Equisetum Xylem: SEM Studies and their Implications

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ABSTRACT.—Scanning electron microscope (SEM) studies of xylem of three species of *Equisetum* reveal numerous details not previously reported on the basis of light microscopy. SEM images of thick (ca. 1 mm) sections reveal pit shapes, cell contexts, and microstructure of pit membranes. Pit shapes are remarkably diverse in comparison to those of ferns or conifers. Nodal tracheary elements are isodiametric to fusiform in shape, and have crowded circular (mostly) to elongate prominently bordered pits and uniformly thick secondary walls. Internodal tracheary elements, by contrast, have relatively large circular pits with inconspicuous borders. Secondary walls of internodal tracheids are thin, with thickenings that are annular, looplike, or of some intermediate form. Metaxylem internodal tracheary elements line the inner surface of carinal canals (= proxylem lacunae), and many of the large circular (often crateriform) pits facing the canals may lack pit membranes, especially in *E. giganteum* and *E. myriochaetum*. Because dye experiments show that carinal canals can conduct water in stems of *Equisetum*, portions of tracheary elements facing the canals may have perforations (many of the pits in *E. myriochaetum* lack pit membranes despite careful handling techniques). This opens the possibility that internodal tracheary elements may, in some species, be vessel elements that permit conduction from the carinal canals of one internode to those of the next internode (carinal canals are not intercontinuous between internodes), aided by metaxylem tracheids of the nodal plates. Such vessel elements would not be the same as those reported by Bierhorst, who found vessels only a few vessel elements long in rhizomes.

KEY WORDS.—carinal canal, conduction, pit dimorphism, tracheids, vessel elements

Bierhorst (1958a, 1958b) offered careful light microscope observations and figures of *Equisetum* xylem. Fine though that work was, it invites additional observations. He did not study the genus worldwide—he concentrated on temperate North American species. He used thin sections, which, by their nature cannot offer three-dimensional views of tracheary elements, individually and in groups. The contours of secondary wall thickenings can be examined only to a limited extent by light microscopy. The appearance and fine structure of pit membranes can be seen clearly in face view with scanning electron microscopy (SEM), whereas staining and high power light microscopy are limited in what they can show. Bierhorst's observations have not hitherto been supplemented by SEM studies.

SEM reveals a hitherto unappreciated diversity and distribution of pit sizes, shapes, pit border shapes, and secondary wall thickenings. By studying these types with relation to location in the shoot systems, we are able to see distribution of tracheary elements types and pitting that suggest patterns of

water conduction in xylem and in the carinal canals. There is considerable polymorphism of tracheary elements within any given section, a diversity that shows that exploration within a plant body of a species of *Equisetum* is justified at present. Our results can point the way, however, to what genus-wide studies might be useful in the future, and what tracheary element features might deserve intense examination with SEM and with transmission electron microscopy (TEM). The diversity of pitting on *Equisetum* tracheary elements is not only considerable, it exceeds the diversity seen in ferns.

SEM is labor-intensive, and the limited observations of the present study cannot be monographic in focus; they are, rather, an exploration of xylary diversity in *Equisetum* xylem. We have selected three species that are taxonomically not closely related, according to the data of Hauke (1978). Plants of *Equisetum hyemale* L. have unbranched aerial stems with terminal strobili, and are typically a meter or less tall; the species belongs to subgenus *Hippochaete*. *Equisetum myriochaetum* Schlecht & Cham. is one of two species of giant horsetails (the other is *E. giganteum*), with plants to five meters tall, in *Equisetum* subgenus *Equisetum*. *Equisetum telmateia* Ehrh. is branched, less than 1 m tall, and belongs to subgenus *Equisetum*. Subgenus *Hippochaete* has strobili at tips of chlorophyllous branches, whereas subgenus *Equisetum* possesses strobili on achlorophyllous branches; the chlorophyllous branches are sterile. These and other distinctions of the subgenera and species are covered in detail by Hauke (1978) and Guillon (2004). We were able to study both aerial and underground stems of *E. hyemale* and *E. telmateia* subsp. *Braunii* (Hilde) Hauke. The rhizomes are slender and elongate in *E. hyemale*, whereas in *E. telmateia* they are comparable in thickness to the aerial stems.

