TYPES OF CAMBIAL ACTIVITY AND WOOD ANATOMY OF STYLIDIUM (STYLIDIACEAE)[1]

SHERWIN CARLQUIST

Claremont Graduate School, Pomona College, and Rancho Santa Ana
Botanic Garden, Claremont, California 91711

ABSTRACT

Three types of cambial activity, two hitherto unreported, are described for *Stylidium*. The four species of sect. Rhynchangium of subgenus *Nitrangium* have woody cylinders in upright stems. In these a cambium formed beneath the endodermis produces a determinate quantity of fibers, vessel elements, and interxylary phloem strands toward the inside but no derivatives toward the outside; this was correctly reported by Van Tieghem and Morot (1884a) but doubted by subsequent workers. The same species have lignotubers in which a cambium produces contorted xylem (mostly vessels) to the inside, phellem toward the outside. In *S. glandulosum* and *S. laricifolium* a cambium formed beneath the endodermis produces an indeterminate quantity of xylem (fibers and vessel elements) and interxylary phloem toward the inside, nothing toward the outside. The xylem is rayless and lacks axial xylem parenchyma. These three modes of cambial activity represent innovations within Stylidiaceae. The family has a wholly herbaceous ancestry if one can judge from the total lack of cambial activity in vascular bundles.

THE FAMILY STYLIDIACEAE appears to have very little secondary growth. Indeed, the family can be said to have a monocotyledonous plan of organization to a remarkable degree. Vascular bundles are devoid of any cambial activity; in most species vascular bundles are not arranged in a single cylinder but are scattered within a zone around the pith; roots are adventitious (Van Tieghem and Morot, 1884a; Mildbraed, 1907).

Stylidium glandulosum Salisb. and *S. laricifolium* L. C. Rich. do have secondary growth evident upon superficial examination. The type of cambial activity which produces wood in these species has not been hitherto analyzed, nor has the wood of this pair of species been described. However, material sufficient for study of both is now at hand.

In addition, the section Rhynchangium Benth. of *Stylidium* subgenus *Nitrangium* Endl. offers some unusual problems. This section consists of *S. adnatum* R. Br., *S. falcatum* R. Br., *S. fasciculatum* R. Br., and *S. rhynchocarpum* Sond. These closely related species are perennials which branch from a lignotuberous base appearing to be a fusion of stems or occasionally a knotlike thickening (Fig. 9). Research has been done on the upright stems of *S. falcatum,* which was widely cultivated in Europe in the 19th century (Van Tieghem and Morot, 1884a). These workers reported a form of cambial activity in the upright stems (Van Tieghem and Morot, 1883,

1884a, 1884b). Doubt was cast on this interpretation by Burns (1900), and the issue has not been resolved. The lignotubers of the Rhynchangium species have not been investigated until now, however. The anatomy of these structures proves to be yet another facet in the story of cambial activity of Stylidiaceae.

Misunderstanding of types of secondary growth in *Stylidium* has resulted in omission of the family from lists of groups which possess interxylary phloem in wood. More importantly, the types of cambial activity in the family pose phylogenetic problems of considerable interest.

MATERIALS AND METHODS—Specimens of four species were collected in the field: *S. adnatum* (Canal Rocks, W. Australia, Carlquist 3842, RSA); *S. falcatum* (Tutanning Reserve, W.A., Carlquist 3856, RSA); *S. laricifolium* (S. of Sydney, N.S.W., Carlquist 1312, RSA); and *S. scandens* R. Br. (near Albany, W.A., Carlquist 6030, RSA). Portions were preserved in formalin-acetic-alcohol. Twigs of *S. glandulosum* were available from an herbarium specimen (Cape Le Grand, W.A., N. N. Donner 1569, AD), courtesy of the State Herbarium of South Australia. Because both hard and soft tissues and their interconnections required examination, sectioning was performed with a sliding microtome. Sections were stained with safranin and haematoxylin in the case of *S. laricifolium,* and with safranin and fast green in the remaining species. Counterstaining proved essential for delineating the strands of interxylary phloem. Macerations of the wood

of stems of *S. glandulosum* and *S. laricifolium*, and of the woody cylinder of *S. falcatum* were prepared and stained with safranin.

