SEM studies of vessels in ferns

14. Ceratopteris

Sherwin Carlquist *, Edward L. Schneider
Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, CA 93105, USA

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Abstract

SEM study of xylem macerations of Ceratopteris thalictroides (L.) Brongn. (Parkeriaceae, or Pteridaceae, tribe Ceratopterideae) has co-occurring vessel elements and tracheids in rhizomes and probably also in roots, a condition newly reported for ferns. Vessel elements with helical thickening in the root have perforation plates that consist of elliptical holes in primary walls, with no thinning or discontinuation of the gyres of secondary wall bands; groups of perforations alternate with smaller zones of intact primary wall. Perforations of this type are not visible with a light microscope, and thus the perforation plates differ from those previously figured for vascular plants [Bailey, I.W., 1944. Am. J. Bot. 31, 421–428] in which helical elements have perforation plates in which the bands show alterations with relation to the perforation plates. End wall facets of rhizome vessels that are perforation plates, have the same morphology as lateral walls; some perforation plates also occur on lateral walls. Weblike pit membranes that contain pores of various sizes are present on some perforation plates and in the transitions between perforation plates and intact lateral wall pitting. The presence of clearly differentiated vessels in Ceratopteris shows that vessels are present even in a submersed aquatic fern. The xylem of Ceratopteris opeus new questions about co-occurrence of vessel elements and tracheids in ferns, relative morphology, distribution, and abundance of the two cell types, and functional and evolutionary significance of vessel element presence in a fern that has maximal moisture availability. ©2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Our studies on tracheary elements of ferns began with Pteridium (Carlquist and Schneider, 1997a) and other genera in which descriptions by White (1962) suggested

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* Corresponding author. Tel.: +1-80-5682-4726; fax: +1-80-5563-0352
E-mail address: eschneider@sbbg.org (S. Carlquist)

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the existence of specialized end walls. White (1962) could not with certainty term these perforation plates, because light microscopy cannot reveal the presence or absence of pit membranes. The genera cited by White (1962) as having tracheary elements with end walls different from lateral walls proved to have vessel elements when studied with SEM. These fern genera include Woodsia (Carlquist et al., 1997; Carlquist and Schneider, 1998a; Schneider and Carlquist, 1998a) Astrolepis (Carlquist and Schneider, 1997b); Phlebodium and Polystichum (Schneider and Carlquist, 1997). The fact that all of these genera proved to have vessels led us to extend our studies into ferns in which end walls of tracheary elements were like lateral walls when seen with light microscopy. In all of the genera we investigated, whether from families regarded as primitive or specialized, or whether from localities with little seasonality or with greater seasonality in moisture availability, vessel elements proved to be present. These genera included such leptosporangiate fern families as Gleicheniaceae (Schneider and Carlquist, 1998b); Osmundaceae and Schizaeaceae (Carlquist and Schneider, 1998b) and even the eusporangiate fern families Ophioglossaceae (Schneider and Carlquist, 1999) and Marattiaceae (Carlquist and Schneider, 1999).

Our findings were novel in several important respects. Genera with tracheary elements that had perforation plates different from lateral walls in morphology characterize habitats in which marked extremes of water availability and temperature occur. All the ferns we studied had lateral wall perforation plates as well as end-wall perforation plates. Several end wall facets can occur on fern tracheids, and any or all of these may bear perforation plates. Vessels were present in rhizomes as well as roots in all the species studied; the rhizome vessels had perforation plates just as specialized as those of roots in most species, only slightly less specialized at most. Pit membrane remnants that are webleike or bear pores, much as in perforation plates of primitive dicotyledons (Carlquist, 1992) were observed in all species at points of transition between lateral wall pitting and perforation plates, or sometimes in less specialized perforation plates, either on end walls or lateral walls (a range in degree of perforation plate specialization may be found within most species). Although we observed vessel elements readily, we were not able to identify tracheids with certainty in the species we studied. Although our macerations for most of these species did not offer maximal clarity (e.g., numerous intact tracheary elements), absence or scarcity of tracheids in most fern species studied is a reasonable conclusion.

Vascular strands of tracheary elements in most ferns appear to be strands of vessels interconnected both terminally and laterally by perforation plates, and are thus like a sort of multiple vessel (vessel groupings in dicotyledons are termed ‘vessel multiples’) but lateral interconnections among dicotyledon vessels are few or none.

We selected Ceratopteris as a genus that offered, on account of its aquatic habitat, possibilities to demonstrate correlations between habit and nature of vessels. If vessels occur widely within terrestrial ferns, do they occur to the same extent in aquatic ferns? Are the vessels less specialized than in other ferns? Are tracheids present, in addition to vessel elements? In order to find correlations in vessel occurrence in ferns between vessel presence and ecology, one must sample ferns from as wide a range of ecological sites as possible. Our earlier studies have indicated that generalizations may be possible, despite the fact that each species has special xylary characteristics not shared with other species in the same family or in the same habitat.
Ceratopteris is a genus of four species from aquatic sites in tropical to subtropical areas of the New World and Old World (Lloyd, 1974). Although often placed in a monogeneric family, Parkeriaceae, some authors (e.g., Tryon and Tryon, 1982) regard Ceratopteris as a monogeneric tribe, Ceratopterideae of Pteridaceae. Evidence for placement in Pteridaceae is offered by the cladograms of Pryer et al. (1995), who incorporate both macromorphology and rbcL studies.

