

WOOD ANATOMY AND SUCCESSIVE CAMBIA IN *SIMMONDSIA*
(SIMMONDSIACEAE): EVIDENCE FOR INCLUSION IN
CARYOPHYLLALES S.L.

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ABSTRACT

Simmondsia chinensis (Link) Schneider, endemic to dry areas of California, Arizona, and adjacent Mexico, is the sole species of Simmondsiaceae. Wood anatomy and cambial activity of this species are analyzed in view of the transfer of the taxon from Buxaceae or Euphorbiales to Caryophyllales s.l. The wood contains features considered primitive in dicotyledons: tracheids, diffuse parenchyma, and rays that are both multiseriate and uniseriate. These features are shared with Agdestidaceae, Rhabdodendraceae, and Stegnospermataceae, families considered basal to most of the Caryophyllales s.s. ("core Caryophyllales"). *Simmondsia* has nonbordered perforation plates and successive cambia, features that occur widely in Caryophyllales s.l. Wood anatomy of *Simmondsia* is congruent with placement of the genus in Caryophyllales s.l. *Simmondsia* wood features reflect the desert habitat both quantitatively and qualitatively. Terminology and ontogenetic concepts of *Simmondsia* secondary xylem, successive cambial action, and periderm are contrasted with those in a previous study, and the problems of analysis of woods with cambial variants are discussed.

INTRODUCTION

Simmondsia chinensis, contrary to its species name, is native to limited areas of arid hills and low mountain ranges of southern California, southern Arizona and adjacent portions of Mexico (Munz 1973). The genus has frequently been placed in Euphorbiales (see Goldberg 1986, Table I, for the treatments of Simmondsiaceae by 11 leading phylogenists). Simmondsiaceae has been placed in the family Buxaceae within the order by many workers. The unisexual flowers and tricoccoid fruits were suggestive of this relationship for many systematists. However, *Simmondsia* was unique within Euphorbiales in having successive cambia. Successive cambia have evolved in several groups independently (e.g., Menispermaceae, Gnetales); consequently, the presence of successive cambia was probably not considered a feature of prime taxonomic value.

Recent phylogenies based on molecular data have opened possibilities in taxonomic interpretation with regard to *Simmondsia* and some other genera with successive cambia. These newer views have effectively supplanted earlier thinking, so various earlier phylogenies are not compared here. An expanded Caryophyllales ("caryophyllids") was proposed by Williams et al. (1994). Most notably, Droseraceae and Nepenthaceae were added, with Dilleniaceae the first branch on this clade. In subsequent phylogenetic constructions (Nandi et al. 1998; Soltis et al. 2000), families added to the expanded Caryophyllales include Ancistrocladaceae, Asteropeiaceae, Dioncophyllaceae, Frankeniaceae, and Tamaricaceae. Santalales are considered the outgroup for Caryophyllales s.l. In all of the phy-

logenies utilizing molecular data, Buxaceae are not adjacent to Caryophyllales s.l., so the concept that *Simmondsia* belongs to Buxaceae is not supported. The present study is designed to compare wood and stem anatomy of *Simmondsia* to that of Caryophyllales s.l. that may be related. That purpose was also basic to the recent study of Rhabdodendraceae (Carlquist 2001a). Successive cambia occur in Caryophyllales s.l. in Agdestidaceae, Amaranthaceae, Aizoaceae, Barbeuiaceae, Basellaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolaccaceae, Plumbaginaceae, Polygonaceae, and Stegnospermataceae. The only families of Caryophyllales s.s. ("core Caryophyllales") that lack successive cambia are Cactaceae, Didieriaceae, and Portulacaceae. Thus, the presence of successive cambia in *Simmondsia* alone is a reason to compare *Simmondsia* to Caryophyllales.

