ORIGINS AND NATURE OF VESSELS IN MONOCOTYLEDONS. 11. PRIMARY XYLEM MICROSTRUCTURE, WITH EXAMPLES FROM ZINGIBERALES

Sherwin Carlquist and Edward L. Schneider
Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, California 93105, U.S.A.

Introduction

In our earlier scanning electron microscopy (SEM) studies on the xylem of monocots (e.g., Orchidaceae; Carlquist and Schneider 2006), we focused on the tracheid-vessel element continuum. We selected families and species that possess pit membrane remnants in perforations of vessel elements, and we compared various degrees of reticulate pit membrane presence in perforation plates of vessel elements and in end walls of tracheids to the intact, nonpore structure of lateral wall pits. Our efforts were directed mainly toward vessels in roots, because in the monocot families that we studied, vessels are present in roots, and xylem cells intermediate between vessel elements and tracheids (as judged by pit membrane presence) are most commonly found in roots, less commonly in stems. We used data compiled by Cheadle (1942; schematically shown in Carlquist 1975, pp. 106, 115) as a basis for exploration, because Cheadle listed families (1942; schematically shown in Carlquist 1975, pp. 106, 115) and to study other Zingiberales in an attempt to see whether the microstructures recorded in Canna were limited to that genus or extended into other genera and families and were thus of potentially broader significance.

Basic to our discovery of distinctive microstructure in protoxylem tracheary was our adoption of minimally destructive methods of preparation. Macerations inevitably involve high temperatures and oxidative solutions using acids—there is no other way of separating xylem cells. This is true whether a chromate solution is used or a hydrogen peroxide solution is employed. Some xylary tissues require longer times of exposure to these solutions if xylem is associated with fibrous sheaths that do not macerate readily. The longer the exposure, the greater the chance of potential damage to primary walls. In order to avoid this problem, we have turned to a minimally destructive method: thick sections prepared from ethanol-fixed material (Carlquist and Schneider 2006, 2007, 2009). By using these methods, we discovered distinctive microstructure patterns in Cannaceae that cannot be detected with macerative techniques. This method opens a new ave-

Using scanning electron microscopy of hand sections of alcohol-fixed material, microstructure of primary xylem of roots, stems, and rhizomes of Canna was studied. Comparable material of selected other species of Zingiberales, representing five families other than Cannaceae was examined. The appearances present in Canna proved to be shared by other Zingiberales. In protoxylem of inflorescence axes, primary walls that experience appreciable elongation bear longitudinally oriented strands facing the lumen. These can be exposed by sectioning and can also be seen in face view on inner surfaces of tracheary elements. Exterior to these strands, primary walls contain reticulate meshworks of cellulosic strands; longitudinal orientation of the strands is present and corresponds to the degree of elongation of the tracheary elements. These meshworks are mostly embedded in amorphous wall materials (primary middle lamella) and are often faintly seen on the inner primary wall surfaces of tracheary elements but may be variously exposed by sectioning. Metaxylem tracheary elements have much the same microstructure but with meshworks composed of randomly oriented fibrils. The meshwork layers can often be sectioned away from the amorphous wall material wholly or in part. Cellulosic strands, mostly longitudinal in orientation and not embedded in amorphous material, were observed in end walls of protoxylem tracheary elements of Canna inflorescence axes; these should be considered intermediate between tracheids and vessel elements. Perforation plates retaining porose pit membrane remnants were observed in stems of Strelitzia. Use of ethanol-fixed material avoids the tendency of macerating fluids to dissolve primary walls and offers many opportunities for further understanding of microstructure of primary xylem, particular in monocotyledons.

Keywords: Canna, cell wall elongation, primary xylem, Heliconia, Maranta, protoxylem, Strelitzia.
patterns of secondary wall in primary xylem in monocots were explored in a series of articles by Cheadle (1942, 1943a, 1943b, 1944). The focus of Cheadle’s work was development of a scheme with phylogenetic significance. The basis of his work was organographic distribution of vessels, surveying monocots as a whole. Later, he and collaborators studied particular families, thereby developing a more detailed knowledge of secondary wall architecture in vessels and tracheids of monocots (e.g., Cheadle and Kosakai 1971, 1980, 1982). This series of monographs was extended by a study of Eriocaulaceae (Thorsch and Cheadle 1996) and of Zingiberaceae (Thorsch 2000), who included data from electron microscopy. The study of primary xylem by Bierhorst and Zamora (1965) emphasized tracing ontogenetic changes in wall patterns of tracheary elements within organs and systematic distribution of tracheary element types. These studies used light microscopy and focused on three-dimensional shapes of tracheary elements.

