

SEM Studies on Vessels in Ferns. 1. *Woodsia obtusa*

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ABSTRACT.—Circular to oval pores are present in primary walls (pit membranes) of end walls of tracheary elements in roots and rhizomes of *Woodsia obtusa* (Spreng.) Torr. (Dryopteridaceae). The pores are at least as abundant in rhizomes as in roots. We regard tracheary elements with end walls bearing these pores as vessel elements (such pores are absent on lateral walls). Pores occur in thin areas of the primary wall of pit membranes; thicker portions of pit membranes, both on lateral and end walls, occur in the form of striations oriented parallel to the long axis of the cell. These striations, recently demonstrated by means of scanning electron microscopy (SEM) for primary xylem of Nymphaeales and the monocotyledon *Acorus*, are likely widespread in vascular plants. Pore formation in *W. obtusa* is only moderate compared to that in Nymphaeales and *Acorus*, and may correlate with the fact that *W. obtusa* occupies habitats relatively more mesic than those in which other species of *Woodsia* grow.

The presence of vessel elements in ferns was claimed over a century ago by Russow (1872), who cited only two species, *Pteridium aquilinum* (L.) Kuhn and *Nephrodium filix-mas* Rich. (= *Dryopteris filix-mas* (L.) Schott), as having true perforations in the end walls. White (1962), who reviewed the history of claimed vessel presence in ferns, demonstrated that opinions varied widely: De Bary (1884) agreed with Russow (1872), whereas Gwynne-Vaughan (1908) thought that vessels could be found in Cyatheaceae, Gleicheniaceae, Hymenophyllaceae, Marattiaceae, Ophioglossaceae, Polypodiaceae, and Schizaeaceae. Bancroft (1911), using a variety of techniques, thought that vessels exist in ferns only in *Pteridium aquilinum*, a conclusion shared by Duerden (1940).

White (1961, 1962) presented clear evidence for the presence of vessels in roots of *Marsilea*. In addition, White (1962, 1963) suspected vessel presence in two other species of ferns, *Woodsia ilvensis* (L.) R. Br. and *Astrolepis sinuata* (Lag. ex Sw.) D.M. Benham & Windham (= *Notholaena sinuata* (Lag. ex Sw.) Kaulf.), although he did not demonstrate perforations. White's (1962, 1963) reason for suspecting vessels in *Astrolepis sinuata* and *Woodsia ilvensis* was marked differentiation (as indicated by distribution of pits and therefore secondary wall) between the lateral walls and the end walls of tracheary elements. In addition, moderate differences between pits of lateral walls and pits (or perforations) of end walls of tracheary elements were claimed by White (1962, p. 75) for 10 other species of *Woodsia*, and one hybrid *Woodsia*. The species we have elected to study here, *W. obtusa* (Spreng.) Torr., was cited by White (1962) as having "slight" differentiation of end walls as compared to lateral

walls of tracheary elements in roots, but he reported no differentiation between end walls and lateral walls in rhizomes of this species.

White's work was done without the benefit of scanning electron microscopy SEM. This instrument is ideally suited to examination of walls of tracheary elements and resolving pores of various sizes in the walls. Pit membrane remnants, some nearly intact, in perforation plates of primitive woody dicotyledons were shown in this way by Carlquist (1992), and the technique was extended to primary xylem of Nymphaeales, in which presence of porosity in end walls permitted designation of the tracheary elements as vessel elements, in *Barclaya* (Schneider and Carlquist, 1995a), *Victoria* (Schneider and Carlquist, 1995b), *Nuphar* and *Nymphaea* (Schneider et al., 1995) of the Nymphaeaceae, and *Cabomba* (Schneider and Carlquist, 1996a) and *Brasenia* (Schneider and Carlquist, 1996b) of the Cabombaceae. We are extending this method to monocotyledons, in which we have demonstrated porose pit membranes in end walls of tracheary elements in *Acorus*, a genus of Acoraceae formerly placed in Araceae (Carlquist and Schneider, 1997). We are also engaged in SEM studies of tracheary elements of a wide range of ferns.