There have been previous accounts of *Simmondsia* wood, notably those of Solereder (1885, reported as *Brocchia*), Van Tieghem (1897), Bailey (1980), and Carlquist (1982a). The present study goes beyond those studies by utilizing scanning electron microscopy (SEM) and by careful attention to ontogenetic phenomena (and associated terminology). Better understanding of successive cambia in *Simmondsia* will lead to a more accurate picture of successive cambia in dicotyledons and Gnetales.

MATERIAL AND METHODS

Stems of *Simmondsia chinensis* from a large shrub cultivated in the Santa Barbara Botanic Garden were fixed in 50% aqueous ethanol. Stems were taken in January and June, 2001, in order to compare degree of meristematic activity in the lat-

eral meristem. The stem of *S. chinensis* is hard enough to be sectioned, without softening, on a sliding microtome, but thin sections contain fractures. Some sliding microtome sections were stained with safranin and used for permanent slides. Some tangential sliding microtome sections were dried between clean glass slides, sputter coated with gold, and viewed with a Bausch & Lomb Nanolab SEM. Portions of "bark" (tissue exterior to vascular bands) were fixed in 50% aqueous ethanol, softened for three days at 60°C in 8% ethylene diamine, infiltrated, embedded with paraffin, and sectioned according to the method of Carlquist (1982b). These sections were stained with a safranin-fast green combination. Macerations were prepared with Jeffrey's Fluid (equal parts of 10% chromic acid and 10% nitric acid) and stained with safranin. The stems studied were between one and three cm in diameter.

Vessel lumen diameter rather than outside vessel diameter was measured; for vessels oval in transection, long and short chords were averaged. The vessel density recorded was based upon scans that did not include conjunctive tissue. If conjunctive tissue were to be included, the number of vessels per mm² would be about 50% lower. Both earlywood and latewood were included in the computation of quantitative vessel data. Terms are in accordance with the IAWA Committee on Nomenclature (1964) and Carlquist (2001b). The term "successive cambia" follows the usage of Schenck (1893) and Pfeiffer (1926).

RESULTS

Secondary Xylem

Growth rings inconspicuous, but evident on the basis of earlywood vessel diameter (Fig. 1); earlywood is usually not initiated at the beginning of each vascular band, but at some point within vascular bands. Vessels are virtually all solitary (Fig. 1); mean number of vessels per group, 1.04. Mean vessel lumen diameter, 21 μm. Mean number of vessels per mm², 260 μm. Mean vessel element length, 163 μm. Mean vessel wall thickness, 2.8 μm. Perforation plates simple, nonbordered (Fig. 3, top). Helical thickenings present on vessel walls, some in the form of pairs of thickenings paralleling helices of pits (Fig. 3). Pit cavities of lateral wall vessel pits about 3 μm in diameter. Imperforate tracheary elements all tracheids, densely covered with fully bordered circular pits about 3 μm in diameter (Fig. 4). Mean tracheid wall thickness, 3.2 μm. Axial parenchyma sparse, diffuse, composed of cells that are not subdivided. Rays uniseriate to wide multiseriate (Fig. 2); uniseriate rays are more common than multiseriate rays. Most uniseriate rays are a single cell in height (and thus not easily seen in Fig. 2). Mean height of multiseriate rays, 212 μm. Mean width of uniseriate rays, 74 μm. Mean ray cell wall thickness, 1.5 μm, walls lignified. Ray cell

wall pits simple. Ray cells predominantly procumbent; square and upright cells relatively uncommon. Starch abundant in ray cells (Fig. 5). Secondary xylem nonstoried.