Equisetum myriochaetum is distinctive by virtue of the fact that most metaxylem tracheary elements are located on the adaxial or inner surface of the carinal canal (= protoxylem lacuna). Few metaxylem tracheary elements occur within the xylem embedded between the pair of phloem strands. In the other species, the reverse is true: most of the metaxylem elements are flanked by the two phloem strands, and a relatively small number (plus protoxylem) occur lining the carinal canal. The occurrence of metaxylem lining the carinal canal has interesting implications for conduction. Studies testing uptake with the use of dyes have shown that the carinal canals are filled with water and do function in upward conduction (Buchholz, 1921; Bierhorst, 1958b). Because of these observations, the potential role of xylem elements facing the canal becomes a question of importance. The carinal canals of one node are not interconnected with those of the next node above: they alternate in successive nodes, and terminate at the tops and bottoms of nodes. Thus, if the carinal canals play a role in conduction, the metaxylem tracheary elements must be responsible for conduction across nodal regions. The conductive pathways in *Equisetum* stems may thus involve carinal canals, internodal tracheary elements, and nodal metaxylem working in conjunction. This has implications for interpretation of tracheary elements as seen by SEM.

Material and Methods

Aerial stems and rhizomes collected for study were cut into nodal and internodal segments and preserved in 50% ethanol. The collections documenting this study are: *E. hyemale* L.: CA, Santa Barbara Co., ditch adjacent to road, Parma Park on Highway 192 north of Santa Barbara, March 18, 2010, *S. Carlquist & E. L. Schneider s.n.* (SBBG); *E. myriochaetum* Schlecht. & Cham.: CA, Alameda Co., cultivated in University of California Botanical Garden greenhouses, Berkeley, (accession number 78.00364); *E. telmateia* Ehrh. subsp. *brauni* (Hilde) Hauke ("*E. telmateia*" in this paper): CA, cultivated near visitor entrance of Santa Barbara Botanic Garden, and adventive there, 25 March 2010, *S. Carlquist s.n.* (SBBG).

Thin longisections of nodes of *E. hyemale*, made into permanent slides in 1958 by the senior author, and transections of E. *giganteum* L. prepared by A. W. Haupt were studied with light microscopy. Free-hand longitudinal sections about 1 mm in thickness were prepared by use of single-edged razor blades. These sections were subjected to three changes of distilled water to remove water-soluble substances and to remove loose starch grains and surface contaminants. Sections were then placed between pairs of clean glass slides with pressure applied by clips to assure flatness and air dried at 60° C on a warming table. Sections were sputter coated with gold and examined with a Hitachi S2600N SEM.

Thick sections cut by hand (e.g., Carlquist and Schneider, 2007; Schneider and Carlquist, 2007) have proved preferable to thin sections cut by microtome because damage from handling is minimized. Thick sections also permit one to see three-dimensional structure of tracheary element surfaces and to see cell groupings, such as the relationship of tracheary elements to each other and to carinal canals.

The term "tracheary element" is used here as an inclusive term for both tracheids and vessel elements, because the entirety of a tracheary element was not visible, and thus we could not designate a xylem cell with certainty as either a tracheid or a vessel element. Bierhorst (1958b) reported a few vessel elements in the rhizomes of several species (based on seeing tracheary element end walls in sectional view), but our findings suggest that vessel elements may be present more widely, hence our choice of the more inclusive term "tracheary element."

