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Scanning electron microscope studies of cycad tracheids

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Abstract

SEM studies of pit membranes of cycad tracheids revealed porosities in some, but not most of the tracheid pits examined. Porosities, where present, are mostly varied in size and density. Porosities do not extend through pit membranes and appear limited to the first-formed portion of the primary wall. Because of inconsistency in porosity presence within a secondary xylem sample, as well as between stems and roots of a species, and among species, interpretation is difficult. Earlier reports of perforations or perforation plates in cycads are not confirmed, and may be the result of oxidative effects of maceration fluids. Forms of helical sculpture on the inner surfaces of tracheids in *Encephalartos* and *Zamia* represent a phenomenon newly reported for cycads. Helical sculpturing on secondary xylem elements of other vascular plants is usually interpreted as a xeromorphic feature, and this interpretation may be valid for cycads as well.

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1. Introduction

Tracheids of cycads as seen by means of light microscopy are familiar, and have been figured in many textbooks and in various accounts of gymnosperm wood. The radial walls of cycad tracheids are covered by circular bordered pits or, in some genera, notably Stangeria and Zamia, scalariform bordered pits (Greguss, 1968). The claim of "perforations" by Greguss in cycad tracheids almost certainly refers to somewhat unusual pitting, judging by his illustrations (Greguss, 1968; Plate 14; Figs 8-10). Based on his illustrations, his statement (1968, p. 125) that "there are only tracheids, however the endings of some tracheids or their lateral walls are very exceptionally perforated," should probably be interpreted as a statement that on such facets, unusual pitting patterns occur. The term "perforation" is never defined directly or indirectly by Greguss (1968), who does apply the term vessel in the case of plant groups other than cycads, such as palms. The possibility that the translator and translation reviser of Greguss' book were not applying the terms precisely must be entertained. With light microscopy, moreover, one cannot observe the nature of

The tracheids of ferns as seen with SEM are pertinent with regard to the present paper. Studies of fern tracheids revealed that four genera (Astrolepis, Marsilea, Pteridium, Woodsia) have vessels as commonly defined: end walls with architecture different from that of lateral walls, with pit membranes absent on the end walls (Carlquist and Schneider, submitted for publication). Many other genera of ferns apparently have tracheids, the end walls of which possess pores or holes in pit membranes (Carlquist and Schneider, submitted for publication). We have interpreted such porosities as prefigurations of perforation plates of vessels. Our earlier claim that these prefigurations can be regarded as perforation plates (Carlquist and Schneider, 2001) has been withdrawn (Carlquist and Schneider, submitted for publication). Our change in opinion was the result of adoption of a more reliable preparation method. The primary walls of fern tracheids can be altered by the maceration process. When materials are merely fixed in alcohol and sectioned without application of any other reagents, more reliable results can be obtained. In this regard, we note reports of

primary walls either in end walls or in lateral walls of tracheids: these primary walls are too thin to absorb sufficient stain. We have turned to scanning electron microscopy (SEM) as a way to observe these primary walls and to see if any features other than ordinary laminar cell wall surfaces could be observed.

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vessel elements in cycads based on maceration techniques (Huang and Zhang, 1999; Lin and Huang, 1999). By turning to non-macerated material, we hope to arrive at maximally reliable results.

A byproduct of the sectioning technique that we have employed is the opportunity to examine interior surfaces of cycad tracheids. Although some optical appearances in the photomicrographs presented by Greguss (1968) suggest some unusual contours for pit apertures, Greguss does not describe these, and light microscopy is not the best tool for exploration of pit contours because the existence of thickenings or grooves in the secondary wall is not clear at given planes of focus. SEM is an ideal tool to reveal structural variations in pits because of the great depth of focus possible with this technique. Therefore, we are figuring and interpreting interior surfaces of tracheid secondary walls as well as variations observed in primary walls.