OBSERVATIONS—*Anatomy of stems with indeterminate cambial activity*—*Sylidium glandulosum* forms small shrubs on open granitic slopes of the Recherche Archipelago and on nearby Cape Le Grand, near Esperance, Western Australia. *Stylidium laricifolium* forms sparsely branched plants, some of which may grow through understory shrubs, on granitics of the Blue Mountains and adjacent regions of southeastern Australia (Mildbraed, 1907). The woody stems of these species can slightly exceed 1 cm in diameter at their bases.

As shown in Fig. 1, *S. laricifolium* wood appears in transection as radial series of cells varying but little in diameter. This pattern begins outside the pith and the adjacent bundles derived from procambium (Fig. 3). There may be some difference in wall thickness denoting growth rings (Fig. 1); these are not annual rings, since they may not extend continuously around a stem. In tangential section (Fig. 2), the wood proves to be rayless, and to consist of vessel elements only a little wider than the fibers. Not readily visible in tangential sections but easily seen in transections are strands of interxylary phloem (Fig. 4). These phloem strands are small, and correspond to the volume which would be occupied by one or two fibers. They consist of sieve tube elements and companion cells chiefly, with only occasional cells identifiable as parenchyma cells. Often only a single sieve tube element and a companion cell comprise a phloem strand. This can also be seen in *S. glandulosum* (Fig. 5), as can the simple sieve plates of sieve tube elements (Fig. 13).

The cambial region of *S. laricifolium* (Fig. 4, 6) lies underneath an endodermis and cortical cells which stretch tangentially as the circumference of the woody cylinder increases. These cells do not divide or collapse. From cells of the outer cortex, a periderm forms. There is no continuity between the radial files of periderm cells and the radial files of xylem cells—the two originate and operate independently. The cambium produces xylem and phloem strands toward the inside of the stem, but it produces no derivatives toward the outside. Thus it is always adjacent to the endodermis, interior to which it originated (Fig. 6). Divisions to accomodate increase in girth of the cambium occur as radial longitudinal divisions, as indicated by storied patterns in groups of fibers or vessel elements (Fig. 2). The vessel elements have bordered simple perforations (Fig. 7) and alternate, never crowded, bordered pits (Fig. 8). Perforation plates are mostly near tips of vessel elements, but vessel elements with long tails are fairly common, suggesting a modest degree of intrusive growth. Vessel elements in *S. laricifolium* average 542 μm in length. Fibers have pits with minute borders and small apertures; the pits are much smaller in diameter than those of vessel elements (Fig. 8). Fibers average 672 μm in length. Because of the minute borders, the fibers might be called fiber-tracheids. I have hesitated to apply this term because the cambium from which they originate is not a vascular cambium in the ordinary sense. The secondary xylem of *S. laricifolium* is entirely devoid of both axial parenchyma (except for a very occasional phloem parenchyma cell, as noted above) and ray parenchyma.

The wood of *S. glandulosum* differs only in having thicker-walled vessel elements and fibers (Fig. 5). Vessel element length averages 528 μm, fiber length averages 720 μm, figures remarkably like those for *S. laricifolium*. Dimensions of wood cells in these two species of *Stylidium* probably do not correlate with water-relation ecology, as do those of most woods (Carlquist, 1975), but represent instead a constant and uniform mechanical function, an interpretation also suggested by lack of rays. The slender upright stems of *S. falcatum* have cells which present much the same figures: vessel elements average 527 μm, fibers 645 μm. The vessel element length is greater than one would expect for plants as xeromorphic as these *Stylidium* species, but the greater-than-expected length would be easily explainable if mechanical function governed length, for longer fibers tend to correlate with increased mechanical strength (Carlquist, 1975). The stems of *S. glandulosum* show unidirectional cambia and periderm derived from cortical cells entirely comparable to the conditions in *S. laricifolium*.