Results

Internodal tracheary elements.—Pits vary greatly within a stem or rhizome of *Equisetum*, depending mostly on location. Tracheary elements may be divided into nodal and internodal. Internodal metaxylem tracheary elements are elongate, and have secondary walls that appear to have annular thickenings forming bands (note contrast between Figs 1 and 2 and Fig. 3A, B) not unlike those of protoxylem (Fig. 1F). Sometimes the bands are looplike or helical
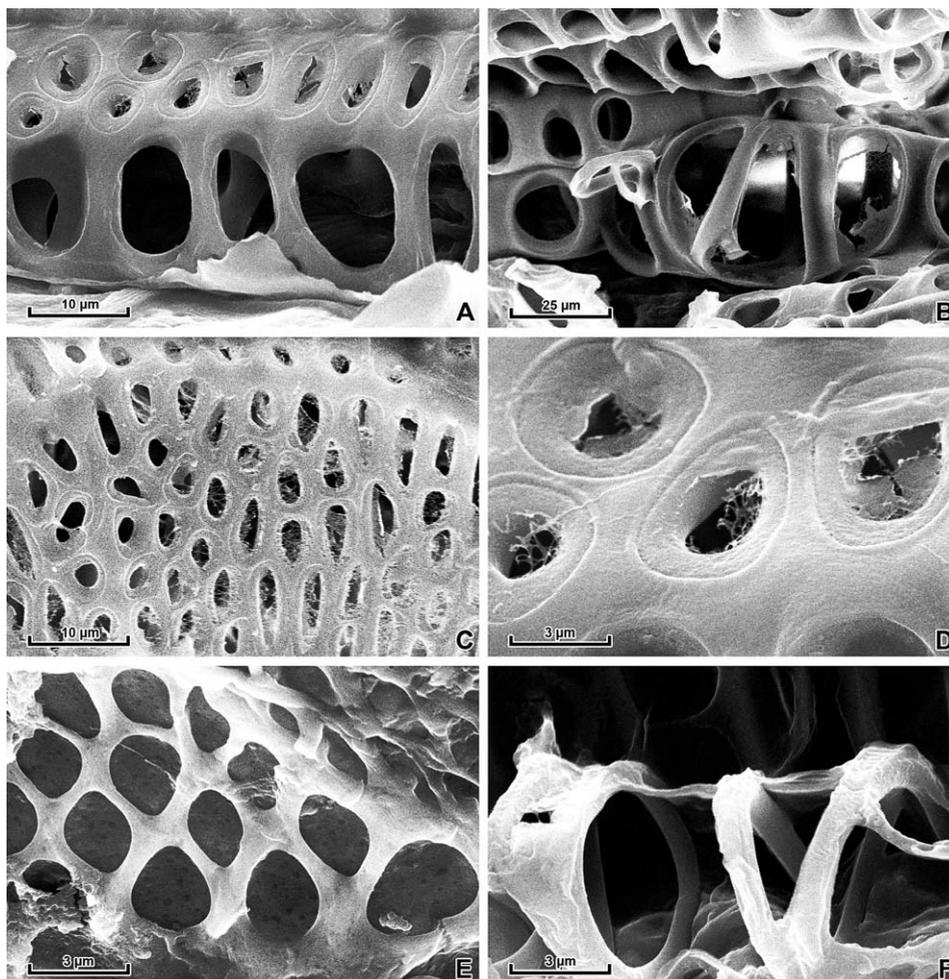
FIG. 1.   SEM micrographs of longisections of stems (A–D) and rhizome (E–F) to show tracheids of *Equisetum hyemale.* A) Internodal tracheid, two adjacent facets that show marked difference in size, shape, and border width of pits. B) Portions of several internodal tracheids; pits are markedly unlike in size and shape. C) Tracheid from node; pits are densely placed. D) Pits from tracheid at node; sectioning has scraped away pit membrane material to various extents, so that amorphous material shows in the upper pit, but the cellulosic reticulum is revealed on the lower pits; note wide borders. E) Metaxylem tracheid portion from node; the densely-placed pits have minute depressions in pit membranes. F) Protoxylem tracheid from node; sectioning has removed part of the primary wall between the annuli. Scales, 10 µm (A, C); 25 µm (B); 3 µm (D–F).

rather than annular (Fig. 3A–C). Pits were observed to lie between such bands. Pits have narrow, but perceptible borders.

