Lateral meristems, successive cambia and their products: a reinterpretation based on roots and stems of Nyctaginaceae

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Anatomical studies of stems and roots with lateral growth from eight species of seven genera of Nyctaginaceae provide material for analysis of meristematic activity and histological products of that activity. Ideas about occurrence of meristems that achieve lateral growth in Nyctaginaceae are reviewed. The interpretation offered for the family differs from those of other workers, although the new interpretation is clearly implicit in Solereder's figure of secondary growth in *Pisonia*. A lateral meristem produces secondary cortex to the outside. To the inside, it produces conjunctive tissue (both parenchymatous and fibrous), true rays (except in *Bougainvillea* and *Heimerliodendron*) and a succession of vascular cambia. As each vascular cambium is produced, the lateral meristem outside of the vascular cambium tends to become quiescent, returning to activity when the vascular cambium internal to it has become less active. Quiescence of the lateral meristem at these points coordinates the amount of tissue produced to the inside in zones without vascular cambia with that produced in zones with cambia. *Heimerliodendron* is rayless; *Bougainvillea* has minimal differentiation between conjunctive tissue parenchyma and ray parenchyma. Vascular cambia do not produce rays in Nyctaginaceae (lateral meristems do), although vascular cambia produce vessels, axial parenchyma and sometimes fibres to the inside and indefinite amounts of secondary phloem to the outside (earlier increments of phloem are crushed). Conjunctive tissue is held to have both parenchymatous and fibrous components and is readily distinguished from products of vascular cambia. Nonbordered perforation plates characterize Nyctaginaceae, as they do many families of Caryophyllales. The anatomical plan of *Heimerliodendron* is markedly different from that of *Pisonia s. s.*, and *Pisonia* sect. *Prismatocarpus* (which includes *Heimerliodendron* in some treatments) may merit generic recognition. The lateral meristems of all Nyctaginaceae studied are storied (stratified), as are products except for fibres, which undergo such extensive intrusive growth that a storied pattern is not achieved at maturity. The terms 'included phloem' and 'interxylary phloem' must be abandoned for descriptions of vascular tissue in families of the order Caryophyllales. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, **146**, 129–143.

ADDITIONAL KEYWORDS: cambial variants – Caryophyllales – conjunctive tissue – lateral growth – vascular cambia – wood anatomy.

INTRODUCTION

Plant anatomists who have dealt with Nyctaginaceae (see Gregory, 1994) have offered satisfactory descriptions of the ground plans of stems and roots, and provided accurate accounts of the histology. An understanding of these ground plans, however, requires appropriate knowledge of the ontogenetic mechanisms that have produced these patterns. Nyctaginaceae that increase in lateral thickness may be said to have successive cambia (although some authors reject that term in Nyctaginaceae). However, the source of these cambia and the way in which they act have been subject to diverse and controversial interpretations. The apparent diversity of ground plans in Nyctaginaceae offers opportunity, as well as difficulty in any attempt at resolution, of interpreting the full range of meristematic activity in axes of the family. A relatively large grouping of genera and species has been assembled here in comparison to other

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studies on meristematic activity in the family, studies that have usually focused on a single genus. The diversity of meristematic manifestations and the diversity of products of these meristems in Nyctaginaceae relate, in my opinion, to variations on a single plan. Evolution of entirely disparate phenomena for achieving lateral growth in plants with similar appearing axes seems unlikely. A comparative method is therefore followed here. Compilation of detailed wood data is not deemed justified because the sampling is not extensive and because data based on different organs of widely different ages are not applicable to comparative studies elsewhere in the order Caryophyllales. The meristematic phenomena shown by Nyctaginaceae may well be of pervasive importance within the order. Just as entirely disparate methods of cambial activity are not to be expected within Nyctaginaceae, a single basic plan (with variations) related to the phenomenon of successive cambia seems likely present throughout Caryophyllales. In turn, the interpretations offered for patterns in Nyctaginaceae seem, at present, applicable to families as diverse as Convolvulaceae and Gnetaceae.

Some introduction to the nature of disagreements is necessary as a background for the present observations. Esau & Cheadle (1969), studying *Bougainvillea spectabilis*, favoured the idea of a succession of cambia which 'arise in centrifugal order . . . each originating among the derivatives of the preceding cambium. Such cambial layers function bidirectionally, producing xylem towards the inside of the axis and phloem towards the outside.' In addition, they believed that 'conjunctive tissue and xylem fibres' are formed 'toward the inside'. This view contrasts sharply with that of Studholme & Philipson (1966), who worked with *Heimerliodendron brunonianum*. Studholme and Philipson claimed that there is a 'region of maximum meristematic activity . . . which progresses regularly outwards', apparently by ceasing to function internal to phloem but then by forming outside of the phloem: 'as the development of the phloem strands proceeds, the cambial cells external to it can be seen to become meristematically more active, so that the phloem appears to be enclosed within two arcs of the most active zone. Finally, the active zone lies entirely outside the phloem.' Stevenson & Popham (1973), working with *Bougainvillea spectabilis*, claimed a single unidirectional meristem which gives rise to 'desmogen strands' (term used earlier by Pfeiffer, 1926), a concept allied to procambial strands. The desmogen strands are claimed to be embedded in a background of conjunctive tissue. Only after phloem and xylem differentiate from cells in these strands does a (vascular) cambium of limited duration become active, according to these authors. Mikesell & Popham (1976) applied the same concept to *Mirabilis jalapa* roots.

The above interpretations all have several plausible features, especially where the species treated by the authors are concerned. All of these studies have the disadvantage of treating only a single genus, and none of them include the distinctive pattern represented by *Neea, Guapira, Pisonia s.s.* and *Torrubia*. The technical difficulties in sectioning woods of these genera may have delayed understanding of meristematic activity in lateral growth of stems of the family. A summary headed 'Wood' was presented by Metcalfe & Chalk (1950) in their account of Nyctaginaceae. Metcalfe and Chalk called parenchymatous background tissue 'conjunctive tissue', but the fibrous background tissue was termed 'wood' and thus they used the term 'interxylary phloem' for phloem strands. Metcalfe & Chalk (1950) used the term 'rays' in some genera of Nyctaginaceae, but 'radial conjunctive tissue' in others. The terms used by various authors reflect their concepts for the origin of the mature tissues that they discuss. Although all of the authors cited attempted to present accurate descriptions of histology, none of them have, in my opinion, presented interpretations for lateral growth mechanisms that are entirely accurate, and a reinterpretation of the phenomenon is therefore needed.

I am hoping that by offering a survey of axes of various Nyctaginaceae, using methods that show both meristems and mature tissues clearly, the nature of meristematic action can be clarified. In addition, I am influenced by my earlier studies on anatomy of Caryophyllales with successive cambia (Carlquist, 1995, 1997, 1999a, 1999b, 1999c, 2000a, 2000b, 2000c, 2002, 2003). The summary of Gibson (1994) has also been helpful. Above all, note should be taken that the pattern of secondary activity in the stem of *Pisonia* illustrated by Solereder *et al.* (1908) and copied by Metcalfe & Chalk (1950) has never been explained in ontogenetic terms. The concepts in the present paper may be considered overdue explanations for this pattern, as well as for others in Nyctaginaceae.

I do not believe that any useful purpose is served by having two terminologies for anatomy of dicotyledons with cambial variants: one for those knowledgeable about these plants and one for those unfamiliar with them. Consequently, a terminology that is accessible to both kinds of workers is attempted here. The term 'successive cambia', used by Schenck (1893), Pfeiffer (1926), and others can readily be applied to dicotyledon axes in which one sees a series of vascular strands or bands, each with both secondary xylem and secondary phloem. Likewise, the term 'conjunctive tissue' can easily be used for the ground tissue in which the secondary xylem and phloem are embedded (secondary xylem is defined by vessel presence, phloem by presence of sieve tube elements and companion cells: vessels absent in some vascular strands of *Mirabilis*). The

terms 'interxylary phloem' and 'included phloem' are not used here because conjunctive tissue, not xylem, is adjacent to the outer faces of strands or bands of secondary phloem.