By using nonoxidative techniques and ethanol-fixed material, we can present rather accurate images of primary walls of xylem in Zingiberales, thereby setting an interpretative baseline for studies in other monocots. The nature of cell wall elongation, the degree to which fibrillar orientation is related to degree of wall elongation, and the nature of wall microstructure in protoxylem and metaxylem are important to our understanding of how surfaces suited for maintenance of water columns are constructed.

Material and Methods

Our studies were based on cultivated specimens of *Canna indica* L. (garden hybrid) and *Canna iridiflora* Ruiz and Pavon (Cannaceae), *Hedychium gardnerianum* Wall. (Zingiberaceae), *Heliconia angusta* Vell. (Heliconiaceae), *Maranta arundinacea* L. (Marantaceae), and *Strelitzia reginae* Banks. Material of all of these were collected in the gardens of the Lotusland Foundation, Montecito, California, where specimens have been accessioned and documented.

Materials were fixed in 50% aqueous ethanol. Appropriate sections (mostly longitudinal) were cut by hand with the aid of single-edge razor blades. These sections were subjected to three changes of distilled water and kept warm on a 50°C warming table in order to remove unwanted compounds soluble in water that might obscure wall surfaces, as well as to wash away larger objects, such as starch grains. Sections were then placed between clean glass microscope slides, subjected to mild pressure to assure flatness during the drying process, and dried on the 50°C warming table. Dried sections were sputter-coated with gold, mounted on aluminum stubs using electroconducting pads, and observed on a Hitachi S2600N scanning electron microscope. These methods have been used earlier (Carlquist and Schneider 2006, 2007, 2009).

Our use of the terms protoxylem and metaxylem corresponds to that of Schneider (1976), in which protoxylem applies to tracheary elements that have annuli or helical gyres of secondary wall material, whereas metaxylem has pitted secondary wall architecture. The scheme of terminology of Schmid (1977) is therefore not employed here. We interpret apparently amorphous wall portions as compound middle lamella and any reticulate appearances as primary wall portions formed later (internal to) the compound middle lamella.

Results

Canna (Figs. 1–3)

Only a small amount of protoxylem is formed in the vascular core of the roots (fig. 1A–1C). Perforation plates of metaxylem vessels are readily discernible and range from long scalariform (fig. 1A) to simple (fig. 1C). Pit membrane remnants can be found in perforations at the ends of scalariform perforation plates (fig. 1B). Alternatively, these can be termed perforations transitional to lateral wall pits or even pits, depending on degree of pit membrane presence.

Stems (rhizomes) of *Canna* (fig. 1D–1F) have a relatively small number of protoxylem tracheids (fig. 1D); no vessels were observed in the material we studied. End walls of metaxylem tracheids have pit membranes with a reticulate pattern of cellulosic microfibrils (fig. 1D). This reticulum is exposed in the micrographs shown by virtue of the sectioning process: various degrees of the amorphous wall portions have been shaved away. Protoxylem tracheids of *Canna* stems (fig. 1E, 1F) have wall patterns that can vary considerably. The primary walls between gyres may have fibrils oriented longitudinally with respect to the tracheary element axes. Various degrees of density of these fibrils (cf. fig. 1E and 1F) are due to the extent to which fibrils have been removed by sectioning.

The inflorescence axes of *Canna* we studied have mostly protoxylem (see definition above). Some protoxylem tracheary elements show apparently fibrillar strands, elongated longitudinally in the elements, between gyres (fig. 2A, 2B). These strands can be exposed by sectioning, so they are visible from the outside of an element (fig. 2A). Tracheary elements in which sectioning exposes inner surfaces (fig. 2B) also reveal the presence of these strands. There are only a few such strands in both kinds of views, probably because some of the strands merge with amorphous portions of the wall.