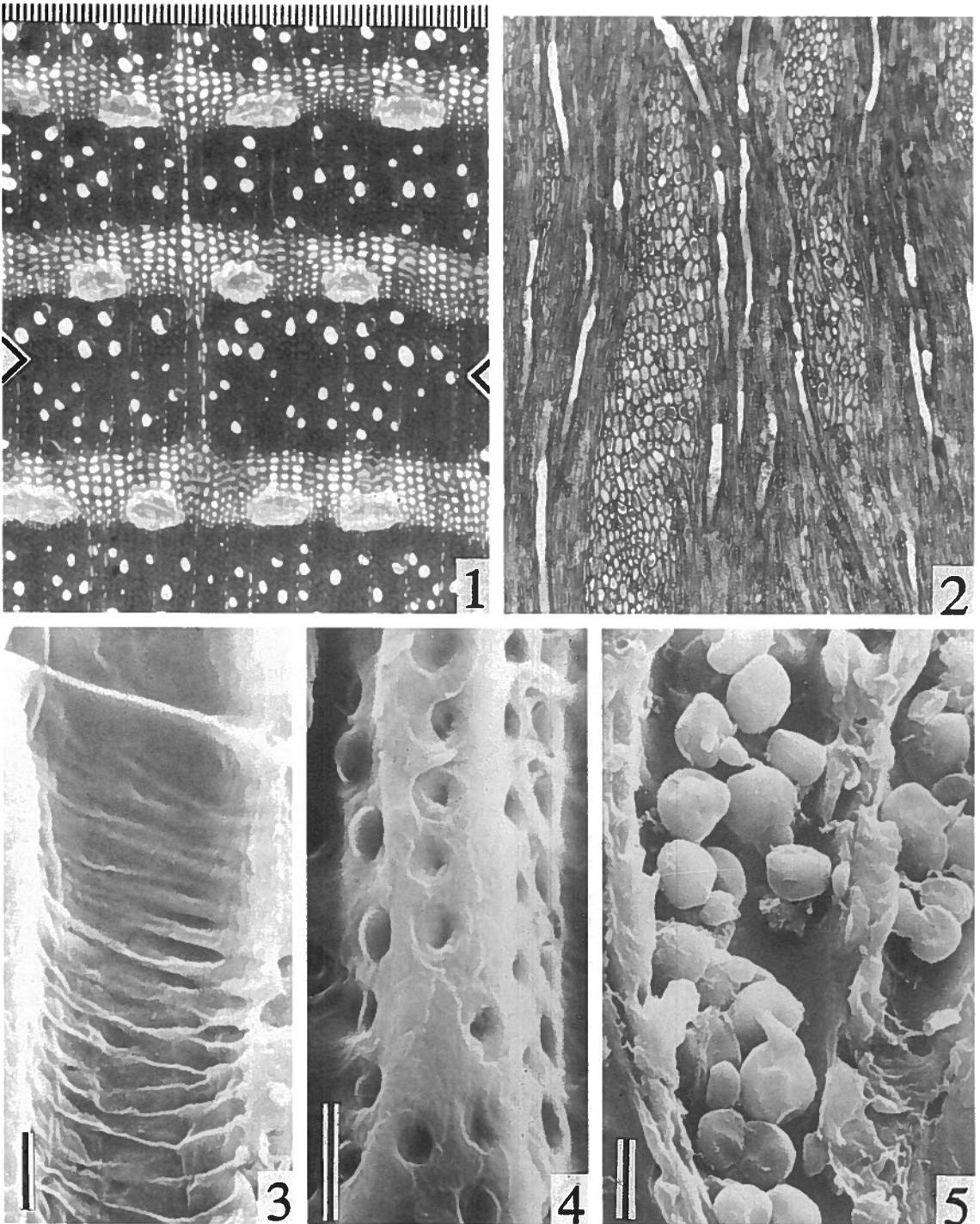
Conjunctive Tissue and Cambial Action

Conjunctive tissue is composed of bands five to ten cells thick radially (Fig. 1: tangential bands of thin-walled radial cells, containing phloem strands, intervening between tangential bands of dark secondary xylem). As seen in radial section (Fig. 6), cells are mostly upright, a few square (and thus contrast with ray cells of the secondary xylem). Cell walls of conjunctive tissue are lignified and about 1.5 μm in thickness, but thin-walled nonlignified cells present on bark side of phloem strands (Fig. 7, above crushed phloem). Functional phloem cells, adaxial to the dense crushed phloem strand, are somewhat compressed due to sectioning rather than polygonal. What portion of the phloem cells that are not crushed are functional could not be determined. Continued production of secondary phloem is possible because earlier-formed phloem is progressively crushed. Solitary rhomboidal crystals are occasional in conjunctive tissue (Figs. 6, 8). Periclinal divisions can be seen in young conjunctive tissue (Fig. 9), but number of cell layers in the meristematic zone of the lateral meristem between the most recently initiated vascular cambium and the previous vascular band varies (e.g., Fig. 8).