Anatomy of upright stems with determinate secondary growth—If one examines transections of stems of sect. Rhynchangium, such as *S. adnatum* or *S. falcatum* (Fig. 10), one finds that a determinate amount of what appears to be secondary growth has occurred. By determinate growth, I mean the secondary xylem is formed pericyclically within the endodermis in a predictable and limited quantity which does not result in stretching of endodermis or distortion or shedding of cortex. To be sure, as shown in Fig. 10, the fibers and vessel elements are not always perfectly aligned into radial rows in these species, but they usually
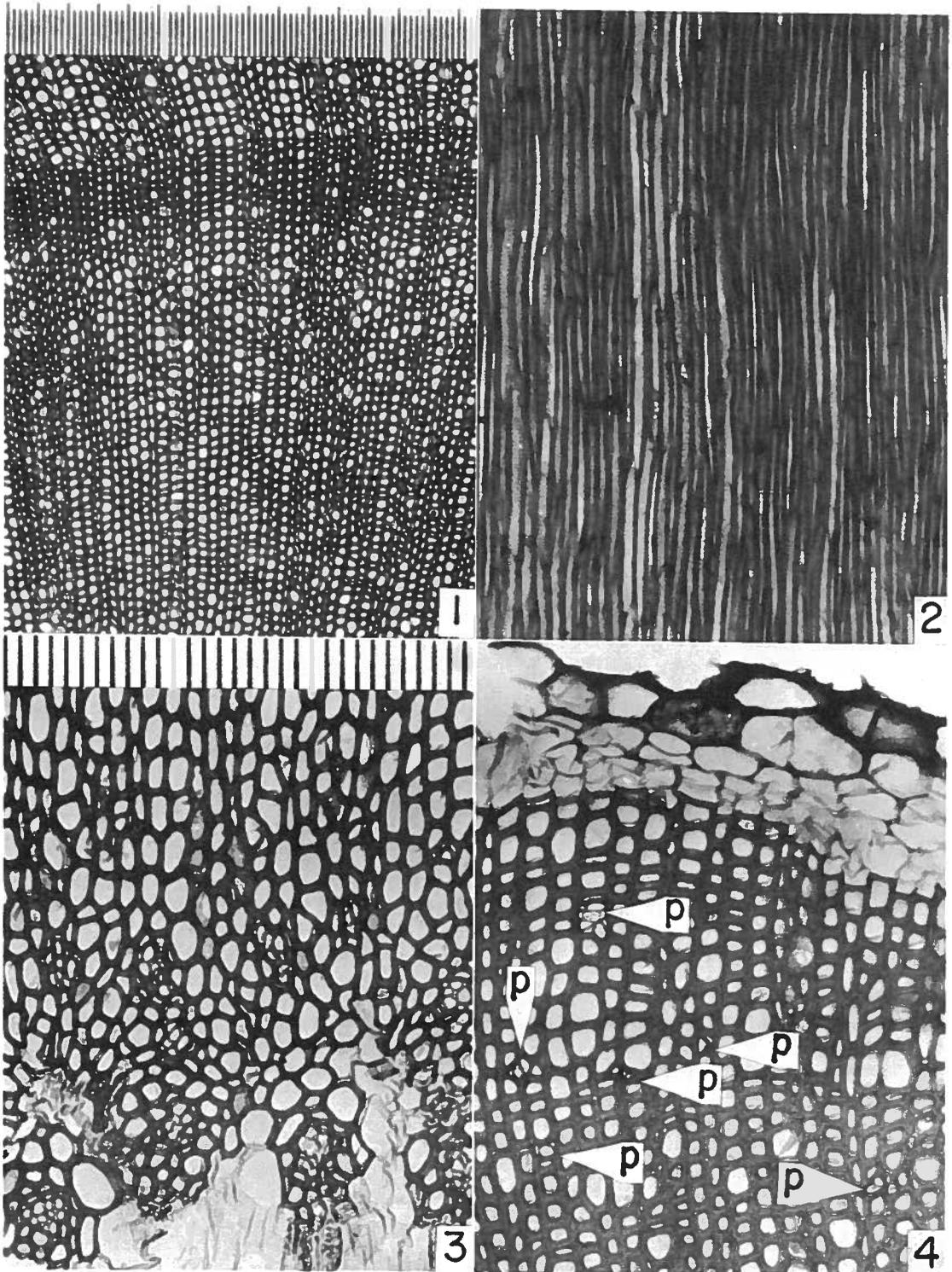
Fig. 1–4.   Secondary xylem in older stem of *Stylidium laricifolium*. **1.** Transection showing homogeneous appearance; growth ring above. **2.** Tangential section; storied structure and lack of rays apparent. **3.** Transection, adjacent to pith: scattered amphivasal bundles below, radial rows of secondary xylem above. **4.** Transection, periphery of stem: endodermis and innermost cortical layer above, xylem below; p = interxylary phloem strands. Magnification scale for Fig. 1 & 2 above Fig 1: finest divisions = 10 μm. Scale for Fig. 3 & 4 above Fig. 3: divisions = 10 μm.