In walls of later protoxylem tracheary elements, gyres are more numerous and elongation is less. The cellulosic pattern of fibrils in such elements is more nearly reticulate, apparently related to the lesser degree of elongation of the primary wall (fig. 2C, 2D). The reticulum is often not very evident because of the degree to which it is embedded in amorphous wall materials (fig. 2C). When the amorphous wall materials are shaved away, the cellulosic reticulum is more evident (fig. 2D). Other appearances that we encountered included denser patterns of longitudinally oriented fibrils between gyres (fig. 2E; the white triangle at bottom left is a gyre portion). Strands between gyres can be disposed somewhat three-dimensionally (fig. 2F). Moreover, strands of the inner wall vary in coarseness (fig. 3F).

End walls of protoxylem tracheary elements from *Canna* inflorescence axes feature longitudinally oriented fibrillar strands (fig. 3A–3E). Viewed from the inside of one tracheary element and looking through into the lumen of an adjacent element, one can see that amorphous material is lacking between these strands (fig. 3A), which were not subjected to any
Fig. 1  SEM micrographs of tracheary elements of *Canna iridiflora* (A, B, D) and *Canna indica* (C, E, F). A–C, Views of metaxylem vessel elements from root. A, Long scalariform perforation plate; fibers at top of micrograph. B, Portion of perforation plate; lateral wall pits (top) have intact smooth pit membranes. The perforations of a perforation plate (bottom) mostly have pit membranes, somewhat fractured; this is characteristic of the end of a perforation plate transitional to end wall pitting. C, Double rim of a simple perforation plate; lateral wall pits of vessel element, variously subdivided by secondary wall material. D–F, Views of rhizome tracheids. D, Pit membrane from end wall of metaxylem tracheid, seen from outside surface. E–F, Wall structure of protoxylem tracheids; adjacent cells have been scraped away, revealing wall microstructure; annular thickening of cell oriented vertically in both micrographs. E, Fibrils embedded in amorphous wall material at left; amorphous wall material mostly sectioned away at right. F, Sectioning reveals longitudinally oriented fibrils. Scales: A = 50 μm; B, C = 20 μm; D–F = 5 μm.
Fig. 2 SEM micrographs of tracheary elements from *Canna indica*. A–E, Protoxylem from inflorescence axes. A, Section of helical element, seen from the outside, in which a primary wall except for longitudinally oriented strands has been sectioned away. B, Section of a tracheary element as seen from the inside; a few longitudinally oriented strands are evident (especially lower right); faint regularly spaced longitudinal streaks are outlines of adjacent parenchyma cells. C, Section of helical element, seen from the inside; at this magnification, the wall appears mostly smooth, although a faint reticulate surface can be seen in places. D, Wall portion of inside surface of a tracheary; a network of reticulate strands may be seen. E, Wall portion of tracheary element (small portion of helical band at lower left), showing parallel fibrillar appearance. F, Portion of inside of helical element from rhizome protoxylem, showing some coarse longitudinally oriented strands. Scales: A, B = 20 μm; C, D = 5 μm; E, F = 2 μm.
Fig. 3  SEM micrographs of helical protoxylem tracheary elements from inflorescence axes of *Canna indica*. A, View of inside of element; most strands are longitudinally oriented, with some interconnections. B, Interface between two elements, the gyres torn apart by sectioning, revealing longitudinally oriented strands. C, View of outer surface of element, adjacent cell sectioned away, thereby revealing nature of interconnecting strands in the primary wall. D, View of wall portion from outside of element, showing that coarse strands are intercontinuous underneath (external to) the border of the gyre. E, View of outer surface of element; wall portions sectioned away except for a few longitudinally oriented strands. F, Inside surface of element; strands of various degrees of thickness appear superimposed on or integral to amorphous wall material. Scales: A–C, E, F = 5 μm; D = 2 μm.
section or scraping action. Likewise, amorphous material is not evident in an end wall that has been split apart (fig. 3B). Presence of amorphous material in nearby lateral wall areas (fig. 3B, left and right) confirms that absence of amorphous material in the end wall is natural. Although the fibrillar strands in these end walls are predominantly longitudinally oriented, there are interconnections among them (fig. 3A, 3C, 3D). The fibrillar complex does not disappear exterior to the gyres but is continuous over the gyres of the secondary wall (fig. 3C, 3D). In sections in which more wall material is removed, fewer fibrils are evident (fig. 3E).