The presentation of ground plans for stems or roots of the seven genera is necessary for locating the meristems and describing their products. This does not constitute an account of comparative wood features for the species in this study. Presentation and discussion of that data will be offered in a systematically directed study. The systematic distribution of features associated with successive cambia will become clearer when detailed anatomical surveys have been carried out for the 'core Caryophyllales', the Centrospermae of earlier authors and the Caryophyllales of Behnke & Mabry (1994). At that time a review can be offered.

MATERIAL AND METHODS

The collections studied are as follows (asterisks indicate liquid-preserved material): *Abronia latifolia Eschsch., beach west of Oso Flaco Lake, California, Carlquist s. n.; *Bougainvillea spectabilis Willd. cv. 'lateritia', cultivated at University of Rhode Island, Cheadle s. n.; *B. spectabilis, cv. unknown, Vavra Garden of UCLA, Carlquist s. n.; *Guapira discolor (Spreng.) Little, cult. Fairchild Tropical Garden, FTG-951320 A; G. guianensis Aubl., SFCw-R1130-70; *Heimerliodendron brunonianum (Endl.) Skottsb., cult. Santa Barbara, Carlquist 8196 (SBBG); *Mirabilis jalapa L., cult. Santa Barbara, Carlquist 8183 (SBBG); Neea macrophylla Poepp. & Endl., J. Schunke V. 4535 (US), USw-40859; *Pisonia rotundata Griseb., Florida Keys, cult. Fairchild Tropical Garden; Torrubia cuspidata Standl., SFCw-R1231-43. Material of Abronia latifolia and Mirabilis jalapa were infiltrated, embedded in paraffin and sectioned on a rotary microtome. The species available in dried form were boiled in water and stored in 50% aqueous ethanol. Both these species and the liquid-preserved specimens other than those of Abronia and Mirabilis were softened in ethylene diamine, embedded in paraffin and sectioned on a rotary microtome (Carlquist, 1982). The sections were stained with a safranin-fast green combination. Cheadle's remarkable preparations of Bougainvillea, given to the author in 1995, were embedded in celloidin, sectioned on a sliding microtome and stained with a haematoxylin combination (Esau & Cheadle, 1969). Macerations were prepared with Jeffrey's solution and stained with safranin.

The term 'fibre' is used rather than 'libriform fibre' because fibres are held here to originate in Nyctaginaceae sometimes from vascular cambia, but more commonly from lateral meristems. The term 'ray' rather than 'vascular ray' is deliberately used here because rays can be produced by lateral meristems, but not by vascular cambia in any of the genera studied. The term 'lateral meristem' is used for the cell layer that forms in the cortex, produces secondary cortex to the outside and conjunctive tissue, rays and vascular cambia to the inside. This term, although perhaps vague, is used here because it is not in conflict with any observed fact and because any more precise term (e.g. 'lateral meristem leading to production of successive cambia') would be awkward and probably would not find acceptance. The term 'stratified' has been used by various authors for cell arrangements in the lateral meristem, but because there is an ontogenetic continuity between a lateral meristem and the successive cambia internal to it, the term 'storied' as typically used for vascular cambia, is employed here.

Binomials mostly follow current usage. The genus Heimerliodendron is used, although Wagner, Herbst & Sohmer (1990) prefer to consider it within Pisonia sect. Prismatocarpus. Anatomical evidence presented below and discussed in the text supports the recognition of Heimerliodendron. The name Pisonia s.s. is applied to species that have the anatomical patterns described here for P. rotundata.

PROPOSED INTERPRETATIONS AND INTERPRETATIVE CRITERIA

Before presentation of descriptions of the sectioned material, a conceptual basis for the analyses presented here is given. Without this framework, application of terms and the content of the descriptions would not be evident. Authors of other papers on Nyctaginaceae describe their material on the basis of assumptions they have already reached from study of those materials. Those assumptions obviously guided their descriptions. I believe that the reader is better prepared for my descriptions if the conceptual basis for them is made explicit. I am presenting this conceptual basis in the form of arbitrary points, each followed by lines of evidence or reasoning, in an attempt to make the hypothetical views expressed more easily viewed than had they been conveyed in a running text. A condensed version of the concepts represented below can be found in Carlquist (2001). However, the presentation below offers more data and a more comprehensive theoretical apparatus needed to support the concepts.

1. Stems and roots of Nyctaginaceae develop, after maturation of the primary vasculature, a lateral meristem within cortical parenchyma. This meristem yields secondary cortex (parenchyma) to the outside and vascular cambia, conjunctive tissue and (in some genera) rays to the inside. All vascular cambia after maturation of the primary vasculature are formed from the lateral meristem.

Correlative observations and comments:

(a) A lateral meristem can be easily distinguished in most axes if growth is occurring actively.

(b) Radially orientated files of cells aligned with the lateral meristem and leading outward from it show that secondary cortex is derived from the lateral meristem.

(c) The lateral meristem cells are radially narrower than the secondary cortex cells derived from them.

(d) No phloem is found (except for leaf traces) external to a lateral meristem.

(e) The lateral meristem is most easily seen prior to initiation from it of a vascular cambium (at which time, a lateral meristem is evident in areas between the vascular cambia also).

(f) The lateral meristem may become quiescent in places or as a whole, but that is not evidence that it does not exist. Study of several sections from actively growing axes may be necessary to establish its presence.

2. The lateral meristem is functionally a single cell wide in radial thickness.

Correlative observations and comments:

(a) If the lateral meristem were functionally more than one cell thick, each of the cells could produce secondary cortex to the outside and vascular cambia (and products of vascular cambia) and conjunctive tissue to the inside.

(b) Tangential (periclinal) divisions may be present in several layers of a zone that includes the lateral meristem. This does not conflict with the concept of a meristem a single cell thick, because immature derivatives of a meristem have been repeatedly reported to divide (Philipson *et al.*, 1971: 18).

3. Vascular cambia in Nyctaginaceae are considered to exist only where secondary phloem is produced to the outside and vessels (with or without production of fibres) to the inside of the vascular cambium.

Correlative observations and comments:

(a) Vascular cambia in Nyctaginaceae may be defined in precisely the same way as vascular cambia in woody dicotyledons with a single cambium are defined.

(b) Patches of fibres formed internally by a lateral meristem are not associated with phloic strands external to the fibres, but rather, with secondary cortex; a vascular cambium cannot be said to exist at such a point.

(c) As shown by production of vessels well after a band or strand of fibrovascular tissue is first evident, and as shown by the occurrence of crushed as well as functional phloem in such a strands, vascular cambia in Nyctaginaceae are functional for indefinite periods of time.

(d) Fibrovascular strands at their first appearance contain phloem plus only a small number of vessels; fibrovascular strands in older parts of the stem have more numerous vessels (and crushed phloem as well as functional phloem). This is considered evidence of origin of xylem and phloem from vascular cambia.

(e) Repeated production of vascular cambia from the lateral meristem results ultimately in the production of numerous vascular strands throughout an older axis; this distribution validates the use of the term 'successive cambia' in Nyctaginaceae.

(f) Radially extending plates of fibrovascular tissue, each capped by a strand of secondary phloem, indicates prolonged activity of vascular cambia (*Heimerliodendron*, Figs 17, 18; see also *Charpentiera* in Carlquist, 2003: figs 23, 24).

4. Conjunctive tissue is produced by the lateral meristem, not by vascular cambia. The term 'wood' is not applicable to conjunctive tissue, but only to 'the xylary portion of a fibrovascular strand' (Panshin & de Zeeuw, 1980).

Correlative observations and comments:

(a) Vessels are absent in conjunctive tissue; vessels are always formed from vascular cambia, and secondary phloem is present external to radial zones containing vessels.