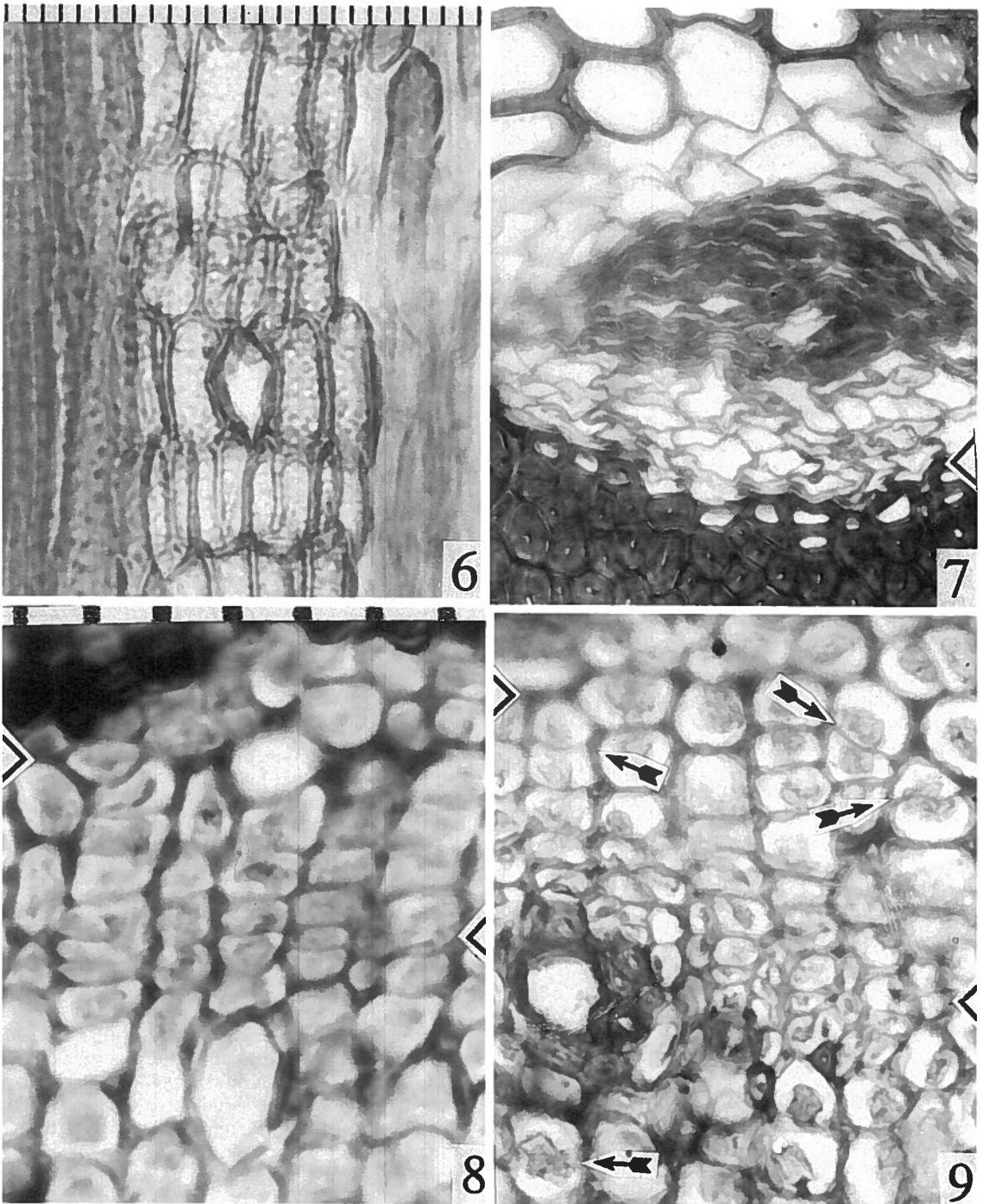
Vascular cambia (pointers at right edge, Figs. 7-9) actively produce secondary phloem and secondary xylem: the secondary phloem occurs as isolated strands, whereas the secondary xylem mostly occurs as unbroken cylinders that extend around the stem. The first tracheids produced by a cambium are polygonal in outline, and their alignment in radial rows is sometimes obscure because during maturation, the interfaces of the several faces shift. As divisions wane in each vascular cambium, a few thin-walled tracheids in radial rows are produced (Fig. 7, below phloem). Cambial activity produces phloem for a prolonged period, probably for several years because the abaxial portion of secondary phloem strands consist of numerous crushed phloem cells (Fig. 7, gray mass in center of photograph) and phloem cells produced by the cambium are in radial rows. Also, crushed phloem cells are always abaxial to apparently functional secondary phloem cells (Figs. 1, 7).