Large circular pits, often crateriform in shape, may be found along the surfaces of internodal metaxylem tracheary elements that face carinal canals (Fig. 2A–D, Fig. 3B–D). The three-dimensional shape of these pits not been
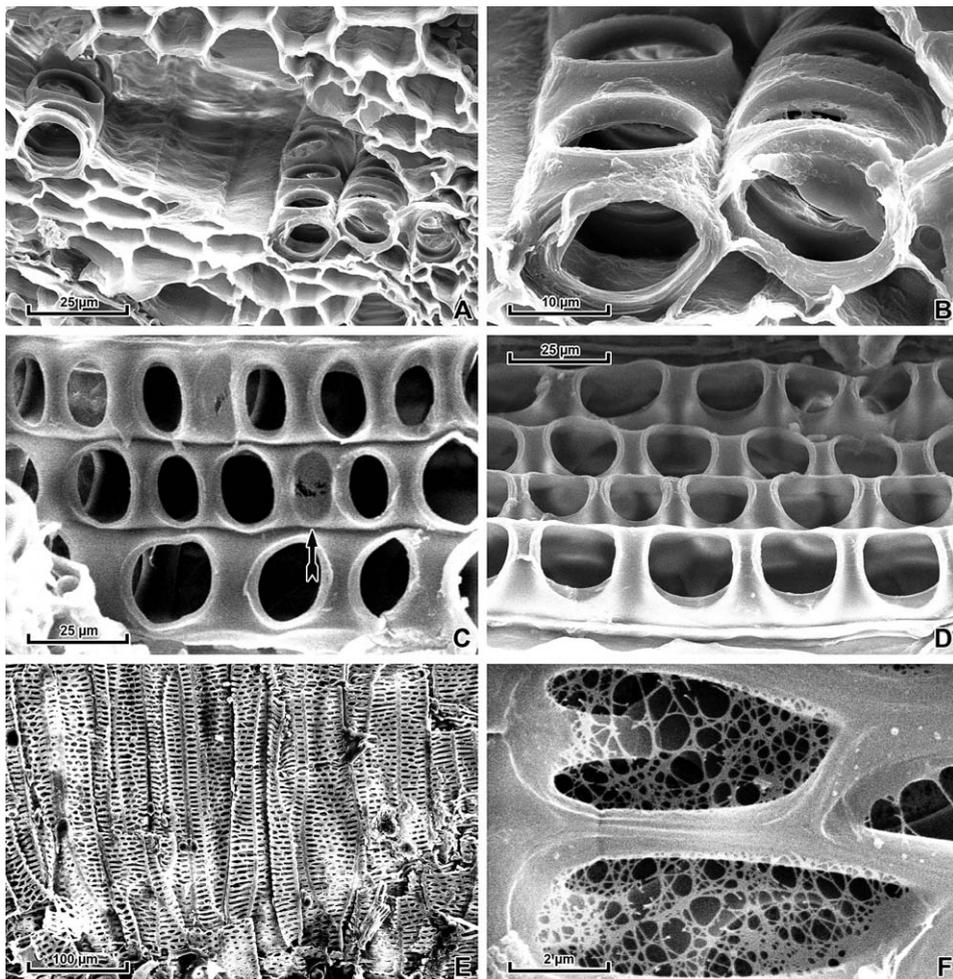
Fɪɢ. 2. SEM micrographs of internodal (A–D) and nodal (E–F) metaxylem tracheids from stem longisections of *Equisetum myriochaetum.* A) Oblique view of carinal canal from end of section; portions of three tracheids (one at center left, two at lower right) face the canal. B) Photo at higher magnification of pits from A, to show crateriform shape of pits (above) and wall thickness of tracheids (below). C) View of the surface of tracheids that face a carinal canal; most pits lack pit membranes, but a few smaller pits (e.g., arrow) retain pit membranes. D) Somewhat oblique view of the portions of several tracheids, facing a carinal canal; all pits shown lack pit membranes. E) Tracheids from nodal region; pits are densely placed. F) Portions of adjacent nodal tracheid; the cellulosic reticulum of the pit membranes is revealed by the sectioning process. Scales, 25 μm (A, C); 10 μm; 100 μm (E); 2 μm (F).