are. A figure in Pfeiffer (1926) purporting to be *S. falcatum* lacks radial rows entirely; however, this figure is actually redrawn from Mildbraed's (1907) figure of *S. scandens* stem, but has been mislabeled by mistake. The stem of *S. scandens* lacks secondary growth in material I have sectioned.

In the upright stems of *S. adnatum* and *S. falcatum*, strands of interxylary phloem are present in the woody cylinder. These can be easily missed (e.g., Vesque, 1878, as noted by Van Tieghem and Morot, 1883, 1884a, 1884b), especially with monochromatic staining. The phloem strands show easily with a counterstain, however.

The above description of stems of *S. adnatum* and *S. falcatum* yields a pattern quite similar to the pattern of *S. glandulosum* and *S. laricifolium* described earlier. However, the woody cylinder of the Rhynchangium species is, at maturity delimited not by a cambium but by a cell layer termed endodermis by various authors (Van Tieghem and Morot, 1883, 1884a, 1884b; Schonland, 1889; Mildbraed, 1907). I have not found a Casparian strip in my material, but I will use the term endodermis anyway. The endodermis of *S. falcatum* (Fig. 10) contains tannins which give a brown color to stems in that species because the cortex sloughs off, exposing the endodermis. Stems of *S. adnatum* (Fig. 9) are straw colored, according with the lack of tannins in the endodermis.

Van Tieghem and Morot (1884a) furnished a figure of a stem transection of *S. adnatum*, showing meristematic action beneath the endodermis leading to the production of the cylinder which contains vessel elements, fibers, and interxylary phloem. This meristematic action leads to the radial arrangement of these elements as seen at maturity, and occurs after the bundles of the primary stem have matured. Thus, there seems no alternative but to consider this "fibrous" or "pericyclic" cylinder a product of determinate secondary growth, a concept that Van Tieghem and Morot (1884a) espoused. Their figures for both meristematic and mature stems of *S. adnatum* seem thoroughly accurate and are in accord with my observations for *S. adnatum* and *S. falcatum*. The figures of Vesque (1878) for *S. adnatum* are accurate except for his omission of the interxylary phloem strands. The figures of Schonland (1889) and Mildbraed (1907) for *S. adnatum* stem transections are copied from those of Van Tieghem and Morot (1884a).

However, Burns (1900), in an extensive account of anatomy of the family, claimed that the woody cylinder of *S. adnatum* was not a form of secondary growth, but merely a pericyclic strengthening ring. The presence of interxylary phloem and of vessels within this cylinder Burns explained in terms of leaf traces. The dissociation of vessels from phloem in primary stem bundles which can be seen in primary stem bundles of some (but not all) stems of *S. calcaratum* may have persuaded Burns that non-associated phloem strands and vessels in the woody cylinder of *S. adnatum* could be considered collectively as disorganized versions of leaf traces. Some species of *Stylidium*, such as *S. scandens* and *S. crossocephalum*, have subendodermal fibers (Mildbraed, 1907), but have no phloem strands among the fibers and very likely the fibers are not produced by cambial activity. The phloem strands of *S. adnatum*, *S. falcatum*, *S. glandulosum*, and *S. laricifolium* not only can be shown to be produced by a cambium, but do not traverse the endodermis, so they have no interconnection with phloem of leaf traces, and thus Burns's (1900) interpretation is invalid. Perhaps Burns had difficulty integrating a concept of finite secondary growth with a concept, suggested by stems such as those of *S. scandens*, of cortical fibers which originate as a result of primary growth.