Our data have potential use in showing the number of times vessels have originated in these groups; the presence of distinctive types of vessel elements, as in *Cabomba* (Schneider and Carlquist, 1996a) and *Brasenia* (Schneider and Carlquist, 1996b); and the alteration of vessel structure with relation to the aquatic habitat (Schneider and Carlquist, 1996b).

In monocotyledons, presence of vessels and their degree of specialization seem clearly related to ecology (Carlquist, 1975, p. 115). The presence of vessels in *Marsilea* seems related to seasonal drying and refilling of ponds in which that genus occurs. *Pteridium*, which has vessels throughout the plant body, occurs in sites that are dry or even frozen for prolonged periods, and it also has extraordinarily high "internal diffusive resistance" (a measure of combined resistance to transpiration from stomata, cuticle, and boundary layer), according to Gates (1968, p. 236), and occurs in localities where freezing of water in xylem in winter is very likely.

We believe that the presence of vessels in *Woodsia* may also be closely keyed to ecology. As a genus, *Woodsia* characteristically occurs in boreal latitudes and altitudes, and is most often found on outcroppings where winter freezing and summer drought may be pronounced (Brown, 1964). Examining the relationship between vessel presence and ecology is therefore a goal of our studies. *Woodsia obtusa* ranges from southern Maine and southern Québec to western Oklahoma and south-central Texas (Brown, 1964). Brown (1964) claims that *W. obtusa* is "more mesic" than its congeners, but that it is "also much more tolerant of high temperatures than other species of the genus." However, Brown's claim may be based on a generalization about elevated summer humidity in the range of *W. obtusa*. In fact, *W. obtusa* habitats may not differ much in this respect from habitats of other *Woodsia* species. *Woodsia obtusa* can characteristically be found in places such as glades and bluffs that become hot and therefore dry in the summer.

MATERIALS AND METHODS

The following collection provided material for our study of *W. obtusa* subsp. *obtusa*: U.S.A., Missouri, Iron Co., along roadcut, State Highway 21, W side of road across from Royal Gorge Natural Area, plants scattered in open crevices of rhyolite cliff, with *Dryopteris*, *Arabis*, *Solidago*, and grasses, 310 m, 37° 32' 30"N, 90° 41' 10"W, 5 Oct 1995, *Yatskievych & Taylor* s.n. (SBBG).