2. Materials and methods

Portions of living cycad stems and roots were fixed in 70% ethanol (hypocotyls rather than stems were available for *Ceratozomia robusta* Miquel). Only portions judged to be free from decay were selected. Roots of various diameters were fixed, but the smallest roots selected were at least 2 mm in diameter. The choice of roots in this size range was to facilitate sectioning. Subdivided portions of fixed material, several per collection, were sectioned with a hand-held razor blade. Only radial sections were cut in order to increase the likelihood of observing tracheid end-walls, which tend to be radially oriented.

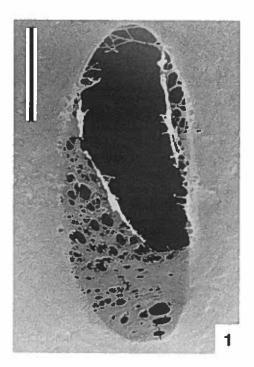


Fig. 1. SEM photograph of tracheid pit membranes, seen from outer surfaces of the tracheids: *Ceratozamia robusta* hypocotyl. Porosities of various sizes and shapes. Scale bar=2 μm.

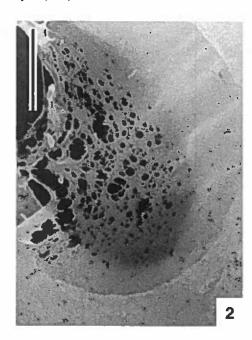


Fig. 2. SEM photograph of tracheid pit membranes, seen from outer surfaces of the tracheids: *Dioön merolae* root; porosities present in central portion of the pit membrane but not over the pit border areas at right and below. Scale bar=2 µm.

Sections were put through three or more changes of distilled water over a period of two days to remove starch and water-soluble compounds from section surfaces. Sections were kept on a 50 °C warming table during distilled water treatment. Sections were then dried between glass slides, in order to insure flatness, on a 50 °C warming table. Dried sections were mounted on aluminum stubs, sputter-coated with gold, and studied with a Hitachi S2600N SEM.

The following species were studied: *Ceratozamia robusta, Ceratozamia sp. (El Mirador, Mexico), Cycas revoluta Thunb., Cycas taitungensis C.F. Shan, K.D. Hill, C.H. Tsou and C.J. Chen, Dioön merolae De Luca, Sabato, and Vásq. Torres, *Dioön purpusii Rose, Encephalartos altensteinii Lehm., Encephalartos horridus Lehm. × Encephalartos natalensis R.A. Dyer and Verdoorn, *Encephalartos senticosus Vorster, *Macrozamia communis L.A.S. Johnson, *Macrozamia macdonnellii (Miq.) Miq., Stangeria eriopus (Kunze) Baill., *Zamia loddigesii Miq., and Zamia aff. neurophyllidia D.W. Stevenson. Stems of all species were available. Roots were available for all species except Encephalartos senticosa and M. communis. In the case of S. eriopus, root material came from a plant cultivated by J.G. Chemnick, whereas stem material was furnished by the University of California, Berkeley, Botanic Garden. Material of all species was from specimens in cultivation and was supplied either by J.G. Chemnick or by Holly Forbes of the U. C. Botanic Garden (*). Identifications were furnished by those two individuals for plants from those respective collections.

As seen with light microscopy, end walls of cycad tracheids cannot be distinguished from lateral walls. The fact that Greguss (1968) does not designate end walls and lateral walls in his photomicrographs attests to this lack of distinction in secondary wall architecture. Likewise, we were unable with SEM studies

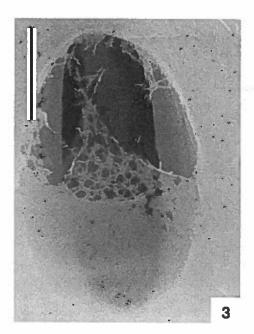


Fig. 3. SEM photograph of tracheid pit membranes, seen from outer surfaces of the tracheids: *Encephalartos altensteinii* stem. Porosities are present in upper portion, which may represent a single layer of the pit membrane, but are absent in the lower portion, which may represent the double primary wall thickness. Scale bar=2 µm.

to establish any difference in end walls and lateral walls. Thus, no attempt was made to identify the surfaces of tracheids illustrated in this paper. The arrangements of illustrations in the pit membrane portion and in the inner tracheid surface portion of the paper, respectively, are alphabetical or nearly so. Materials are presented and discussed in these sequences rather than in terms of roots or stems, respectively, because no