Nevertheless, Burns's interpretation proved persuasive to subsequent authors, such as Solereder (1908) and Mildbraed (1907). Pfeiffer (1926) cited Stylidiaceae under the heading "Unique and doubtful or mostly insufficiently known cases of pericyclic meristems." Chalk and Chattaway (1937) did not cite Sylidiaceae in their summary of anomalous cambial types, nor do Metcalfe and Chalk (1950) list it under families in which interxylary phloem is known. However, the secondary growth of *S. glandulosum* and *S. laricifolium* cannot possibly be discounted as merely "pericyclic fibers" among which disorganized leaf traces are distributed. This pair of species forces us to recognize that secondary growth which produces secondary xylem containing interxylary phloem strands, a secondary growth which originates exterior to the vascular bundles, does indeed occur in Stylidiaceae.

Secondary growth in lignotubers—The only species which can be said to have woody lignotubers are the four species of section Rhynchangium, although other species have fleshy stems which survive fire (e.g., *S. carnosum*). The Rhynchangium species have several stems branching from the base; adventitious roots originate a short distance below the juncture of the stems. The tissue between stems and roots takes the form of an inconspicuous
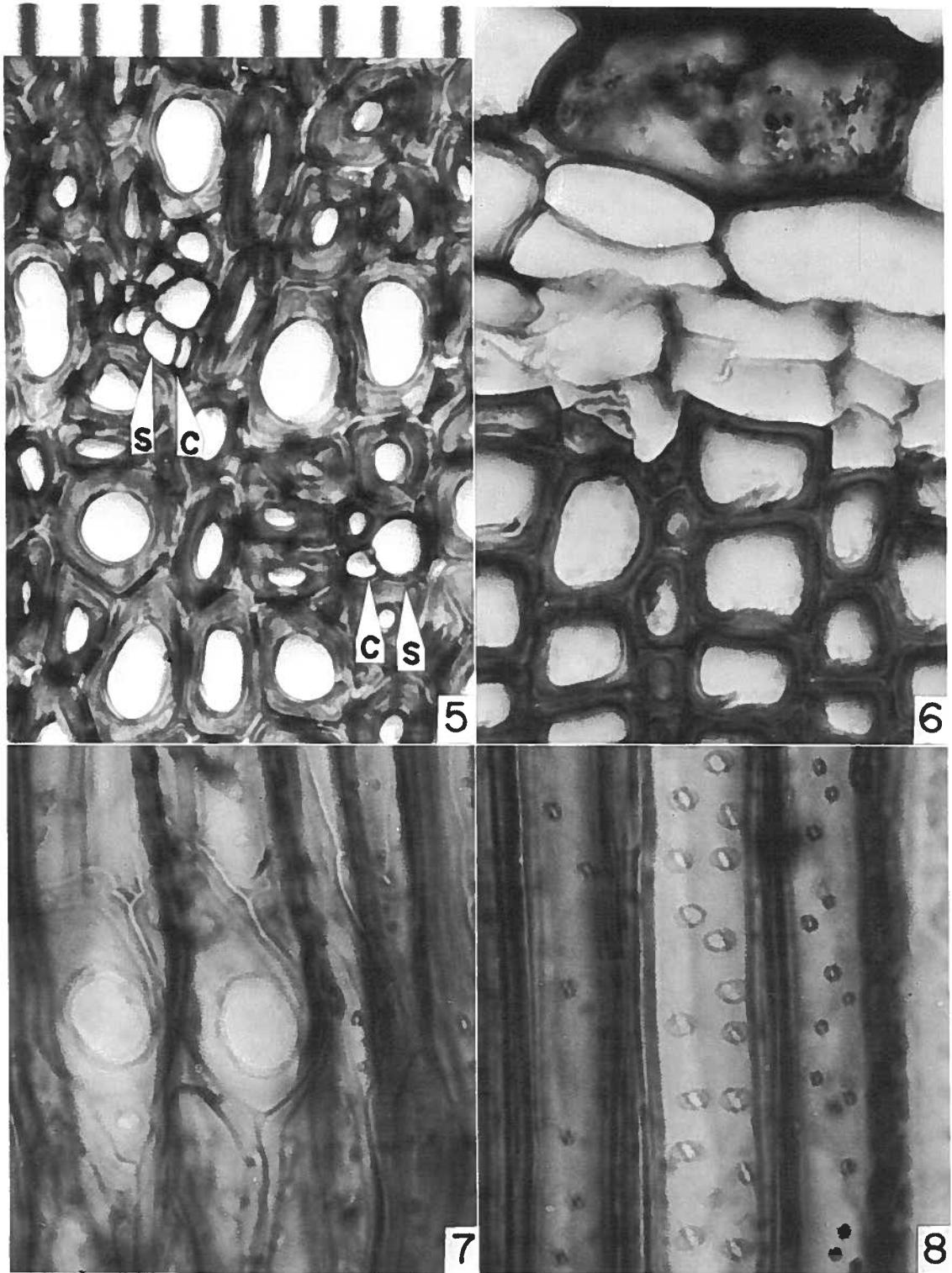
Fig. 5–8. Secondary xylem of older stems of *Stylidium*. **5.** *S. glandulosum,* showing two strands of interxylary phloem; c = companion cell, s = sieve tube element. **6–8.** *S. laricifolium.* **6.** Transection. Top cell layer = innermost cortical layer; second layer = endodermis; third layer = cambium. Interxylary phloem strand originating from cambium, left of center. **7.** Simple perforation plates in vessel elements, from radial section. **8.** Radial section: vessel, showing wide-bordered pits, flanked by fibers with small, reduced pits. Magnification scale for Fig. 5–8 shown above Fig 5: divisions = 10 μm.
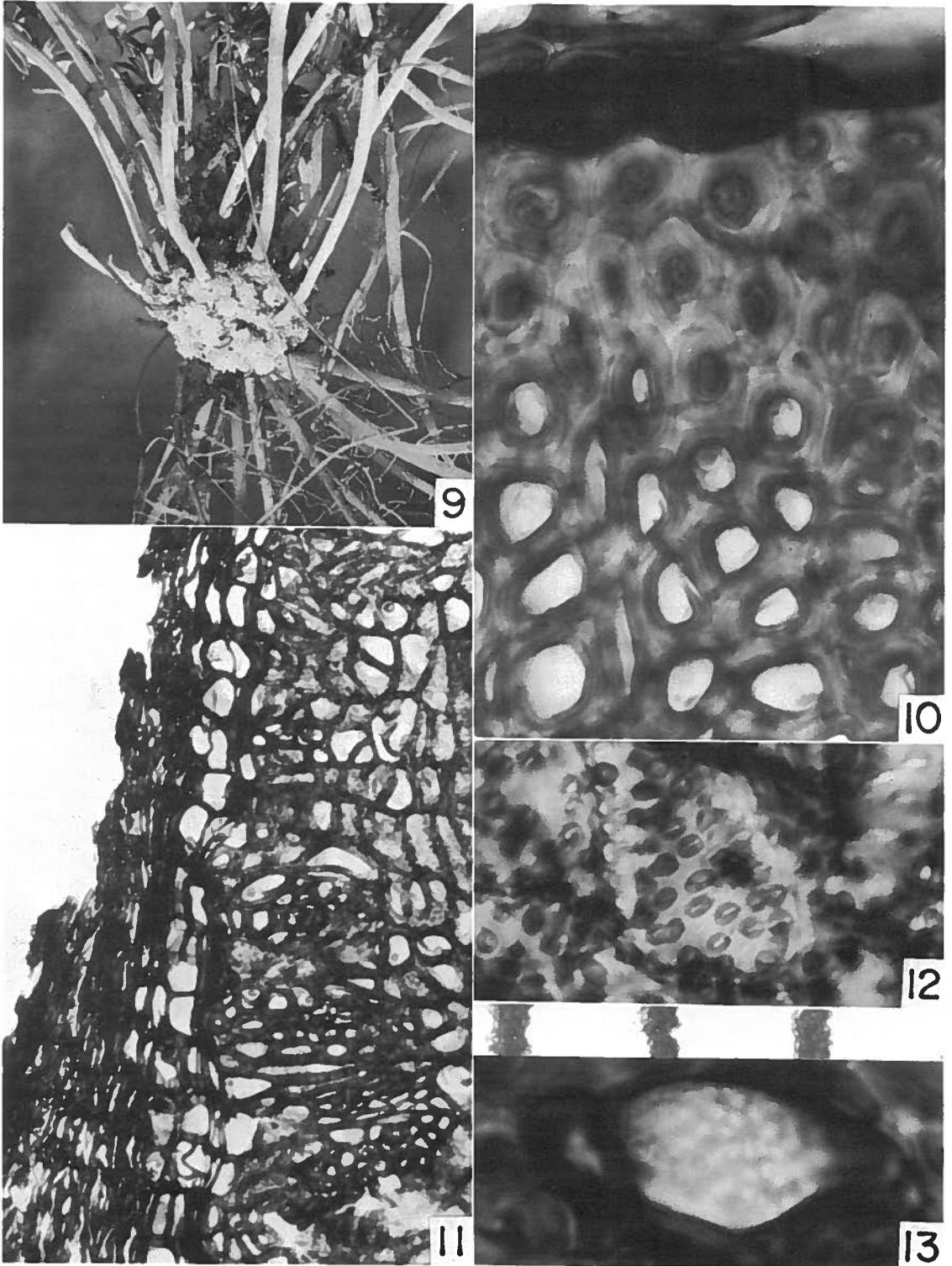
Fig. 9–13. **9.** *Stylidium adnatum*, base of plant collected at Canal Rocks, Western Australia: lignotuber about 1 cm in diameter at center. **10.** *S. falcatum*, transection of woody cylinder; tannin-filled endodermis at top. **11.** *S. adnatum*, transection of lignotuber, phellem at left. **12.** *S. falcatum*, contorted vessel element with bordered pits, from longisection of lignotuber. **13.** *S. glandulosum*, wood transection, sieve plate from interxylary phloem. Scale for Fig. 10, 12 above Fig. 5; scale for Fig. 11 above Fig 3; scale for Fig. 13 above Fig. 13: divisions = 10 μm.