described hitherto, probably because it is obviously only when imaged by means of SEM. The crateriform pits mostly lacked pit membranes in our preparations, except for those depicted in Fig. 3C. These pits should not have been affected by sectioning, because they lie within the carinal canals, and
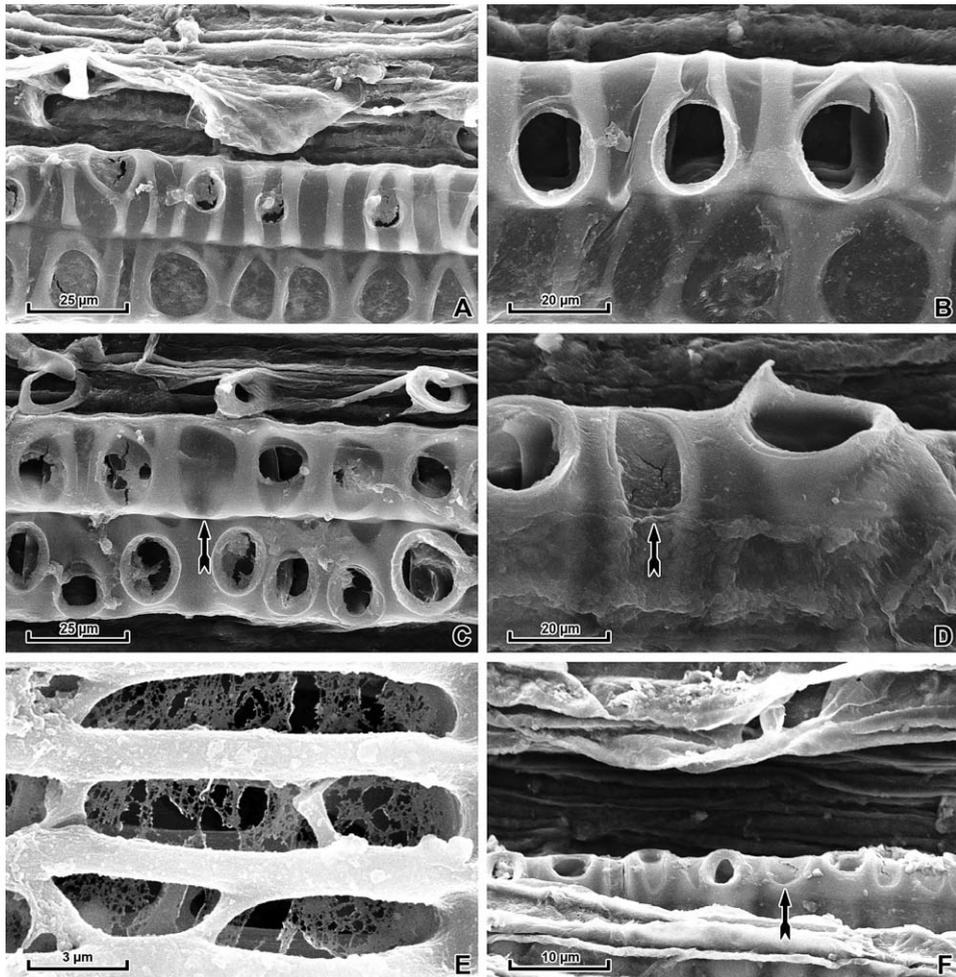
F$_{IG}$. 3.   SEM micrographs of internodal (A–D, F) and nodal (E) tracheids from stem (A–E) and rhizome (F) longisections of *Equisetum telmateia.* The tracheid surfaces depicted in A–E and F face carinal canals. A) Portions of two adjacent tracheids; pits are smaller, and lie between wall annuli in the upper tracheid. B) Three pits lacking pit membranes, located between annular wall thickenings. C) A protoxylem tracheid (top) and, below it, two metaxylem tracheids; Pit membrane portions are present in the metaxylem tracheids, only the pit indicated by the arrow has a pit membrane. D) Two crateriform pits lacking pit membranes (= perforations?) and, between them, a non-crateriform pit (arrow) with an intact pit membrane. E) Pits from cut-away surface of nodal tracheid; reticulate cellulosic pit membrane portions are revealed. F) Portion of internodal tracheid that faces a carinal canal (the canal, center of photograph, is dark gray). A non-crateriform pit with an intact pit membrane (arrow) and four crateriform pits that lack pit membranes (= perforations?) are shown. Scales, 25 μm (A, C); 20 μm; 3 μm (E); 10 μm (F).