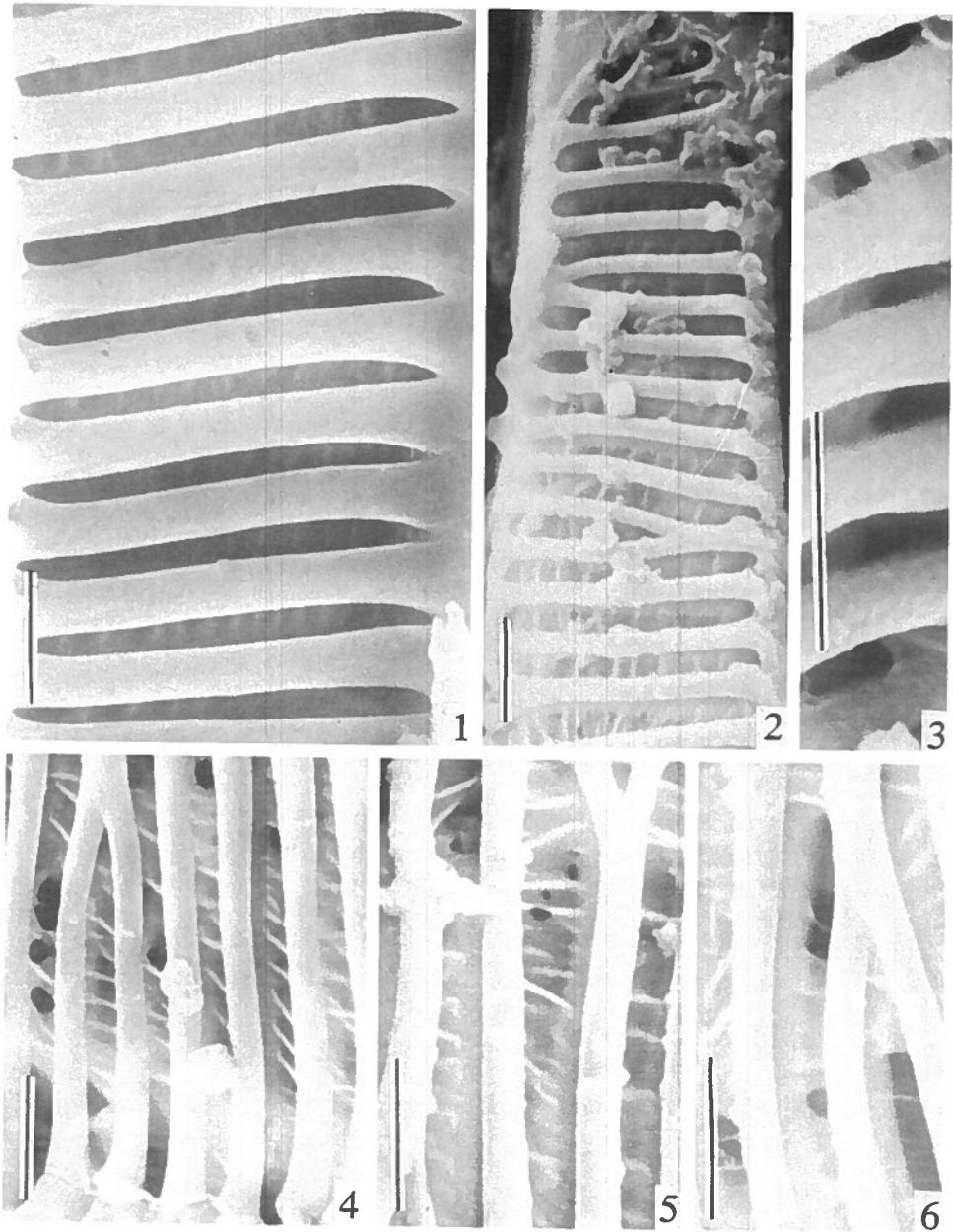
Entire plants were preserved in 50% aqueous ethanol. Portions of roots and rhizomes from these plants were infiltrated by means of the tertiary butyl series of Johansen (1940), embedded in paraffin, and sectioned on a rotary microtome. Some of the sections were mounted on glass slides and stained with a safranin-fast green combination corresponding to Northen's modification of Foster's tannic acid-ferric chloride method (Johansen, 1940). Other sections were mounted on aluminum stubs, using methods similar to those for mounting sections on glass slides, and were cleansed of paraffin and then sputter-coated for examination with SEM.

We are using the term vessel element for any tracheary element in which the presence of pores appreciably larger than those of plasmodesmata can be found with SEM in primary walls of end walls of tracheary elements, but in which such pores are absent on lateral walls.

RESULTS

In the root of *W. obtusa*, lateral walls of tracheary elements have moderately narrow pits (Fig. 1). Faint vertical striations that indicate thicker places in the pit membranes of lateral walls can be found. In the end walls of tracheary elements or roots (Figs. 2–6), pit areas are relatively wide. In the portion of an end wall shown in Fig. 2, pores are relatively few: A line of pores can be seen in each of two pit areas, the fourth and fifth from the top end of the end wall. Striations are pronounced in the pit membranes of Fig. 2, especially in the bottom two-thirds of the photograph. Larger pores are evident in the photograph of Fig. 3, which represents a longitudinal strip of an end wall. Figures 2 and 3 represent extremes in degree of pore presence; Figs. 4–6 are more typical. The end walls of Figs. 4–6 show abundant striations that represent thickenings in the primary walls. Pores of various sizes occur in the primary walls: larger in Fig. 4, smaller in Fig. 5 (Figs. 5 and 6 are portions of the same end wall).

In the rhizome of *W. obtusa* (Figs. 7, 8), pores are present in the end walls, as are the thickenings termed striations here. The tendency for pores to be circular or oval in outline is clearly shown in Figs. 7 and 8. Also, the coalescence of smaller pores close to each other into larger pores, perhaps because of progressive wall lysis that begins at particular points on the primary wall, is evident. There is no doubt that pores are present on end walls of tracheary elements of *W. obtusa*. The pores are somewhat more evident in the rhizomes than in the roots in the material we examined, but our sampling was not extensive.