Strelitzia (Fig. 4)

SEM micrographs of Strelitzia reginae tracheary elements form a series remarkably similar to those of Canna. In roots, scalariform perforation plates are presence in metaxylem vessels (fig. 4A). There are only small vestiges of pit membranes present within the perforation plate. Lateral walls of metaxylem vessels in roots have pit membranes that are composed of a cellulosic reticulum embedded in an amorphous matrix. The degree to which the reticulum is revealed is related to how much of the amorphous matrix is shaved away by sectioning (fig. 4B).

Stems of Strelitzia have metaxylem composed of vessels with long scalariform end walls (fig. 4C). Tomlinson (1959, p. 332), very likely referring to such a cell as that illustrated in figure 4C, stated that the vessel elements in the stems of Strelitzia are the “least specialized” of the vessel elements found in Strelitziaae. Certainly they exemplify the transition between tracheids and vessel elements, because the end walls have wide bars and there are extensive remnants of porous pit membranes (fig. 4C). Lateral walls of stem metaxylem vessel elements (fig. 4D) bear pits much like those of lateral walls of root vessels.

Inflorescence axes of Strelitzia reginae are composed predominantly if not wholly of protoxylem tracheids. The inner surfaces of such tracheids (fig. 4E) bear prominent longitudinally oriented strands (fig. 4E). In some places and at high magnifications, one can see that these strands merge into a reticulum embedded in the amorphous matrix (fig. 4E, 4F).

Heliconia (Fig. 5A–5C)

Our studies of Heliconia were limited to tracheids of the inflorescence axes. The inner surfaces of protoxylem tracheids have elongate strands (fig. 5A, 5B). These can be readily seen where the inner surface is exposed by sectioning (fig. 5A). The strands are also apparent in a section where the outer amorphous wall matrix of a tracheid is removed, with various degrees of the wall scraped away by the process (fig. 5B). A reticulate layer is present on the lumen face of the tracheids. The pit membranes of metaxylem tracheids (fig. 5C) have randomly oriented fibrils that form a reticulum, evident to the degree that the amorphous matrix is sectioned away.

Maranta (Fig. 5D–5F).

When paired with the pit membranes from the Heliconia metaxylem inflorescence axes, equivalent pit membranes from stem metaxylem of Maranta (fig. 5E) are remarkably similar. The reticulum has been exposed in both cases by sectioning away of the amorphous wall material. Protoxylem tracheids of Maranta (fig. 5E) have elongate wall strands much like those figured for Heliconia (fig. 5A) or Canna (fig. 1A). A very dense reticulum is shown for a protoxylem element in figure 5F. This represents the typical cellulosic primary wall seen when the amorphous matrix of the wall is pared away by sectioning. Some longitudinally oriented strands are seen at left; the lack of pores at right is a result of less removal of the amorphous material by sectioning.

Discussion and Conclusions

Protoxylem that has undergone appreciable degrees of elongation (yet not enough for breakage of the primary wall) proved frequently, in materials of Zingiberales, to have fibrillar structures, facing the lumen side of the element. These strands are elongate in the direction of the long axis of the tracheary element and do not extend over the secondary wall gyres, nor are they attached to the borders of the gyres. When one views the inner wall surface, these strands may be seen variously emergent from the flat surface of the primary wall. Protoxylem that has experienced considerable elongation (but without primary wall fracture) may also show a reticulum, presumably of cellulosic fibrils, embedded in the amorphous primary wall material. Such a reticulum shows various degrees of stretching that seem to correspond with the degree of elongation that a protoxylem tracheary element has experienced. Late protoxylem tracheary elements thus are more likely to show a meshworklike reticulum instead of strands oriented longitudinally in the cells.

Lateral wall pits in metaxylem tracheary elements have cellulosic reticula composed of randomly oriented fibrils. The degree to which this reticulum is exposed in our preparations depends on the degree to which the amorphous wall material (compound middle lamella) is removed by the sectioning process.

Peculiar coarse strandlike structures were reported in xylem of all genera of Nymphaeaceae and Cabombaceae (e.g., Carlquist and Schneider 2009; Schneider et al. 2009). These strands differ from those we report from Zingiberales by having the following characteristics: they occur in metaxylem only, not protoxylem; they are three-dimensionally disposed and grade from strands into reticula much coarser than those of Zingiberales; they occur on end walls of metaxylem tracheids only; and they are not attached to pit borders. As far as is known, the coarse strandlike structures of metaxylem stem tracheids of Nymphaeaceae are unknown elsewhere in vascular plants.

