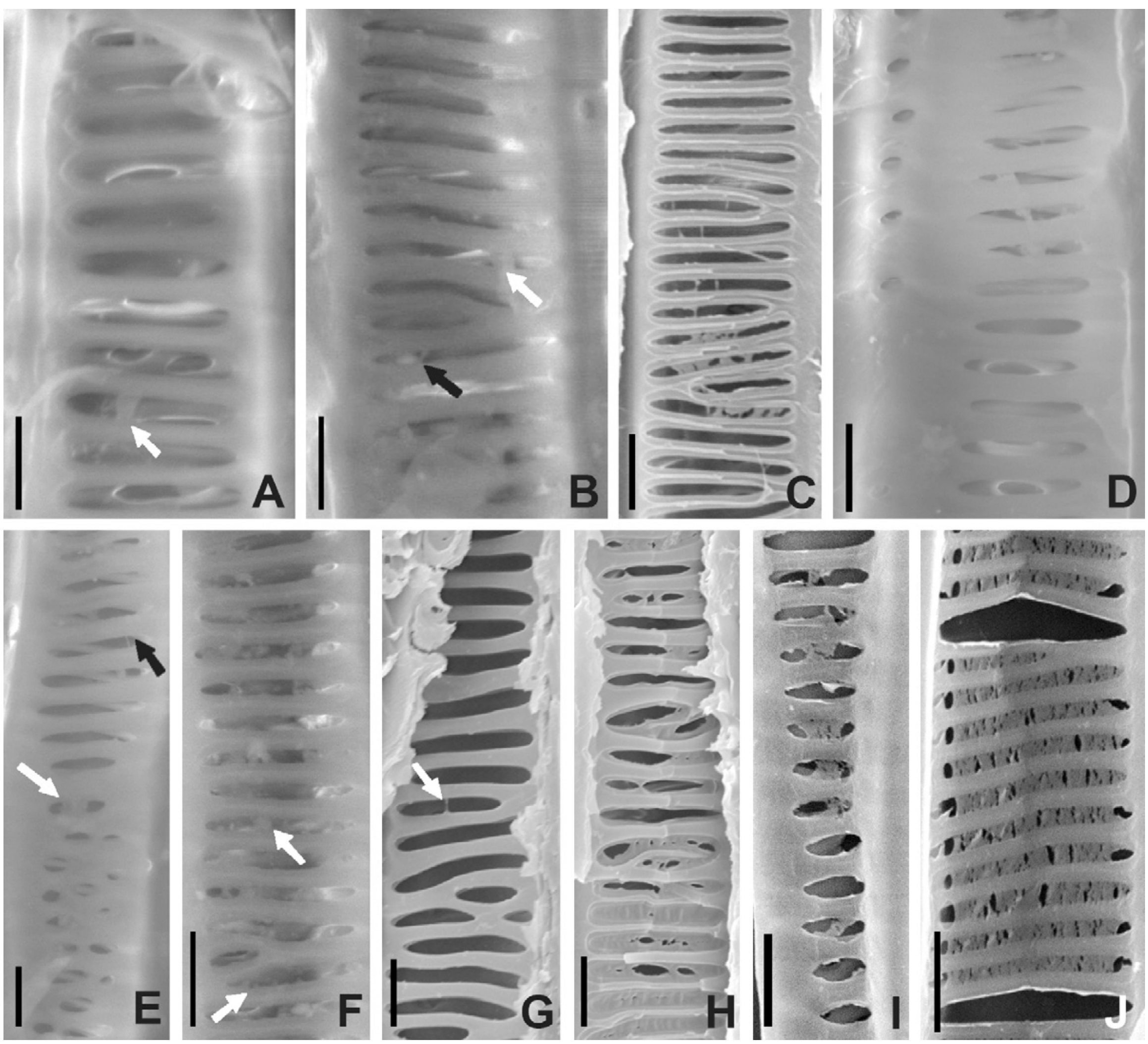

Fig. 1. SEM micrographs of perforation plates in vessels from secondary xylem of *Illicium henryi*, showing pit membrane remnants in different treatments with ESEM. (A) From fresh materials in low-vacuum, strip-like remnants in perforations (arrow). (B) From air-dried materials in low-vacuum, strip-like (white arrow) and thread-like (black arrow) remnants in perforations. (C) From air-dried materials in high-vacuum, showing different transition remnants. (D) From fixed materials in 70% ethanol in low-vacuum, strip-like remnants in perforations. (E) From fixed materials in FAA in low-vacuum, strip-like (white arrow) or thread-like (black arrow) remnants in perforations. (F) From fixed materials in Karnovsky's Fixative in low-vacuum, showing different remnants in perforations (arrows). (G and H) From technique using paraffin sections. (G) Threadlike remnants in perforations (arrow); (H) variations of remnants. (I and J) From materials that were treated by Jeffrey's Fluid. (I) Pit membrane remnants in elliptical perforations; (J) perforation plant portion with more than half area covered by membrane remnants. Scale bars = 10 μm.