(b) Conjunctive tissue may have thin or thick walls, lignified or nonlignified, and cells may be fibre-like or parenchymatous, with every transition between the two types: fibrelike nature is thus not evidence of vascular cambium presence (*Bougainvillea*, Figs 5–7)

(c) Lateral meristems may shift from production of fibres to production of parenchyma (*Abronia*, Figs 2, 3).

(d) Rays occur in parenchyma adjacent to phloem strands (*Guapira*, Figs 13–16; *Pisonia,* Figs 21–24), but not in the phloem itself, so this parenchyma can be readily identified as conjunctive tissue parenchyma rather than phloem parenchyma.

(e) Conjunctive tissue may contain rays, but conjunctive tissue is not equivalent to rays; *Bougainvillea* (Fig. 9) may, however, have an incipient form in differentiation of rays from conjunctive tissue parenchyma ('radial plates of conjunctive tissue' in the terminology of Metcalfe & Chalk, 1950).

(f) Axial parenchyma can occur in fibrovascular strands of Nyctaginaceae, but this is easily distinguished from conjunctive tissue by its location as well as histology.

5. Rays can be produced by the lateral meristem in some genera, and can readily be distinguished

from conjunctive tissue in these genera (*Guapira, Neea, Pisonia s.s.*, *Torrubia*).

Correlative observations and comments:

(a) Rays are radially disposed, and have finite vertical extent in the Nyctaginaceae that have rays.

(b) In these genera, rays extend from the pith to the lateral meristem (or part of this distance) but do not extend through the lateral meristem into the secondary cortex.

(c) Histological features associated only with rays occur in these genera. Cells referable to procumbent and square, and to tip cells of a ray, are present. Bordered pits are present in tangential-walls of ray cells in these genera.

(d) Rays are not produced by vascular cambia of Nyctaginaceae, only by lateral meristems.

GROUND PLANS OF GENERA STUDIED

Abronia (Figs 1–4): The root transection illustrated is from a hypocotyl, which seen externally appears to be the top of a taproot. There is a circle of primary bundles (each with some secondary growth) in the centre, and a complete second cycle of vascular bands and strands occurs outside of that circle (Fig. 1). There is meristematic activity associated with the second cycle (Figs 2, 3). Although I cannot delimit exactly how much of the second cycle of bundles was the product of a lateral meristem, certainly a lateral meristem is at work (Figs 1–3, pointers). Vascular cambia can be identified within the second cycle (Fig. 2, arrow). Presence of radial files of parenchyma external to the lateral meristem shows the existence of a secondary cortex (Figs 1–3). The secondary cortex is not to be confused with periderm, which is easily identified at the top of Figure 1. The periderm is separated from the secondary cortex by parenchyma cells that are randomly arranged rather than in radial files. The lateral meristem is most easily seen because of recent divisions (Figs 2, 3, pointers) in areas between those that contain products of vascular cambia. The lateral meristem produced fibres as well as thin-walled parenchyma, and may shift from one to the other (Fig. 3). The products of vascular cambia include secondary phloem, and, in the secondary xylem, vessels, axial parenchyma and a few fibres (Fig. 2, zones to left of arrow tip).

Meristematic action encircling each of several deactivated vessels was seen on one side of the root studied (Fig. 4). These meristems have not yielded any secondary xylem or secondary phloem. In fact, the cells formed look much like periderm, especially periderm that has been formed as a wound response at a particular place (not shown) near the surface of this root. Lacking evidence to the contrary, the meristematic action encircling the deactivated vessels is considered to be phellogen action.

Bougainvillea (Figs 5–12): Older stems (Figs 5, 6) resemble older roots (Figs 7, 8) in plan, although there are histological differences. One can easily find the lateral meristem in these axes (pointers, Figs 5–7). The lateral meristem lies external to zones of tissue in which a vascular cambium has added secondary xylem and phloem as well as in areas that contain only conjunctive tissue and no secondary xylem and secondary phloem (Figs 5–8, and most conspicuously, Fig. 6, pointers). The lateral meristem is rather uniformly active around the stems and roots studied.

Areas where a vascular cambium has been active (Figs 5–8, arrows) show abundant production of secondary phloem: crushed phloem is present at the external faces of phloem strands (Figs 5, 6, 7, 8, 10). The vessels are produced by the vascular cambium. The basis for this statement is that when activity of vascular cambia is first apparent (Fig. 10), some secondary phloem and only one or two vessels are present, yet as vascular cambia yield more products (further inward in the axes), more numerous vessels are present and much more secondary phloem is evident. The secondary xylem in Figure 10 shows a radial file of vessels, the one nearest the cambium still differentiating, an appearance consistent with the idea that all of the vessels there have developed from a vascular cambium. Thus, the action of vascular cambia in Nyctaginaceae is viewed to be exactly like that of vascular cambia in woody dicotyledons with a single bidirectional vascular cambium, and the appearance (Fig. 12, arrow) is also the same.

In Figure 11, both a vascular cambium (arrow) and a lateral meristem (pointers) are present: the two types of meristems coexist. The products of the vascular cambium are sieve-tube elements, companion cells and phloem parenchyma in secondary phloem, and vessel elements, axial parenchyma and fibres in secondary xylem. The axial parenchyma has lignified walls.

The roots of *B. spectabilis* (Figs 7, 8) differ from the stems (Figs 5, 6) by having more abundant parenchyma. The parenchyma of the roots (Fig. 8) is rich in starch, whereas it is not conspicuous in parenchyma of stems (Fig. 6). The parenchyma other than that produced near vessels by vascular cambia is considered here to be conjunctive tissue parenchyma, and the fibres not produced by the vascular cambia are considered to be conjunctive tissue parenchyma. All conjunctive tissue is considered to be produced by the lateral meristem. Fibres and parenchyma of the conjunctive tissue occur in patchworklike fashion (Figs 5 and 6, especially at left; Fig. 7, especially lower right), demonstrating the ease with which the lateral meristem can shift from production of one cell type to production of the other. In *Bougainvillea*, the conjunctive tissue parenchyma can be distinguished from fibres by the
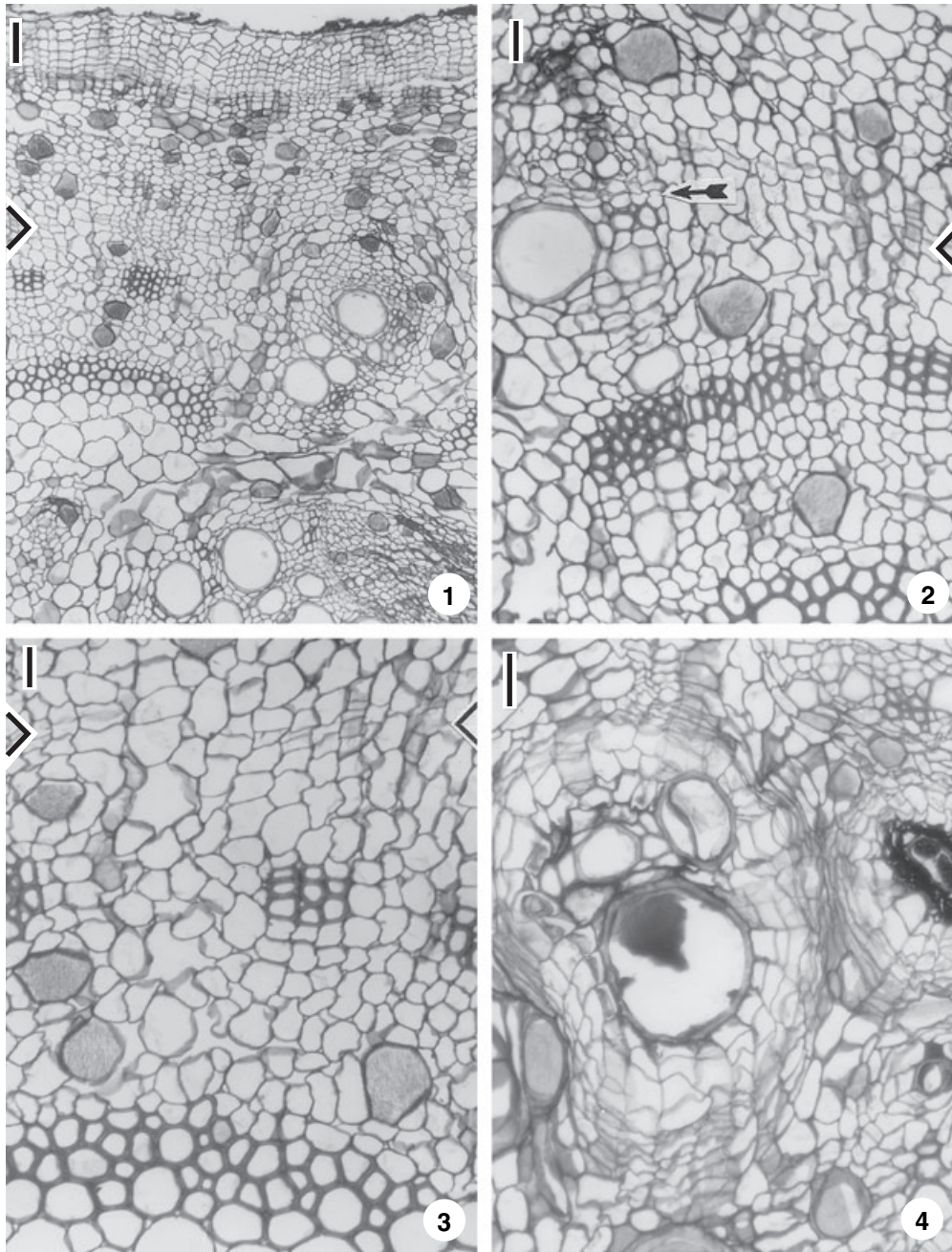
Figures 1–4. Transections of upper root of *Abronia latifolia*. Fig. 1. Section extending from periderm to inner cycle of vascular strands; pointer = lateral meristem. Scale bar = 60 µm. Fig. 2. Area showing lateral meristem (pointer, right) plus vascular cambium (arrow, left). Scale bar = 30 µm. Fig. 3. Area showing lateral meristem (pointers) and products, which include both parenchyma and fibres. Scale bar = 30 µm. Fig. 4. Area with deactivated vessel group (left) and solitary vessel (right), meristematic activity surrounds both. Scale bar = 30 µm.