were exposed to view but not touched by the sectioning process. Somewhat narrow, non-crateriform pits were observed occasionally on the surfaces of internodal tracheary elements that face carinal canals. These non-crateriform pits were observed to retain pit membranes (Fig. 2C, 3C, 3D, 3F, as indicated by arrows). The pit membranes of the non-crateriform pits appeared to be thinner and more easily ruptured than the secondary wall portions that lie between the annular thickenings.

The precise location of particular tracheid types in the nodal-internodal continuum is best studied with light microscopy and was not a goal of the present study.

Nodal tracheary elements.—Nodal metaxylem tracheary elements have more densely placed pits, and the pits tend to be polygonal to elongate, with wide borders (Fig. 1C–D, 2E–F). Strips of secondary wall material were observed to traverse or subdivide some elongate pits (Fig. 3E).

Pit membranes.—Pit membranes of metaxylem tracheary elements that are not affected by sectioning appear to be uniform in thickness and non-porose, except for the metaxylem tracheary elements depicted in Fig. 1E and Fig. 3E. However, sectioning often did separate, remove, or partially scrape away layers of the pit membranes. Where that happened, the amorphous layer between adjacent tracheids was partly or wholly lacking, and a reticulum of cellulosic fibrils was revealed (Fig. 1C, 1D, 2F). The pits at upper left in Figure 1D retain the amorphous materials (fracturing in the pit membrane is an artifact).

Stretched primary walls were observable on protoxylem tracheary elements (Fig. 1F, 3C, top). In both of these instances, the front half of the primary wall has been sectioned away, affording a view of the inside of the primary wall. In neither instance was any cellulosic network exposed, even on the sectioned edge of the primary wall (Fig. 1F, above).

DISCUSSION AND CONCLUSIONS

Pit polymorphism.—All of the species of *Equisetum* studied, despite their differing habits, share the basic plan, in both aerial stems and underground stems (rhizomes) of two distinctive types of metaxylem tracheary elements: internodal and nodal. The former are markedly elongate, with narrowly bordered circular pits and walls thickened with annular bands or variations thereof. Nodal tracheary elements, by contrast, are isodiametric to moderately elongate and have uniformly thick walls and densely arranged pits (smaller than those of the internodal tracheids) angular to circular in shape, with wide borders (Bierhorst, 1958a, 1958b). Tracheary elements with intermediate characteristics may be found at the juncture between nodal and internodal xylem. The dimorphism in tracheary element types is striking, because one does not, for example, find such dimorphism in angiosperms with basal meristem growth, such as bamboo (Carlquist and Schneider, 2011).