Perforation plates in protoxylem of angiosperms at large are little known. The most extensive compendium of illustrations can be found in Bierhorst and Zamora (1965). Perforation plates that they illustrate include ones that are simple, with no bands of secondary wall material on end walls. Also, they illustrate perforation plates that are oblique and differ from lateral walls in that the secondary wall gyres are attenuate across the end walls. These types were illustrated earlier by Cheadle (1942). These results are difficult to extend into what one observes with SEM, because macerated tracheary el-
Fig. 4  SEM micrographs of tracheary elements from primary xylem of Strelitzia reginae. A, B, Metaxylem vessel elements from root. A, Portion of a scalariform perforation plates. B, Lateral wall pit portions seen from outside of the cell; sectioning has shaved away the amorphous wall portion, revealing the reticulate nature of the inner wall layer. C, D, Metaxylem tracheary elements from stem. C, End wall; note wide bars and remnants of pit membranes. D, Lateral wall pit portion seen from outside of the cell; sectioning has removed amorphous wall portions, revealing a reticulate pattern. E, F, Portions of inner wall surface from helical protoxylem elements of inflorescence axis. E, Secondary wall gyre portion (center), plus strands oriented longitudinally in the element. F, Secondary wall gyre portion (at extreme left) plus strands of wall material with various thicknesses and orientations on the inner wall surface. Scales: A, C = 20 μm; B, E = 5 μm; D, F= 2 μm.
Fig. 5  SEM views of tracheids from Heliconiaceae and Marantaceae. A–C, Tracheids from inflorescence axis of *Heliconia bihai*. A, View of protoxylem tracheid inner surface, showing strands of wall material running between gyres. B, View of protoxylem outer surface, amorphous wall portions shaved away by sectioning, revealing reticulate pattern and strands running between gyres. C, Metaxylem tracheid; portions of three pit membranes, seen from outside of cell, outer wall scraped away by sectioning. D–F, *Maranta zebrina*; portions of tracheid walls from upright stems (canes). D, Portions of pits from metaxylem, viewed from outside of cell; amorphous wall portions shaved away by sectioning, revealing reticulate pattern. E, F, Wall portions of protoxylem tracheids, seen from outside of cell; amorphous wall portions removed by sectioning. E, Strands running between gyres of a helical element. F, Reticulate wall layer with randomly oriented fibrils. Scales: A, B, E, F = 5 μm; C, D = 2 μm.
lements, on which most earlier work has been done, have walls affected by hydrolytic action of the acidic and oxidative macerating fluids. Macerations do have the merit of revealing the contours of entire cells, whereas sections at best reveal only portions of cells. The use of thick sections in our work does help to develop three-dimensional understanding of tracheid structure, but entire perforation plates are rarely revealed.

This study reports an interesting phenomenon not hitherto mentioned for monocotyledons: cellulosic strands, predominantly longitudinal in orientation, crossing end wall pits that lack an amorphous matrix (e.g., *Canna* in fig. 3A–3D). These end walls are thus intermediate between vessels with scalariform perforation plates (primary wall material absent, mostly as a result of hydrolysis) and tracheid end walls (primary walls present, even if somewhat porose, in pits of end walls), and they help one understand the origin of perforation plates in monocotyledons. Likewise, presence of extensive porose pit membrane remnants in end wall pits of metaxylem stem tracheary elements of *Strelitzia* reveals a condition intermediate between tracheids and vessel elements.

We believe that various degrees in porousness of the end wall of tracheary elements may be found in monocotyledons, and that there is no line that can be drawn between vessel elements and tracheids in some portions of some monocotyledons. One could use, as did Cheadle (1942), the criterion of whether India ink particles can pass through end walls. Today, suspensions composed of microspheres of uniform size are available and could provide a more refined version of the India ink experiment (uptake of microspheres can be tracked with SEM studies of sectioned xylem). However, one is still left with the question of what particular degrees of porousness mean. What size of pores enhances the conductive flow to an appreciable extent? What size of pores prevents transmission of air bubbles from one tracheary element to the next in a linear series? Identification of these physiological processes in relation to wall structure represents the significant task in future studies of the transition between tracheids and vessel elements.

**Literature Cited**


Queries

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