occurrence of wider cell diameter and thinner (but lignified) walls in the parenchyma. Metcalfe & Chalk (1950) apparently regard fibres in *Bougainvillea* as xylem components, regardless of their origin or distribution. This usage is followed by Esau & Cheadle (1969), who used the term 'xylem fibers'.

Metcalfe & Chalk (1950) use the term 'raylike radial sheets of conjunctive parenchyma' for radially arranged parenchyma tissue in *B. spectabilis*. However, if one looks at tangential sections of stems in this species (Fig. 9), one finds fibres (thin fusiform nonstoried cells with thick dark-staining walls) as well as
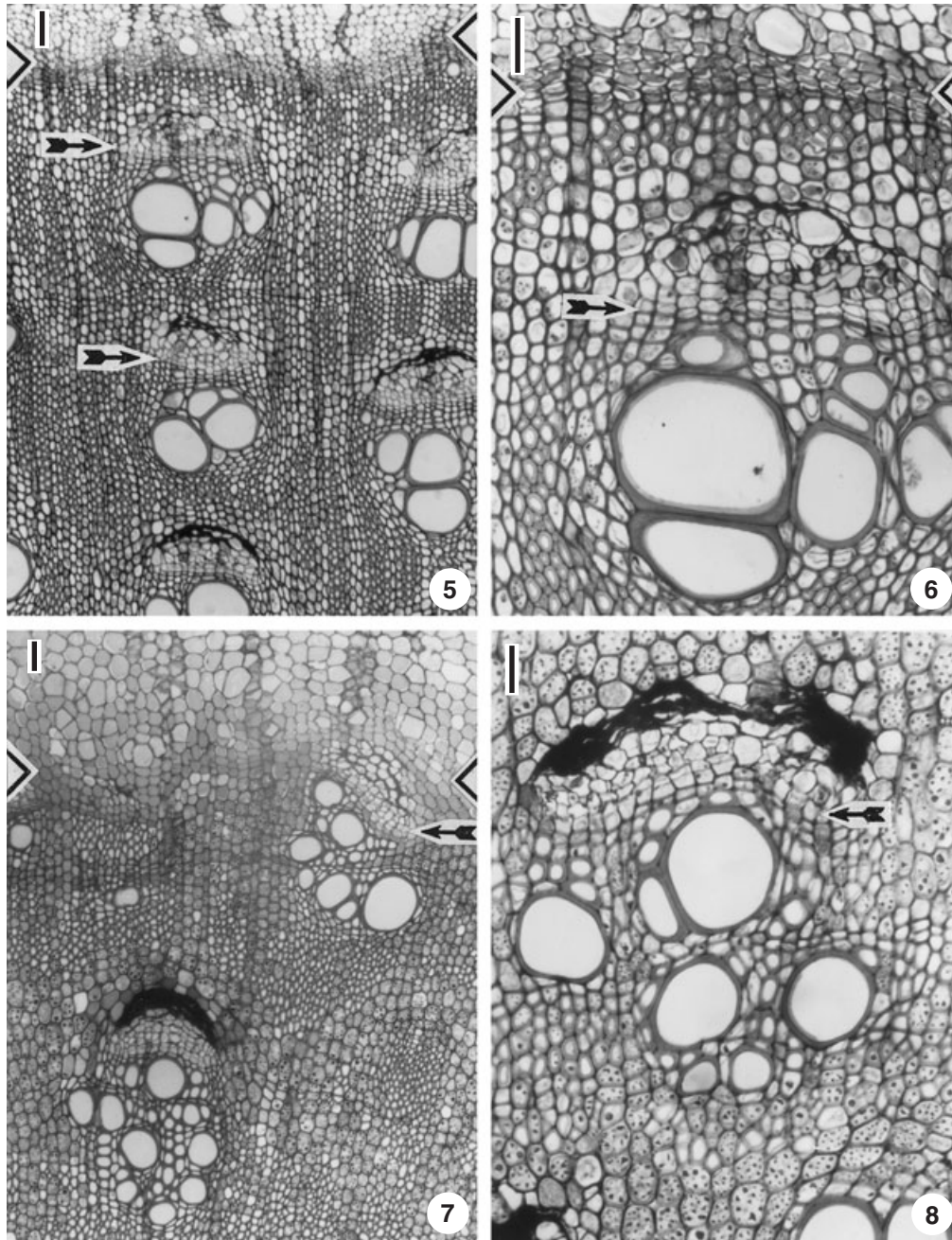
Figures 5–8. Transections of stem (Figs 5, 6) and root (Figs 7, 8) of *Bougainvillea spectabilis* (Cheadle s.n). Fig. 5. Area to show location of lateral meristem (pointers) and two of the vascular cambia (arrows). Scale bar = 60 µm. Fig. 6. Area showing that a lateral meristem (pointers) is active outside of a vascular strand in which the vascular cambium (arrow) is active. Scale bar = 30 µm. Fig. 7. Area to show location of lateral meristem (pointers) and a vascular cambium (arrow); fibres and parenchyma of conjunctive tissue distributed irregularly, lower right. Scale bar = 60 µm. Fig. 8. Area to show vascular cambium (arrow), crushed phloem (dark band, near top) and numerous starch-filled cells in conjunctive tissue surrounding the vascular strand. Scale bar = 30 µm.