Phellogen and Lateral Meristem Action

The outer layers of the relatively mature stems studied here lack the cortical fiber strands reported by Bailey (1980) in the relatively young stems he studied. Such fiber strands had been shed from the stem I studied. Phellem, which consists of cells filled with dark-colored contents, is present on the stem surface. The phellem cells are narrower than cells in what is here termed diffuse lateral meristem



FIGS. 1-5. Stem sections of *Simmondsia chinensis*. 1. Transection (abaxial side above), showing alternation of bands of secondary xylem (each with associated phloem strands) and conjunctive tissue; vascular bands are not annual in extent; the pointers demarcate part of the earlywood of one year (above pointers) from part of the latewood of the preceding year (below pointers), several vascular bands are produced per year. 2. Tangential section of secondary xylem; a few large multiseriate rays are apparent (center), uniseriate rays are mostly inconspicuous because of their very small size. 3-5. SEM photographs from a tangential section of secondary xylem. 3. Portion of inner surface of a vessel, showing nonbordered perforation plate (top) and helical thickenings. 4. Outer surface of a tracheid, showing bordered nature and density of pits. 5. Starch grains in ray cells. Figs. 1, 2, magnification scale above Fig. 1 (divisions = 10 μm ; Figs. 3-5, scale bar in each figure = 5 μm).



FIGS. 6-9. Sections of stem of *Simmondsia chinensis*. 6. Radial section of conjunctive tissue to show cell shape and (center) rhomboidal crystal (secondary xylem at extreme left, secondary phloem at extreme right). 7. Strand of secondary phloem and surrounding tissues (conjunctive tissue above, secondary xylem below); crushed secondary phloem in abaxial part of phloem strand (pointer indicates site where vascular cambium was prior to cessation of active division). 8, 9. Transections of lateral meristem zone at periphery of stem; pointers at left in each indicate offset between outermost cells of the radial lateral meristem (radial files) and the innermost periderm cells; pointers at right indicate vascular cambium location. 8. Lateral meristem zone in which a vascular cambium has recently formed (no secondary xylem or phloem elements identifiable yet); rhomboidal crystal at bottom center. 9. Lateral meristem zone in which vascular cambium has yielded a vessel (extreme left) and some tracheids as well as some secondary phloem (right). Diagonal arrows denote recent divisions in the radial files of the lateral meristem zone; horizontal arrow (lower right) denotes a cell plate (obscured by cell contents adherent to it) that indicates a recent division in conjunctive tissue that is still somewhat meristematic. Figs. 6, 7, magnification scale above Fig. 6 (divisions = 10 μ m). Figs. 8, 9, scale above Fig. 8 (divisions = 10 μ m).