"weld" or, as in *S. adnatum* (Fig. 9) a woody cushion. When one sections the lignotubers of either *S. adnatum* (Fig. 11) or *S. falcatum,* one discovers a distinctive mode of cambial activity. The cambium produces numerous layers of phellem to the outside. To the inside, it produces variously contorted xylem cells and strands of interxylary phloem. The vast bulk of xylem tissue, especially in *S. falcatum,* consists of cuboidal vessel elements (Fig. 12) bearing bordered pits like those of vessel elements in upright stems. These vessel elements have simple perforation plates. A few cuboidal cells with thick lignified walls like those of vessel elements bear pits which are simple or nearly so, and can be considered the homologs of fibers. In *S. adnatum* lignotubers, a few thin-walled parenchyma cells were observed. Not all xylem cells of the lignotubers are cuboidal: there is every transition from cuboidal to fusiform, and fusiform vessel elements in strands winding through a mass of cuboidal vessel elements may be seen at many points.

One might be tempted at first to compare the cambial activity of the lignotubers to the lateral meristems of monocotyledons. However, no vascular strands can be designated in the *Stylidium* lignotubers. There is no ground tissue of parenchyma, but rather a background of cuboidal or variously-shaped vessel elements. The few sclerenchyma or parenchyma cells within this background cannot really be likened to conjunctive tissue between vascular bundles.

The phellem undoubtedly aids by insulating living tissues of the lignotuber from fire or excessive heat. I have seen burned plants of *S. falcatum* producing shoots from lignotubers in the Tutanning Reserve, Western Australia. One may speculate that living cells in the cambial zone can furnish the sites of origin of new shoots or roots.

CONCLUSIONS—The stems of certain *Stylidium* species contain an array of unusual or unique cambial types. Many species in the genus have no cambial activity, but do have a sclerenchymatous cortex, consisting of fibers beneath a cell layer that may be termed an endodermis. Among such species one could name *S. crossocephalum, S. repens,* and *S. scandens.* Presumably the longitudinal divisions in procambial cells that lead to this cylinder of fibers occur at random in most species, because no radial rows of fibers are apparent. Occurrence of tangential divisions in procambial zones in various dicotyledon stems, leading to production of cells in radial rows, is not a rare phenomenon. From such a tendency,

the production of radial rows of xylem cells in *S. adnatum* and other Rhynchangium species might conceivably be derived. However, one must stress that in these species the process is quite orderly, that interxylary phloem strands are formed, and that vessel elements are produced along with the fibers. The cambium is, functionally, the cell layer inside the endodermis. Although endodermal cells are tangentially stretched by the development of the woody cylinder in the Rhynchangium species, the cylinder size is quite predictable and secondary growth is finite. These concepts are in line with the findings of Van Tieghem and Morot (1883, 1884a, 1884b) and this interpretation should be resurrected despite the comments of Burns (1900). One can consider that this cambium is somewhat like a primary thickening meristem in the sense of Stevenson and Popham (1973) or Mikesell (1979), but it does not lead to production of desmogen strands as in the cases given by those authors. The similarity lies in the fact that both meristem types originate in pericyclic regions.