parenchyma (mostly storied, vertically elongate, tips of cells blunt, walls thinner). One also sees some cells transitional between the two types. There are some parenchyma areas that are entirely unlike rays (top third of Fig. 9) but one also sees parenchyma that is organized into fusiform groups of cells that are suggestive of rays (right half of Fig. 9, mostly below centre). Where a group of fusiform cells is clearly enclosed
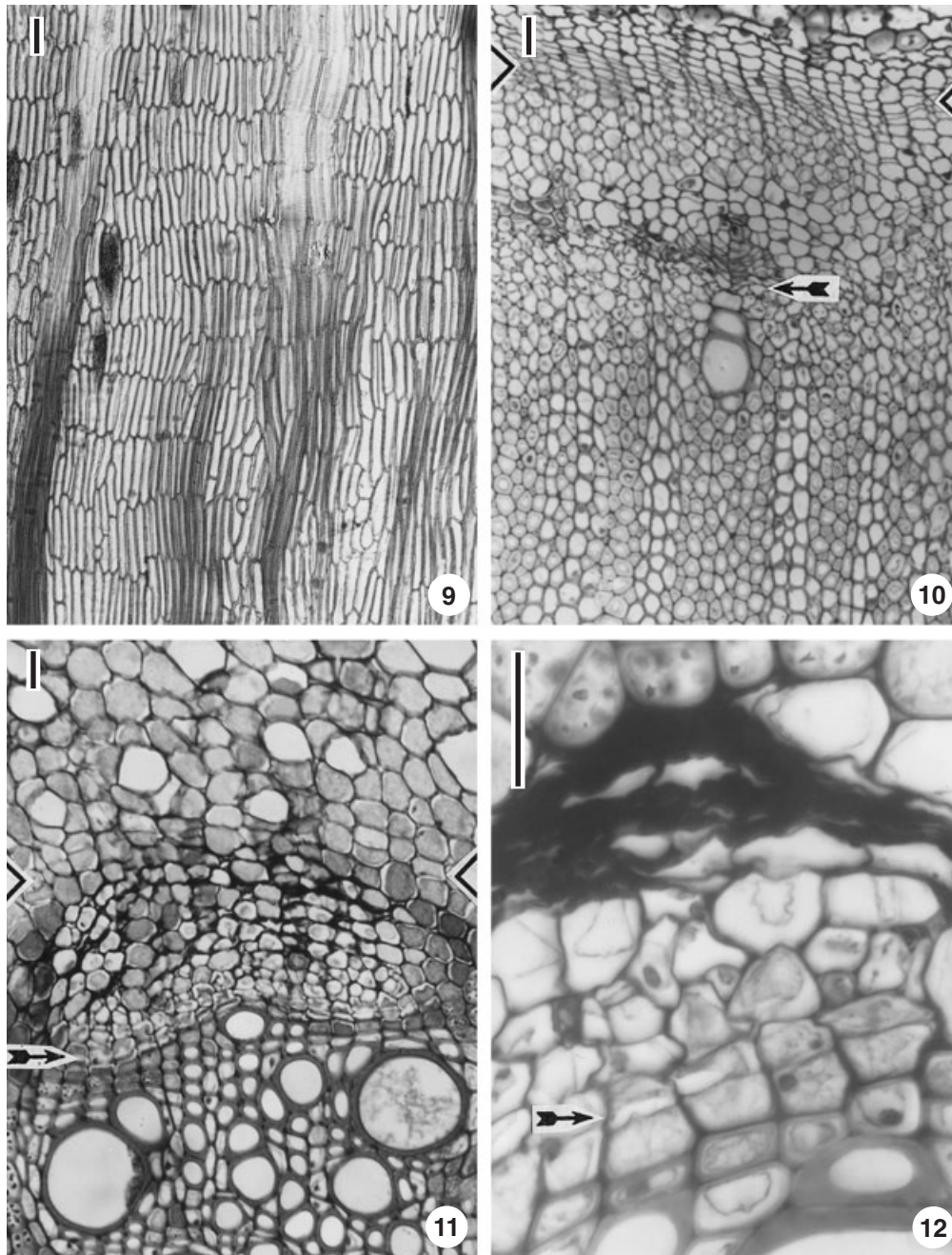
Figures 9–12. Sections from longisection of stems (Figs 9–11) and root (Fig. 12) of *Bougainvillea spectabilis* (Fig. 9, Carlquist s.n., Figs 10–12, Cheadle s.n). Fig. 9. Area showing somewhat ray-like tissue (lower right), fibres (medium grey vertical streaks) and several packets of raphides (dark grey, left); most cells are storied conjunctive tissue parenchyma. Scale bar = 60 μm. Fig. 10. Area showing lateral meristem (pointers) and a vascular cambium (arrow) that has produced some secondary phloem and a few vessels. Scale bar = 30 μm. Fig. 11. Lateral meristem (pointers) external to vascular strand; arrow indicates vascular cambium. Scale bar = 30 μm. Fig. 12. Area to show histology of vascular strand. From top to bottom: starch-filled parenchyma, crushed secondary phloem, functional secondary phloem, vascular cambium (arrow), recent secondary xylem derivatives. Scale bar = 10 μm.

within fibre strands, from top to bottom of a group (instances observed but not shown), one could justify the designation of rays. However, there are many places where there is no such demarcation between radially arranged parenchyma and other parenchyma, and the histology of the cells in ray-like areas is like that in the groupings of parenchyma cells that are tangentially or diagonally disposed. Thus, the distinction between ray-like and non-ray parenchyma seems incomplete or various, and is best described as such.

Guapira (Figs 13–19): When wider stems (Figs 13, 14) are compared with narrower stems (Figs 15, 16), the former have wider vessels, more numerous vessels per strand of secondary xylem, and a broader arc of conjunctive tissue parenchyma exterior to each strand of secondary phloem. The parenchyma is considered conjunctive tissue rather than phloem parenchyma because it contains rays, and in *Guapira*, as in *Neea*, *Pisonia s.s.* and *Torrubia*, rays are produced by the lateral meristem, not by vascular cambia (Figs 14, 16). This localization of conjunctive tissue parenchyma adjacent to secondary phloem in these genera is striking in comparison to conditions seen in *Bougainvillea* and *Heimerliodendron*. The conjunctive tissue parenchyma has primary walls. Vascular cambia are readily identifiable (Fig. 14, arrow). Lateral meristem activity (Fig. 13, pointers) is also present. A few layers of secondary cortex are present external to the lateral meristem. Axial parenchyma occurs among the vessels (Fig. 14) and should therefore be termed paratracheal. A few fibres are produced from the vascular cambium (Fig. 14); these are adjacent to vessels. Fibres of the conjunctive tissue have thick secondary walls (Figs 14, 16) with simple pits.

Rays are mostly uniseriate, like those of *Pisonia s.s.*, but biseriate rays are not uncommon. Ray cells have lignified walls. Ray cells are mostly procumbent; the rays would qualify as homogeneous or homocellular. Borders were observed on some pits of ray cells as well as some pits of axial parenchyma cells (when pits are viewed either in face view or in sectional view). The two species of *Guapira* studied are alike in qualitative features, although perceptively different in quantitative features.

Heimerliodendron (Figs 17–20): The stem of *Heimerliodendron* has a background tissue of alternating bands of fibres and parenchyma (Figs 17–20). The difference between these two cell types can be seen in longisection; the portion on the right in Figure 20 is not a ray, but conjunctive tissue parenchyma; conjunctive tissue fibres occupy the remainder of the photograph. The parenchyma cells have wider diameter, blunt ends, are shorter, and have nonlignified walls; alternative character states typify the fibres of the

conjunctive tissue. Sections closer to the outside of the stem show fibre bands (Fig. 17) narrower than those further toward the centre of the stem (Fig. 18).