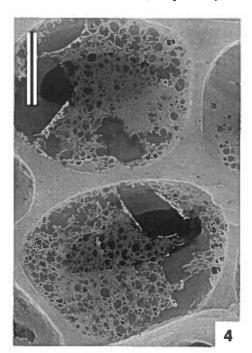


Fig. 4. SEM photograph of tracheid pit membranes, seen from outer surfaces of the tracheids: *Encephalartos horridus* × *Encephalartos natalensis* stem. Porosities are common on two pit membranes, but sparser on the pit membrane portion shown at upper right. Scale bar=2 μ m.

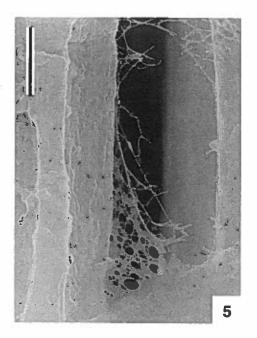


Fig. 5. SEM photograph of tracheid pit membranes, seen from the outer surfaces of tracheids: Stangeria eriopus root. Pit membrane in which threadlike porosities are present above, but porosities occur in the portion near bottom. Scale bar= $2 \mu m$.

consistent differences between tracheid structure in roots and stems were observed.

3. Results

The vast majority of tracheid pit membranes observed in this study showed no porosities regardless of age of plant, diameter of

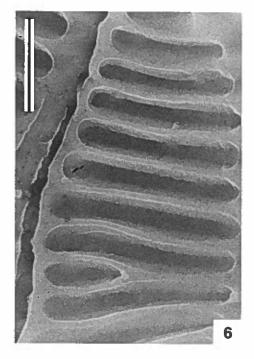


Fig. 6. SEM photograph of tracheid pit membranes, seen from the outer surfaces of tracheids: $Stangeria\ eriopus\ stem.$ Numerous pits on a tracheid tip; porosities are absent in the scalariform pitting. Scale bar=10 μm .

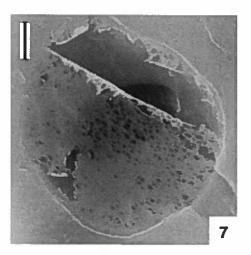


Fig. 7. SEM photograph of tracheid pit membranes, seen from the outer surfaces of tracheids: Cycas taitungensis stem. Small porosities are distributed across a circular pit membrane. Scale bar= $2 \mu m$.

plant, or organographic source of the portion sampled. Porosities were not observed at all in material of Ceratozamia sp. (El Mirador), C. revoluta, D. purpusii, E. senticosus, M. communis, and M. macdonnellii. Pit membranes selected for illustration (Figs. 1-10) do show porosities, with the exception of Figs. 6 and 10, which have been included in order to demonstrate the nonporose nature of most tracheid pit membrane surfaces. In all of the pit membranes figured, some tearing of pit membranes is evident. The term "porosities" excludes obvious tearing of pit membranes. In C. robusta (Fig. 1), porosities vary from large, separated by threadlike remnants of the pit membrane, to very small. In most of the pit membranes illustrated, only a few porosities are visible on pit membrane areas overlying the pit border; most are on pit membrane areas overlying the pit aperture (Figs. 1 and 2). In D. merolae (Fig. 2), a very similar distribution of porosities with relation to the pit border and the pit aperture is illustrated. The pit membrane shown for E. altensteinii (Fig. 3) has porosities in one half, but the remainder is essentially devoid of porosities. The pores in pit membranes illustrated for E. horridus × E. natalensis (Fig. 4) are less diverse in size, but differences may be found within the three membranes shown. The portion of pit membrane at upper right in Fig. 4 has only a few porosities. The pit membrane of a S. eriopus root tracheid (Fig. 5) shows threadlike pit membrane remnants at one end (top), but a

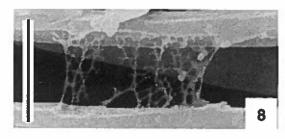


Fig. 8. SEM photograph of tracheid pit membranes, seen from the outer surfaces of tracheids: Zamia loddigesii root. Threadlike pit membrane remnants. Scale bar=2 um.