2. Materials and methods

Our studies are based on material of Ceratopteris thalictroides (L.) Brongn. collected in the San Marcos River near San Marcos, Texas. Water temperature in this river varies little from 21°C and clarity of the water is very high. Ceratopteris is infrequently found near the water surface entangled with other aquatic vegetation. The history of the introduction of this species to the San Marcos River has been detailed by Morton (1967), Hannan (1969) and Petrik-Ott (1976). The constant temperature of the San Marcos River (Hannan and Dorris, 1970) has very likely permitted establishment of tropical to subtropical species in this locality.

Roots, stems, and leaf bases were preserved in 50% aqueous ethanol. Macerations were prepared with Jeffrey’s fluid and stored in 50% ethanol. Macerations were spread onto aluminum stubs, dried on a warming table, sputter-coated, and examined with a Nanolab scanning electron microscope (SEM). Obvious artifacts, such as tears in pit membranes, can be identified in preparations. Drying of porose pit membranes might possibly result in enlargement of some porosities, but these areas are thin and transitional to perforations in any case, as is evident from the complete absence of pores in most lateral wall pitting. The absence of pit membranes in most perforations in our preparations takes the form of large openings that are not artifacts. Delicate weblike membrane remnants can be torn and destroyed by the electron beam while one observes them under high power. Care has been taken to minimize beam exposure of areas of interest as a way of avoiding artifact formation.

3. Results

3.1. Tracheary elements in roots

Tracheary elements in roots have helical secondary wall bands (Figs. 1, 2 and 3) or scalariform secondary wall frameworks (Figs. 4 and 5). Vessels with helical secondary wall thickenings (Fig. 1, center and right) have elliptical openings in primary walls. These may form perforation plates that are discontinuous, with groups of adjacent openings forming intermittent perforation plates, a phenomenon shown well in Fig. 2. These perforation plates occur on apparently lateral walls (Figs. 1 and 2) or on end walls. In some tracheary elements, perforation plates were not seen (Fig. 1, left; Fig. 2, right; Fig. 3). These cells may be tracheids, although we were unable to see the full length of such cells clearly enough to establish absence of all perforation plates. The frequency of tracheary elements in which no perforation plates were observed, supports the possibility that some tracheids
Figs. 1–5. Tracheary elements from macerations of roots *Ceratopteris thalictroides*. (1) Tracheary elements with helical secondary walls; at left, an element with two imperforate facets to show lateral walls; at right, facets to show the nature of perforation plates, probably on lateral walls, which are subdivided into portions by gyres between which intact primary wall material is present. (2) Portion of perforation plate (left) interrupted in two places, and an intact lateral wall (right). (3) Lateral walls of two adjacent helical elements; primary walls are intact. Scales for all figures = 5 μm. (4) Scalariform tracheary element on which the facets at extreme left and right are lateral-wall perforation plates; on one of the facets, center, are five pits with elliptical openings that may be natural. (5) Lateral wall of a scalariform element, showing intact pit membranes.

may be present in the roots. Tracheary elements with scalariform walls (Figs. 4 and 5) are present in metaxylem. In the scalariform elements, some wall facets are clearly perforation plates (Fig. 4, extreme left and right), whereas others appear to have no perforations or only occasional perforations (Fig. 4, center 3/4 of photograph; we cannot rule out a possible artifact formation in the five openings shown). We observed extensive areas of lateral walls in which no perforations or tears due to handling were present (Fig. 5).

3.2. *Tracheary elements in rhizomes*

In rhizomes, which may include some leaf base tissue, a high proportion of tracheary elements were well intact in our macerations. All tracheary elements described here are
Figs. 6–11. Tracheary elements from macerations of rhizome and rhizome-leaf base transition of *Ceratopteris thalictroides*; all elements shown are scalariform metaxylem elements. (6) Terminal portion of a vessel, several facets of which are perforation plates; at extreme right are wall facets in which pit membranes are present. (7) Vessel element; two facets, above center and left, are perforation plates. (8) Tracheid; lateral wall pitting is visible. (9) Two facets of lateral wall of vessel element; facet at the left contains porose pit membranes; membranes of facet at the right are striate and have few, if any, porosities. (10) Portion of lateral wall of vessel with thin, weblike pit membranes that contain pores or openings of various sizes. (11) End wall of vessel element with perforation plate; a transition to intact pit membranes is present by virtue of presence of threadlike and porose pit membranes at bottom. Scale in all figures = 5 μm.

Scalariform metaxylem elements (Figs. 6–11) and the relatively strong nature of walls in scalariform elements may account for the intact nature of so many elements. Both end walls and lateral walls of vessel elements may be perforation plates (Fig. 6). In the vessel element shown in Fig. 6, the central facet bears about nine pits at the bottom of the photograph, but above those are perforations; the cell facets at extreme right have few openings in pits, and represent lateral wall pitting rather than perforations.