The dimorphism of metaxylem tracheary elements may be related to conductive patterns. The nodal tracheary elements are placed so as to

interconnect xylem of the node above with xylem of the node below and with the xylem of lateral branches, if any are present. Barratt (1920), Jeffrey (1899) and Eames (1936, p. 97) give helpful three-dimensional reconstructions of these xylem patterns. Nodal tracheary elements are relatively strong and undergo virtually no elongation, whereas the secondary walls of internodal metaxylem tracheary elements are relatively thin. Protoxylem tracheary elements, like metaxylem tracheary elements, have continuous and intact primary wall cover. Xylem is not, however, of primary significance in the mechanical strength of *Equisetum* stems, because sclerenchyma is relatively abundant (Jeffrey, 1899; Hauke, 1978). Bierhorst (1958a) covered the range of pitting types in *Equisetum* well from the standpoint of light microscopy. The three dimensional views afforded by SEM provide a valuable supplement, particularly in the case of the large circular pits of metaxylem tracheary elements on the surfaces of carinal canals (Figs. 3A–D).

Microstructure of pit membranes of nodal metaxylem pits was well revealed by sections. The razor-blade sectioning method employed shaved away portions of pit membranes. Amorphous material was present in some pit membranes, whereas in others, that material was scraped away, so that a cellulosic network is revealed. Such a cellulosic network is common in pit membranes of tracheary elements of ferns (Carlquist and Schneider, 2007), cycads (Schneider and Carlquist, 2007) and angiosperms (Carlquist and Schneider, 2010). This cellulosic network is embedded within the amorphous (non-cellulosic) substances of the pit membrane, and is not evident unless exposed by sectioning. We observed such a reticulum only in pit membranes of internodal tracheids, not in the nodal tracheids in *Equisetum*. In ferns (Carlquist and Schneider 2007) and monocots (Carlquist and Schneider 2010), pits of end walls of tracheary elements may bear pores that clearly interconnect one tracheary element with another in a series. Those may be considered pre-vessels, or even vessels (if one were to expand the definition of vessel). We did not observe such end walls in tracheary elements of *Equisetum*.

Vessel elements.—Bierhorst (1958b) reported vessel elements in the nodal metaxylem of stolons of a few species of *Equisetum* His report was based on absence of pit membranes between files of two to three tracheary elements. There is no reason to question these observations. However, such short vessels, uncommon in occurrence, would have limited significance at best with respect to conduction.

In the present study, we encountered, on surfaces of internodal tracheary elements that face carinal canals, a large number of crateriform circular pits that lack pit membranes. Because most of these tracheary elements were below the cut surface of sections, we are reluctant to attribute the absence of pit membranes to sectioning. Indeed, scattered among the crateriform pits are occasional non-crateriform pits in which pit membranes are intact. The consistent presence of pit membranes on these non-crateriform pits also suggests that ageing or the action of micro-organisms is not responsible for absence of pit membranes on the crateriform pits. We therefore entertain the possibility that absence of pit membranes on crateriform pits may not be an

artifact. The possible conductive nature of the carinal canals has been mentioned by Westermaier (1884), Sykes (1906), and Barratt (1920), and the dye experiments of Buchholz (1921) and Bierhorst (1958b) reinforce this possibility. Bierhorst (1958b) further enhances this possibility by mentioning ''The very intimate connection between the nodal metaxylem and the carinal canals…''. If carinal canals do indeed function in conduction, the water column pathway may lead from one carinal canal upward to metaxylem tracheary elements and thence to a carinal canal in the next internode above through the crateriform pits of the internodal tracheary elements. We open the possibility, based on our observations of large numbers of crateriform pits without pit membranes, that at least many of the crateriform pits may be perforations, and that at least some of the internodal tracheary elements may be vessel elements. This possibility seems most prominent in *E. myriochaetum*. The occurrence of such possible perforations facing carinal canals and their extent within the genus need confirmation and further study.

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