FIGS. 1-6. SEM photographs of tracheary elements from roots of *Woodsia obtusa*. 1) Lateral wall pitting; faint light streaks in pits are striations (thickenings). 2) Tip of end wall, with only a few small pores in pit membranes (fourth and fifth pits from top); striations conspicuous at bottom of photograph. 3) Strip from end wall, showing pores. 4) End wall with striations and relatively large pores. 5, 6) Two portions from the same end wall. 5) Portion with small pores (above) and striations in pit membranes. 6) Portion with two coalescent pores (center, above). Scale bars = 5 μ m.

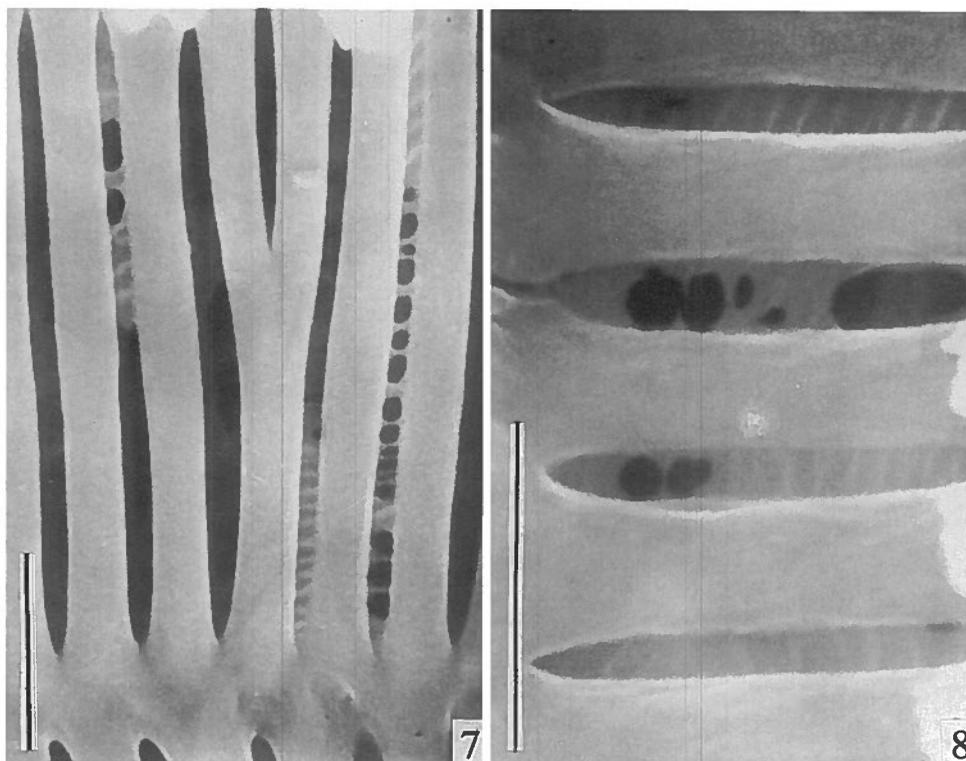


FIG. 7-8. SEM photographs of end wall portions from tracheary elements of rhizome of *Woodsia obtusa*. 7) Portion of end wall showing circular pores and striations; pits that are entirely clear of pit membranes may represent removal of membranes by sectioning. 8) Porose pit membranes, showing various degrees of coalescence; striations are also present. Scale bars = 5 μ m.

CONCLUSIONS

Relatively large pores are present in end walls of tracheary elements of both roots and rhizomes of *W. obtusa*. We believe these tracheary elements should therefore be termed vessel elements, although one may wish to describe the degree of presence of pit membranes in these end walls when using that term. Although White (1962) found slight differentiation between lateral and end walls of roots with respect to width of pits and bars in *W. obtusa*, he apparently found no such differentiation of rhizomes of *W. obtusa*. In our future studies of vessel presence in ferns, we will be searching for presence of vessels in ferns where there is little differentiation between end walls and lateral walls on the basis of light microscopy, as well as those species in which end walls are markedly different from lateral walls as seen with the light microscope. Interestingly, in two species of *Acorus*, a monocotyledon, we found vessels in both roots and rhizomes, although Cheadle (1942), using light microscopy, reported vessels in roots only. We also found vessels in both roots and stems of two genera of aquatic dicotyledons that have only primary xylem, *Brasenia* (Schneider and Carlquist, 1996b) and *Nelumbo* (Schneider and Carlquist,

1996c); no vessels had hitherto been reported in *Brasenia*, and vessels had been reported only for roots in *Nelumbo*.