(it could also conceivably be termed secondary parenchyma). The diffuse lateral meristem cells are in radial rows; the term "diffuse" is used because divisions do not occur synchronously in a single layer as in a vascular cambium, but are randomly distributed throughout the meristematic zone (see Carlquist 1999a). A lateral meristem that consists of a single layer was observed in *Barbeuia* (Carlquist 1999b) and also occurs in Nyctaginaceae (Carlquist unpublished data; data in earlier papers vary in interpretation and will be discussed in a later paper). More numerous divisions were observed in the stems collected in June than in those collected in January. The radial rows of lateral meristem cells are offset from the periderm which consists of a single layer of phellogen (narrow, like the phellem cells, but devoid of dark-colored compounds). In some places, there is a layer of parenchyma between the phellogen and the lateral meristem files. No phellogen cells were identified unequivocally. The offset between the radial rows of the diffuse lateral meristem and the periderm is indicated by a pointer at the left in Fig. 8 and Fig. 9. The entirety of the periderm is illustrated in Figs. 8 and 9, which were selected to show primarily lateral meristem and vascular cambia.

Origin of vascular cambia occurs within the radial files of cells produced by the diffuse lateral meristem. Although only a small portion (for reasons of clarity) could be illustrated, study of the entirety of sections validates this interpretation. The origin of a vascular cambium (Fig. 8, pointer at right) can be distinguished from divisions of the lateral meristem because divisions of the vascular cambium form a single meristematic layer of divisions that are synchronous in tangential bands around the stem. Each vascular cambium soon produces secondary xylem internally (adaxially) and secondary phloem abaxially (Fig. 9, pointer at left; vessels and a few tracheids in secondary xylem). The terminal products of a vascular cambium (Fig. 7) are described above.

CONCLUSIONS

Phylogenetic Position

The occurrence of successive cambia is a character widespread in Caryophyllales s.s. ("core Caryophyllales") so its occurrence in families now added to an expanded Caryophyllales-Rhabdodendraceae (Carlquist 2001a) and Simmondsiaceae is not surprising. "Caryophyllales: s.s." corresponds to the betalain-containing families plus Achatocarpaceae, Barbeuiaceae, and Molluginaceae, and the genera *Limeum* and *Lophiocarpus* (Clement et al. 1994). *Simmondsia* has characters generally considered primitive in dicotyledons: presence of tracheids, presence of diffuse axial parenchyma, and presence of both multiseriate and uniseriate rays (Metcalfe and Chalk 1950: xlv, "fibres with distinctly bordered pits;" Kribs 1935, 1937). All of

these features are present in Rhabdodendraceae (Carlquist 2001a), now placed at the base of Caryophyllales s.l., and in genera now placed at or near the base of Caryophyllales s.l. (Soltis et al. 2000). *Simmondsia* is placed by Hoot et al. (1999) and Soltis et al. (2000) near the base of Caryophyllales s.s. The other genera with the primitive features listed above include *Agdestis* (Carlquist 1999c) and *Stegnosperma* (Carlquist 1999a); *Barbeuia* has tracheids, but not the other character states mentioned above (Carlquist 1999b).

One feature of possible ordinal significance is the presence of nonbordered perforation plates. These have been demonstrated in most Caryophyllales s.s. (see Carlquist 1999a, b, 2000). Nonbordered perforation plates are newly reported here for *Simmondsia* (Fig. 3), and have recently been reported for some Caryophyllales s.l. such as Rhabdodendraceae (Carlquist 2001a). Nonbordered perforation plates may be a symplesiomorphy in Caryophyllales s.l. according to the above data and other observations (Carlquist 2001b).

Ecology

Simmondsia is a desert shrub with only slight succulence in the leaves (Bailey 1980); not surprisingly, it has xeromorphic wood. The Mesomorphy Ratio (vessel diameter times vessel element length divided by vessel diameter) was reported to be 27.8 for *Simmondsia* by Carlquist and Hoekman (1985). A very similar value (24.4) can be derived from the present data if conjunctive tissue is not excluded. The similarity of the two reports is even closer if one notes that outside vessel diameter, rather than lumen diameter was used by Carlquist and Hoekman (1985).