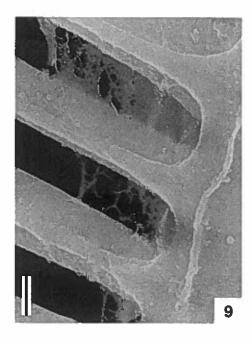


Fig. 9. SEM photograph of tracheid pit membranes, seen from the outer surfaces of tracheids: *Zamia loddigesii* root. Various degrees of pit membrane presence in portions of three scalariform pits. Scale bar= $2~\mu m$.

diminution in size and frequency of porosities at the other end. In the *S. eriopus* stem tracheids (Fig. 6), no porosities were observed, and the tracheid surfaces illustrated are typical of all secondary xylem studied. Only scalariform pits were observed in the secondary xylem of *S. eriopus*. A tracheid pit of a *C. taitungensis* stem (Fig. 7) shows small porosities across the extent of the pit membrane. Tenuous strands of pit membrane material

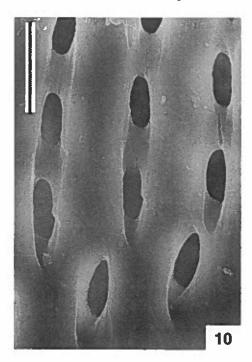


Fig. 10. SEM photograph of inner surfaces of tracheids, showing various types of sculpturing in the secondary walls: *Encephalartos altensteinii* root, long axis of tracheid oriented horizontally. Groovelike extensions of pit apertures, some interconnecting pil apertures, are present. Scale bar=5 µm.

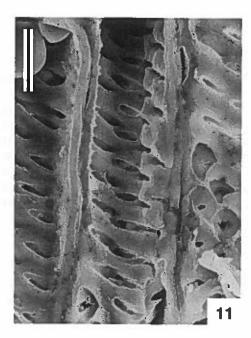


Fig. 11. SEM photograph of inner surfaces of tracheids, showing various types of sculpturing in the secondary walls: *Encephalartos senticosa* stem, long axis of tracheids oriented vertically. Groovelike lateral extensions of pit apertures are present. Several circular pit apertures may be seen on the outer surface of the tracheid at right. Scale bar= $10 \ \mu m$.

are illustrated in a pit of a Z. loddigesii tracheid (Fig. 8), but on another tracheid (Fig. 9), pit membrane remnants range from threadlike in some places to nonporose in other portions.

We did not observe porosities in any pit membranes of tracheids in C. revoluta, C. sp. (El Mirador), D. purpusii, or

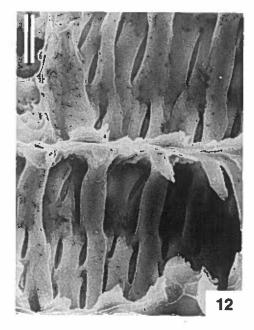


Fig. 12. SEM photograph of inner surfaces of tracheids, showing various types of sculpturing in the secondary walls: $Zamia\ loddigesii$ root; helical thickenings on the wall surface. Portions of two adjacent tracheids, long axis of tracheids oriented horizontally. The slitlike pit apertures run at an angle slightly different from that of the thickenings. Scale bar= $10\ \mu m$.

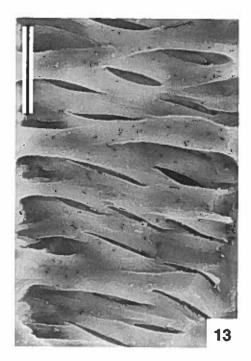


Fig. 13. SEM photograph of inner surfaces of tracheids, showing various types of sculpturing in the secondary walls: *Zamia loddigesii* root; helical thickenings on the wall surface. Wall surface of a tracheid, long axis oriented vertically. Thickenings are not continuous, but rather fade into the wall surface at places; the very narrow pit apertures run parallel to the thickenings. Scale bar=10 µm.