White (1963) gave figures for pits and bars of end walls of tracheary elements in various families of ferns. He organized his figures in terms of root, rhizome, and petiole. In a table prepared from averages of end wall pit diameters for the ferns White considered (Carlquist, 1975, p. 40), the headings of two columns were by error reversed. The table should be corrected so that the pit diameters read: roots, 2.03 μm ; rhizomes, 1.49 μm ; petioles, 1.67 μm . The wider pits in end walls of root tracheary elements might be considered prefigurations of vessel origin in roots of ferns, at least in the sense that greater pit membrane area offers greater potential conductivity, just as a porose pit membrane offers more potential conductivity than a pit membrane without pores. If this reasoning is correct, the wider pits of end walls or root tracheary elements in ferns would be in agreement with White's (1962) generalization, which is admittedly based on limited data, that vessels tend to originate in roots in ferns and progress into stems and then petioles. This sequence is the same as that hypothesized for monocotyledons by Cheadle (1942).

The presence of lateral or adventitious roots in both ferns and monocotyledons, a condition related to lack of secondary growth in vascular bundles, may be related to this organographic sequence. Lateral or adventitious roots in these two groups of vascular plants are relatively ephemeral compared to the stems that bear them, and the lack of secondary growth in ferns and monocotyledons is correlated with this behavior: new roots must originate as older stem portions senesce. In ferns and monocotyledons, therefore, tracheary elements of roots are matured well after tracheary elements in the stem portions that bear those roots have matured: perforation plates (as recognizable with the light microscope) interconnecting stem and root tracheary elements cannot be differentiated in such a situation. Lysis of pores in pits interconnecting tracheary elements of roots with those of stems is conceivable once the root tracheary elements mature in contact with the stem tracheary elements. In dicotyledons, vessels are believed to have originated simultaneously in secondary xylem of stems and roots of particular species (Bailey, 1944). The presence of taproots intercontinuous with stems in dicotyledons, with the consequent intercontinuity of cambium and secondary xylem of stems and roots, correlates with the differential sites and sequences of vessel origins in ferns, monocotyledons, and dicotyledons, respectively. However, our knowledge of primary xylem of all three groups is very limited, because so few SEM studies have been done, and new patterns may emerge when such studies have been performed.

We note with interest the presence of thickenings that we term striations in the primary walls of pit areas both end walls and lateral walls of tracheary elements of *W. obtusa*. We believe the report of these structures in primary xylem of *Euryale* and *Victoria* (Schneider and Carlquist, 1995b) to be the first for dicotyledons, and the report of striations in *Acorus* vessels (Carlquist and Schneider, 1997) to be the first for monocotyledons. Our report of striations in *Woodsia* tracheary elements is evidently the first for ferns. The fact that stri-

ations can be found with SEM in such distantly related groups suggests that striations in primary walls of primary xylem are likely to be found much more widely. Although we do not propose a function for these structures, we note that pores in vessel elements of primary xylem, where present, occur between the striations, if striations are present. The origin of pores thus occurs in the thinner portions of the primary wall.

The pores in end walls of tracheary elements of *W. obtusa* are not large, abundant, or frequently coalescent compared to those of other vascular plants (notably those of woody dicotyledons; Carlquist, 1992). *Woodsia obtusa* represents a minimal to moderate presence of pores in end walls of tracheary elements. In future studies, we will focus on comparison of degree of pore presence and size of pores in primary walls of end walls of tracheary elements of other species of *Woodsia*, as well as in tracheary elements of ferns other than *Woodsia*. Correlation of ecology with various degrees of pore presence and perforation plate morphology will be a prime goal of our studies.

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