Tracheids are conductively safe (excellent at confining embolisms to a single cell) compared to vessel elements. Fiber-tracheids and libriform fibers, by contrast, are nonconductive (see discussion in Carlquist 2001b). The presence of tracheids in *Simmondsia* is a feature of value in a xeromorphic habitat. Because of the presence of tracheids, vessel grouping in *Simmondsia* is virtually nil (1.04), in agreement with the correlation for dicotyledons as a whole claimed by Carlquist (1984). The value of tracheids in promoting conductive safety exceeds the value of vessel grouping (Carlquist 2001b).

Ontogeny and Terminology

The terminology in papers and books that deal with successive cambia is remarkably diverse, but more significantly, different interpretations often underlie the terms used. The present paper is not a proper venue for a review of this situation. However, the paper by Bailey (1980) on *Simmondsia* is appropriate for comparison in view of the ontogenetic interpretations as well as mature structures detailed in both the present paper and Bailey's.

In the present interpretation, a diffuse lateral

meristem forms outside of the vascular cylinder, near the stem periphery. This lateral meristem produces radial files of cells, producing parenchyma cells with primary walls, cells which remain relatively meristematic judging from recent divisions to be found in this region. Within the lateral meristem zone, a new vascular cambium is formed (usually while the preceding vascular cambium is still actively producing secondary xylem and phloem). In Bailey's (1980) interpretation, the zone I have termed lateral meristem is called conjunctive tissue (despite its lack of lignified secondary walls as found in conjunctive tissue in older parts of the stem). Bailey (1980) uses the term "extrafascicular cambium" for what I term the vascular cambium in each of the concentric vascular bands.

Bailey (1980) claims that "the phellogen is actually a region of transition where the peripheral conjunctive parenchyma of previous extrafascicular cambia undergoes further cellular subdivision; a true phellogen is lacking." In the relatively young stems illustrated by Bailey (1980), phellogen might well be formed from cortical parenchyma as it is in many dicotyledons, but my studies indicate the existence of a self-perpetuating phellogen, as so frequently described in dicotyledons. This phellogen is distinguished from the lateral meristem (outermost conjunctive parenchyma of Bailey) not only by its tangentially narrower cell diameter but by an offset between the periderm files and the files of cells in the radial parenchyma (Figs. 8, 9). These two differences would be difficult to explain if periderm were ontogenetically continuous with the files of cells of the lateral meristem.

The vascular cambia produce strands of secondary phloem externally and cylinders of secondary xylem internally. In my interpretation, quite ordinary rays are produced by each cambium. In Bailey's (1980) interpretation, "conjunctive tissue initials produce raylike structures of conjunctive tissue; true vascular rays are absent." This interpretation has not, to the best of my knowledge, been offered in any genera with successive cambia other than in Bailey's study of *Simmondsia*.

The differences in interpretation detailed above show that careful analyses of successive cambia and other cambial variants still need to be undertaken. The diversity of interpretations and terms for the anatomical phenomena is still considerable. The number of different interpretations and terms within instances of successive cambium occurrence seems unlikely to be matched by an equal diversity of ontogenetic mechanisms. Rather, cellular arrangements have been viewed differently by different workers. In part, the diversity of interpretations and terminology may derive from microtechnical considerations. Thin sections such as can be cut with a rotary microtome, are desirable for revealing cell lineages and histological details clearly. The hardness of many stems with successive cambia has, on the contrary, led to preparation of sliding micro-

tome sections in which soft tissues do not section well or are too thick for cell development sequences to be revealed clearly. The use of rotary microtome sections of material that has been chemically softened to a suitable degree seems the best solution to this dilemma. Embedding in resin or plastic is an alternative microtechnical possibility.

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