Vascular cambia are active for prolonged periods of time, producing fibre flanges that extend abaxially from the fibre bands (Figs 17, 18). The lateral meristem extends around the entire stem (Figs 17, 19, pointers), both external to phloem strands and external to areas between phloem strands. The presence of fibres in conjunctive tissue and also in the flanges internal to phloem strands does not blur the distinction between products of lateral meristem and products of vascular cambia. The presence of phloem and of vessels in radial zones internal to the phloem strands serves to define the action of vascular cambia. Where conjunctive tissue fibres occur, there is no phloem external to them, but rather, parenchyma. No vessels occur in conjunctive tissue fibres bands.

Heimerliodendron is rayless (Fig. 29).

Mirabilis: The root of *Mirabilis* is not illustrated. The figures of Mikesell & Popham (1976) are entirely satisfactory. There is a lateral meristem that encircles the root. The lateral meristem produces secondary cortex to the outside. Inside the meristem is background conjunctive tissue composed of parenchyma; fibres are absent. Concentric cycles of vascular strands are located within the conjunctive tissue background. Many of these strands are exclusively phloic; vessels but no fibres are present in the remainder. Despite lack of fibres, vascular strands are readily distinguishable from conjunctive tissue by cell size and shape (see also the features cited in 4a–c in 'Proposed interpretations and interpretative criteria', above). Rays are absent: conjunctive tissue parenchyma between the vascular strands is not ray tissue, but radially stretched background parenchyma. Parenchyma is rich in starch. Although origin of the vascular strands is difficult to trace, I observed no evidence that would contradict an interpretation that they are products of vascular cambia which are, in turn, derived from the inner side of the lateral meristem.

Neea (not shown): In transectional view, the stems of *N. macrophylla* resemble those of *Guapira discolor* in that large sheaths of conjunctive tissue parenchyma surround the phloem strands. However, many of these sheaths are laterally coalescent with each others, so that a pattern intermediate between that of *Guapira* and that of *Heimerliodendron* with respect to conjunctive tissue parenchyma is present.

Rays in *N. macrophylla* are uniseriate almost exclusively; a few rays are biseriate. Ray cells have lignified walls.

Pisonia s.s.: This generic concept is represented here by *P. rotundata* (Figs 21–24). The plan of this stem
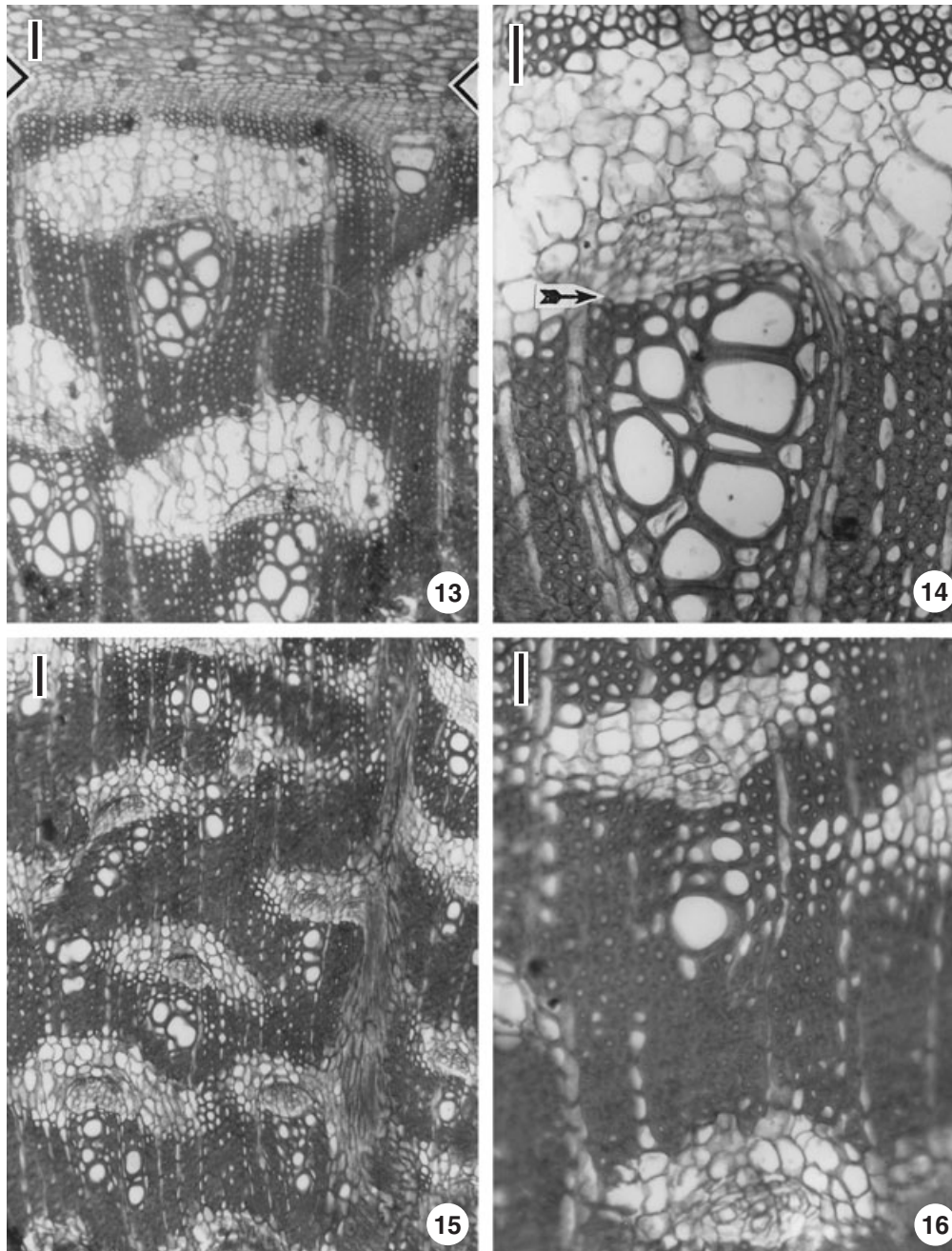
Figures 13–16. Transections from large (Figs 13, 14) and smaller (Figs 15, 16) stems of *Guapira discolor*. Fig. 13. Area to show lateral meristem (pointers) and vascular strands produced by successive cambia. Scale bar = 60 μm. Fig. 14. Vascular strand containing a vascular cambium (arrow); large-celled conjunctive tissue parenchyma above the secondary phloem; note that rays occur at periphery of vessels, but do not occur within secondary xylem or secondary phloem. Scale bar = 30 μm. Fig. 15. Vessels are narrow, arcs of conjunctive tissue parenchyma are relatively small. Scale bar = 60 μm. Fig. 16. One vascular strand and portions of others; vessels and secondary phloem limited in extent. Scale bar = 30 μm.

(Fig. 21) is much like that of *Guapira*. A parenchyma sheath around a phloem strand will occasionally fuse with an adjacent sheath (Fig. 22). Cells of the arcs of conjunctive tissue parenchyma have thin primary walls. The remainder of conjunctive tissue paren-

chyma has thick, lignified walls with simple pits (Fig. 22). A lateral meristem (not shown) much like that of *G. discolor* encircles the stem.