E. senticosus. Porosities, where present, were observed only on the outer surfaces of tracheids. As seen from the inside (Fig. 10) all tracheid pit membranes observed were smooth and lacking in porosities.

Inner surfaces of tracheids in the cycads studied were mostly smooth, lacking in any notable form of sculpturing. In two genera, however, types of secondary wall sculpturing were observed. In *E. altensteinii* (Fig. 10), grooves extend laterally from pit apertures. Some of these intersect grooves related to nearby pit apertures, and thus form a pattern known in the literature as "coalescent pit apertures". Such grooves are observable in the pit apertures of *E. senticosus* (Fig. 11) as well. In the section of *E. senticosus*, one can see that pit cavities are circular, not oval, in outline (Fig. 11, lower right), and that pits are absent on tangential walls of the tracheids (Fig. 11, smooth appearance of the walls seen in sectional view).

In sections of *Z. loddigesii* roots, a second form of sculpturing is present on inner surfaces of tracheids (Figs. 12 and 13). Helical thickenings are present. The sectioned portions of the wall show the degree to which these thickenings are raised above the wall surface (Fig. 12). The elliptical pit apertures vary slightly in orientation from the angles of the helical thickenings, but tend to run similarly. The thickenings do not continue in extensive helices around the tracheid surface, but tend to fade into the wall surfaces here and there (Fig. 13).

4. Conclusions

In monocotyledons (Carlquist and Schneider, 2006) and dicotyledons (Carlquist, 1992), there are pit membrane remnants

in end walls of vessel elements. In fern tracheids, porous pit membranes may be seen in end walls of tracheids (Carlquist and Schneider, submitted for publication). Porosities consistently extend through both of the adjacent pit membranes of a pit pair in fern tracheids, whereas they do not in cycad tracheids. When viewed from the inside of tracheids, pit membranes of cycad tracheids do not show porosities. Tracheid pit membranes in some sectioned preparations when seen from the outer surfaces of tracheids do show porosities but more frequently the pit membranes in such views are nonporose. Because of inconsistencies in the nature of pore presence in tracheid pit membrane presence in cycads, porosity presence is not a characteristic relating to improvement of conduction in tracheid end walls. The porosities may merely represent patterns of microfibril deposition in the first-formed layers of the primary wall. Alternatively, the porosities may represent artifacts of some sort. Certainly there are irregularities in the sizes and distributions within the pit membranes in the collections in which porosities are present. Various degrees of shaving away of the pit membranes by the sectioning process may be responsible for some patterns. Tension due to the drying process may account for some patterns of porosity enlargement. There was no observable pattern of occurrence of the porosities with respect to organography or to the phyletic relationships of the genera. Porosities were not more common in tracheids of roots than in those of stems. The nature of the porosities observed in cycads may represent a phenomenon different from that observed in fern tracheids. Further studies comparing various preparation techniques and using several types of electron microscopy are needed to reveal the significance of the textural appearances we have recorded in cycad tracheid pit membranes. Such studies will probably also demonstrate whether porosities are present consistently with respect to organography or systematics.

The use of the term "perforation" by Greguss (1968) is considered by us probably to be the result of a misapplication of the term or a mistranslation. Reports of vessels in cycads based on SEM studies (Huang and Zhang, 1999; Lin and Huang, 1999) are probably inaccurate because of oxidative removal of pit membranes from tracheids by the maceration process. On the

basis of the 14 collections we studied, we conclude that cycads are vesselless.

Pit apertures that form groovelike shapes on inner secondary wall surfaces of tracheids are newly reported for *Encephalartos*. Likewise, helical thickenings on inner surfaces of *Zamia* tracheids represent a first report for cycads. Helical sculpturing on vessels and tracheids of dicotyledons increases with increased xeromorphy (Carlquist, 1966, 2001). Cycads are mostly xeromorphic with respect to foliage, but the succulence of cycad roots and stems could be considered as a factor that would modify the impact of ecology on design of the conducting system. Attention should be paid to exploring helical sculpturing in inner surfaces of tracheids in further studies of cycad xylem.

Acknowledgements

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