In the rhizome, our macerations provided isolated tracheary elements that could be classified as either vessel elements or tracheids with reasonable certainty. The vessel element in Fig. 7 has at least two perforation plates at one end (top third of element, left and center) and at least another perforation plate (bottom left). We could not see all facets, and additional perforation plates may be present. The tracheary element in Fig. 8 had no discernible...
perforation plates; lateral wall pitting covered all surfaces visible to us in this element, and thus we designate it as a tracheid.

Incipient perforation plates are present on some vessel elements. In the portions shown in Fig. 9, the striate wall facet shown at the right may have a few porosities in pit membranes, but these may be artifacts. The left wall facet in Fig. 9 has pit membranes, most of which bear porosities of various sizes. Thus, the wall facet at left is a perforation plate with relatively abundant pit membrane remnants. A greater degree of loss of pit membranes is shown in Fig. 10; in this facet, holes, pores, and webbing indicate a perforation plate with a greater degree of loss of pit membranes. The end wall shown in Fig. 11 is a perforation plate, but facets to the left and the right of the perforation plate represent lateral wall pitting. The perforation plates shown in Fig. 11 is a short one, and ends with partially lysed or intact pit membranes at the bottom of the photograph, center.

4. Discussion and conclusions

In our series on tracheary elements in ferns, we have hitherto not studied a species in which we could affirm coexistence of vessel elements and tracheids; we report this for rhizomes of *Ceratopteris thalictroides*; the two cell types may coexist in roots of the species also. This condition contrasts with *Pteridium*, in which we reported all tracheary elements in roots and rhizomes to be vessel elements, although some vessel elements have perforation plates better defined than others (Carlquist and Schneider, 1997a). Thus, some ferns may have both vessel elements and tracheids coexisting in xylem and some may have vessel elements only. The opportunity is thus opened to discover relative presence of vessel elements and tracheids in particular fern species, the distribution within xylem, and differential morphology and dimensions of the two cell types. Hitherto, tracheids coexisting with vessel elements in particular xylem portions have been reported in Gnetales and in dicotyledons, but not in monocotyledons, ferns, or other pteridophytes (Cheadle, 1942; Bailey, 1944; Bierhorst, 1960; Bierhorst and Zamora, 1965; Carlquist, 1996).

Vessel elements in primary xylem tracheary elements with helical thickening have hitherto been figured as having identifiable perforation plates because (1) gyres of secondary wall material are attenuated across the perforation plate, which is thus scalariform-like; (2) gyres of secondary wall material taper at the edges of the plate and are absent in the plate, so that a simple plate is present; or (3) a circular strip of secondary wall material interconnects gyres so as to outline a simple perforation plate. Drawings corresponding to these three alternatives are presented by Bailey (1944). These three conditions are visible with light microscopy. Additional configurations have been revealed with SEM, and one of these is shown in *Ceratopteris thalictroides*: perforation plates in which the gyres are identical to those of other areas on the cell, but in which elliptical perforations are present. Because the presence or absence of the primary wall is visible only with SEM, this type of perforation plate cannot be identified with light microscopy. We have found this type of perforation plate in *Araceae*, subfamily *Colocasioideae* (Carlquist and Schneider, 1998c).

Another type of perforation plate (or perforation plate portion) visible only with SEM consists of pit membranes that are porose, weblike, or otherwise partially lost, perhaps due to an incomplete version of the lysis that removes pit membranes entirely in most perforations.
This type of perforation plate or transition between perforation plate and lateral wall has been reported in most of the ferns we have studied (see papers cited in Section 1) Pit membrane remnants are common in perforation plates of Ceratopteris, and may relate to lack of strong selective pressure for a morphology conducive to conduction of large volumes of water per unit time.

Perforation plates in Ceratopteris occur on both end walls and lateral walls. Tips of tracheary elements in ferns may contact numerous other tracheary elements in ferns, and thus multiple ‘end walls’ may be present in a fern tracheary element. We have been able to observe perforation plates on lateral walls as well as on end walls of most of the ferns we have studied, beginning with Pteridium (Carlquist and Schneider, 1997a).

The morphology of end walls is like that of lateral walls in vessel elements of Ceratopteris. This is true of many of the ferns we have studied, although appreciable specialization of perforation plates is visible in some genera, such as Pteridium and Woodsia. Ceratopteris is therefore not unusual in lacking such differentiation.

The presence of vessels in Ceratopteris is perhaps remarkable in view of the aquatic habitat of this fern. However, the vessels do not have specialized perforation plates, and perforation plates bearing pit membrane remnants are common. The coexistence of tracheids with vessel elements may also be indicative of a lowered selective value for a xylem configuration suitable for conduction of large water volumes per unit time. The correlation of vessel element presence and the morphology of those vessel elements, with ecology, will be illuminated by future studies of ferns from a variety of habitats, and with a variety of habits.

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References