Secondary phloem is produced for an indefinite period by each vascular cambium, as evidenced by the
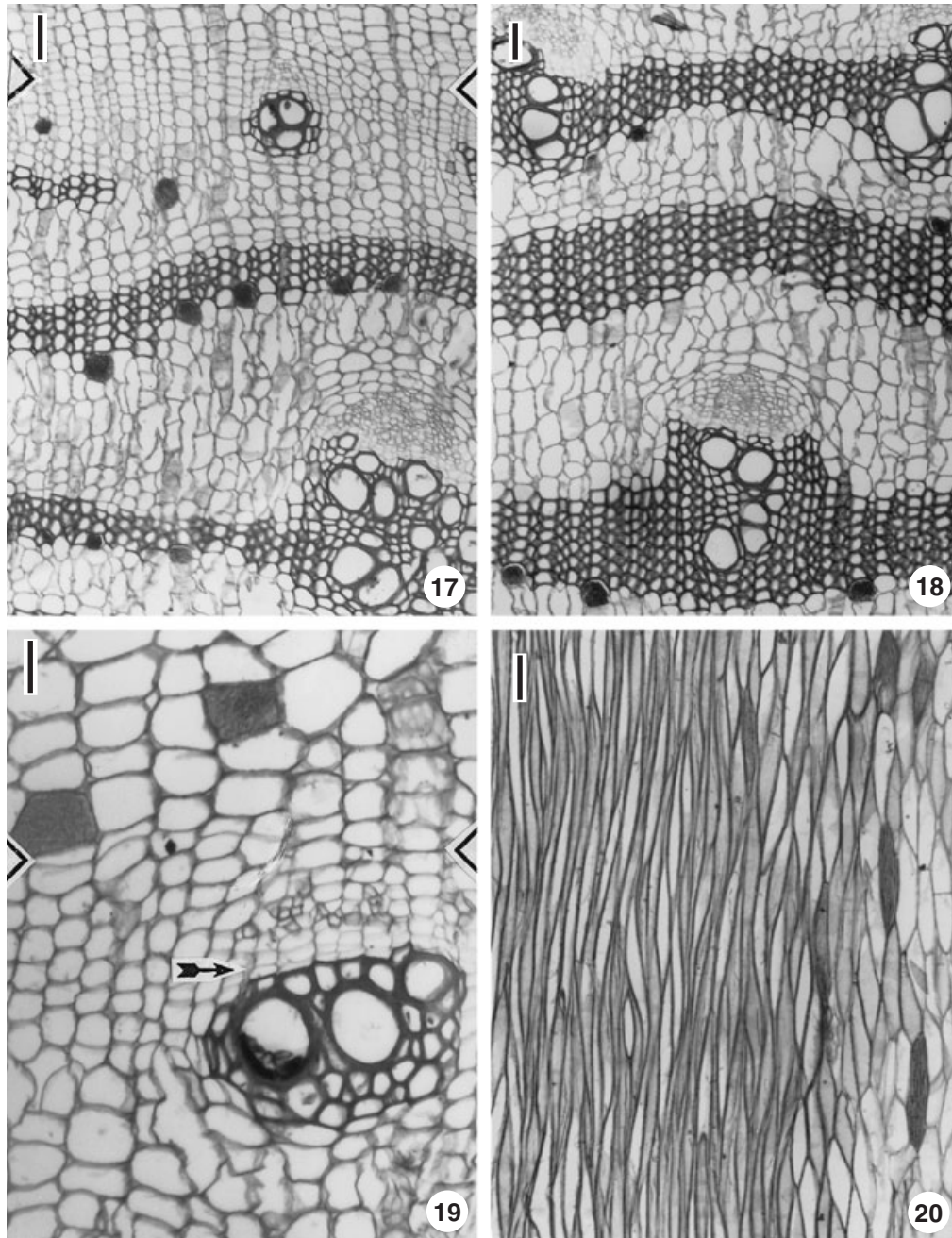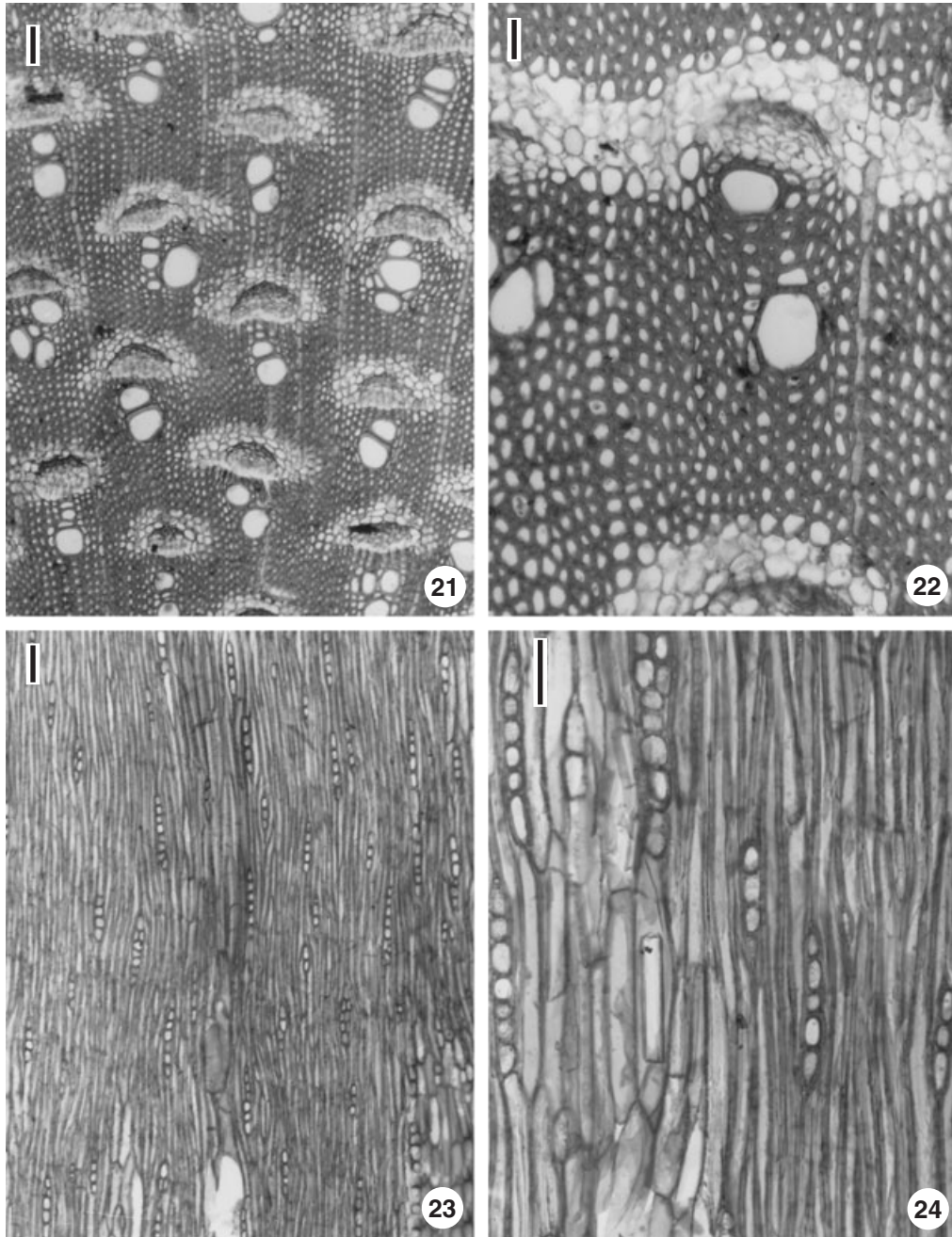
Figures 17–20. Transections (Figs 17–19) and tangential section (Fig. 20) of stem of *Heimerliodendron brunonianum*. Fig. 17. Area including lateral meristem (pointers) and some relatively newly formed bands of conjunctive tissue fibres. Scale bar = 60 μm. Fig. 18. Area from an older portion of stem, showing that fibre bands are thicker than those in Fig. 17. Scale bar = 60 μm. Fig. 19. Lateral meristem (pointers) and relatively young vascular strand in which the vascular cambium (arrow) is active. Scale bar = 30 μm. Fig. 20. Conjunctive tissue fibres (left 3/4 of photograph) and conjunctive tissue parenchyma (right 1/4); some cells of the parenchyma are vaguely storied. Scale bar = 60 μm.

accumulation of crushed phloem. The number of xylem vessels produced by each cambium is, however, relatively small. Some of the vascular cambia produce fibres adaxially after the last vessels mature (Figs 21, 22).

Rays are mostly uniseriate (Figs 23, 24) although a few biseriate rays are also present. Ray cells have lignified walls (Fig. 24).

Torrubia (not illustrated): The anatomical plan of the stem of *T. cuspidata* is like that of *Guapira discolor.*

Figures 21–24. Transections (Figs 21, 22) and tangential sections (Figs 23, 24) of *Pisonia rotundata*. Fig. 21. Area to show scattered nature of vascular strands, each with crushed phloem indicating secondary growth from the successive cambia. Scale bar = 60 μm. Fig. 22. Conjunctive tissue parenchyma of two vascular strands coalesced into a tangential band (above). Scale bar = 30 μm. Fig. 23. Area showing vaguely storied fibres and numerous rays. Scale bar = 60 μm. Fig. 24. Uniseriate rays with lignified ray cells and, below biseriate ray (top, left of centre) a conjunctive parenchyma cells containing a prismatic crystal. Scale bar = 30 μm.

DISCUSSION AND CONCLUSIONS

Analysis of stem transections leads inevitably to the conclusion that there are two kinds of meristems operative in axes of Nyctaginaceae: lateral meristems and vascular cambia. The lateral meristem occurs as a ring around an axis. Not all portions are equally active within a given axis, although this does occur in some. The vascular cambia arise on the inner face of the lateral meristem and, soon after origin, begin to

produce secondary phloem externally and secondary xylem internally. The lateral meristem is not used up in giving rise to a vascular cambium, a fact evident in selected sections such as that shown in Figure 6. The lateral meristem may become quiescent, but it does not cease to exist. Once a vascular cambium produced by it has yielded secondary phloem and secondary xylem, divisions soon begin outside secondary phloem, as shown in Figure 11. The pace of division seems not to slacken in areas of the lateral meristem that give rise to conjunctive tissue and rays (in the case of ray-bearing genera). The slackening of divisions in the lateral meristem in areas external to a vascular cambium and its products accords with the fact that the vascular cambium will produce an appreciable volume of tissue. Thus, the degree of activity in the lateral meristem coordinates the amount of tissue addition (indirectly) from lateral meristem areas external to vascular cambia with the amount of tissue addition from lateral meristem external to areas lacking vascular cambia. The concept of a lateral meristem which gives rise to vascular cambia and to conjunctive tissue internally is not new (see Carlquist, 2001: 273). One consequence of the concepts in the present paper is that the terms 'included phloem' and 'interxylary phloem' must be abandoned for species that have successive cambia. Terms such as 'stem with successive cambia' should be used instead: mention of successive cambia inevitably implies presence of phloem external to each vascular cambium (or where a vascular cambium was prior to cessation of function).

If a vascular strand is encased in fibres (mostly conjunctive tissue fibres), obviously addition of cells within that strand is limited. Addition of secondary phloem is possible because older phloem can be crushed to a fraction of its volume when functional; addition of vessels is mostly spatially not possible (the fibre-free roots of *Mirabilis jalapa* provide an exception). Where conjunctive tissue occurs in the form of indefinitely wide and thick tangential bands of parenchyma, as in *Heimerliodendron*, vascular cambia can add vessels and fibres, forming flanges that extend radially outward into the parenchyma (this has been shown dramatically for *Charpentiera* in Carlquist, 2003: figs 23, 24).

One can see in the above account the appearance that may have led Studholme & Philipson (1966) to the idea of a lateral meristem that moves progressively outward. However, the mechanism by which a meristem moves progressively outward would be much more difficult to achieve than would the occurrence of a meristem that becomes quiescent but can become active again without shift in location of the activity.

The occurrence of secondary cortex has been neglected by most workers. Unilateral meristems are very rare in vascular plants, and do not occur in Caryophyllales in my experience. In fact, radial files of secondary cortex cells outside the lateral meristem are readily identified and demonstrated. If one follows a radial file of cells inward from its first appearance adjacent to primary cortex, one can locate the lateral meristem layer because lateral meristem cells are radially the narrowest cells in such a file. Also, meristem cells show cell plates indicative of recent divisions. The term 'primary thickening meristem' should not be used to refer to meristematic activity in Caryophyllales. That term has been pre-empted for a phenomenon seen in some monocotyledons.

Earlier, the author used the term 'diffuse meristem' with respect to lateral meristem activity (Carlquist, 1999c), and contrasted it to 'confined meristem' (Carlquist, 2000a, b). The former refers to lateral meristems in which recent divisions can be seen in several layers, the latter to a lateral meristem in which recent divisions appear mostly within a single layer. This distinction still seems a valid one, but it does not necessarily indicate that the lateral meristem is functionally more than a single layer in thickness. Divisions can occur in recent derivatives of the cambium, as has been reported repeatedly (Philipson, Ward & Butterfield, 1981: 18).

Rays in the Nyctaginaceae studied originate not from the vascular cambium, but from the lateral meristem, as is implicit in the drawing by Solereder (1908) for a stem transection of *Pisonia nigricans* Swartz (drawing reproduced in Metcalfe & Chalk, 1950: 1064). Solereder's drawing is correct in most details. It is incorrect in showing twice as many files of secondary cortex cells as conjunctive tissue fibres. The number of files in the two tissues should be the same. The drawing also fails to show differentiation between secondary phloem and conjunctive tissue parenchyma arcs adjacent to the secondary phloem. With the exception of these relatively minor flaws, Solereder's drawing forces one to recognize that there are both successive cambia (which have given rise to the numerous vascular stands containing secondary xylem and secondary phloem) and a lateral meristem (which lies between conjunctive tissue fibres and secondary cortex). Solereder's drawing also forces one to recognize that in *Pisonia s.s.,* rays have been derived from the lateral meristem (and, interestingly, occur in the conjunctive tissue but not in the secondary cortex). The present paper really does not represent a departure from anatomical thinking (although it does differ from ontogenetic interpretations of various authors on anatomy of Nyctaginaceae); it represents an acceptance, overdue by nearly a century, of concepts one is forced to accept on the basis of Solereder's drawing. The same pattern as in *Pisonia s.s.* occurs in *Guapira*, *Neea* and *Torrubia*, which have been neglected also.

Rays in *Guapira*, *Neea*, *Pisonia s.s.* and *Torrubia* are composed almost exclusively of procumbent cells, and are histologically identical with vascular rays in woody dicotyledons with a single cambium. The idea that ray production has been transferred, in these genera, from a vascular cambium to a lateral meristem, may seem a novel interpretation, but once again, it is a conclusion that should have been reached by anyone who studies Solereder's drawing of *Pisonia nigricans*. If Solereder's drawing had been larger and more detailed, it hopefully would have shown that, as in the present study, rays in *Guapira*, *Neea*, *Pisonia s.s.*, and *Torrubia* are not produced by vascular cambia. *Bougainvillea* offers a very weak differentiation between ray-like areas (composed wholly of upright cells) and conjunctive tissue parenchyma (composed wholly of upright cells, although more frequently storied than the cells of ray-like areas). *Heimerliodendron* is rayless according to the present account and all previous authors.

Conjunctive tissue is differentiated into fibres and parenchyma portions. In *Heimerliodendron*, these two cell types alternate as concentric bands. In *Guapira*, *Neea* and *Pisonia s. s.*, conjunctive tissue parenchyma forms arcs adjacent to phloem parenchyma strands. The occurrence of rays (identical to and intercontinuous with rays of the fibrous conjunctive tissue) in these parenchyma arcs but not in the secondary phloem or secondary xylem is evidence that the parenchyma arcs are not phloem parenchyma. A second line of evidence is offered by the fact that crushed secondary phloem is produced by the vascular cambia and does not occur within the parenchyma arcs. A conjunctive tissue parenchyma distribution intermediate between that of *Heimerliodendron* and that of *Guapira* is present in *Torrubia*.

Because all of the genera of Nyctaginaceae studied here have vascular cambium action as well as lateral meristem activity, and because these cambia are numerous in mature stems or roots (as opposed to a single vascular cambium) and randomly distributed in these stems and roots, the term 'successive cambia', as used by Schenck (1893) and Pfeiffer (1926), is applicable to Nyctaginaceae.

ACKNOWLEDGEMENTS

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