The cambial activity in *S. glandulosum* and *S. laricifolium* is indeterminate, although stems in these species never exceed approximately 1 cm in diameter. One can imagine the cambium in this pair of species as having originated from the abovementioned determinate type. Both types are unidirectional, producing no cells toward the outside. A periderm does develop from outer cortical cells in *S. glandulosum* and *S. laricifolium.* Despite continual action of the vascular cambium, the endodermal cells are not ruptured. Since the cambium produces no cells to the outside and thus no second periderm origin site is possible, the cambium could be expected to be limited by the ability of the endodermis or the inner cortex to remain intact. Also, the xylem is rayless and lacks axial xylem parenchyma. Lack of these photosynthate-conducting systems is not a problem for woody cylinders of limited diameter, but it would be a limiting factor for larger stems. In fact, this pair of *Stylidium* species appears to have a wood in which heightened mechanical strength leading to a shrubby growth form is the factor of importance. The length of vessel elements is identical in *S. glandulosum, S. laricifolium,* and *S. falcatum;* so is the length of fibers. The vessel elements, as mentioned above, are longer than one would expect to find in plants of such xeric habitats, but the length would not be surprising if selection for longer, and therefore stronger, fibers is of prime importance in this wood. A low (ca. 1.3) ratio between fibers and vessel elements is characteristic of primitive woods,

with few exceptions (Carlquist, 1975). The occurrence of this low ratio in the two *Stylidium* species seems to be a special case, in which the long vessel elements are a byproduct of high selective value for long fibers and therefore long fusiform cambial initials. One should also remember that the cambial acitivity in *Stylidium* does not arise in vascular bundles as it does in most dicotyledons, but rather in a pericyclic region. Consequently, a departure from quantitative patterns typical for woods produced by ordinary vascular cambia is not surprising.

The lignotuber of the Rhynchangium species has a cambium in which contorted xylem (mostly vessel elements) containing interxylary phloem strands is produced toward the inside, whereas phellem is produced toward the outside. That this cambium is different from that of the upright stems on the same plants is not surprising: the lignotuber expands considerably in volume as it grows; it produces living cells which yield new shoots and roots, and it produces phellem which can resist fire and excessive heat. These functions could not be accomplished by a unidirectional cambium. The lignotuber cambium, however, seems a variant on the cambium of the upright stem, and indeed, cambia and conductive tissues of these organs are intercontinuous.

The phylogenetic direction envisaged above runs counter to the concept some botanists tend to have that woodiness should be considered a primitive character in dicotyledonous families, herbaceousness a derived one. Above all, one must remember that no species of *Stylidium* have been reported to have any cambial activity *within* vascular bundles. The pericyclic cambium does not appear primitive within the family either. The three genera *Phyllachne, Forstera,* and *Oreostylidium* have been hypothesized to have the bulk of features primitive for the family on morphological and phytogeographical grounds (Mildbraed, 1907; Carlquist, 1969). Other lines of evidence supporting this have come from palynology (Bronckers and Stainier, 1972) and cytology (James, 1979). The stems of these three genera have only a single cylinder of bundles and no

pericyclic cambial activity. Most species of *Stylidium,* such as those in this paper, have several cycles of bundles, presumptively a derived condition. Origin of cambia within a few species of *Stylidium* may be envisaged as one feature of the adaptive radiation in this large (ca. 150 spp.) genus.

LITERATURE CITED

BRONCKERS, F., AND F. STAINIER. 1972. Contribution à l'étude morphologique du pollen de la famille des Stylidiaceae. Grana 12: 1–22.

BURNS, G. P. 1900. Beiträge zur Kenntniss der Stylidiaceen. Flora 87: 313–354.

CARLQUIST, S. 1969. Studies in Stylidiaceae: new taxa, field observations, evolutionary tendencies. Aliso 7: 13–64.

———. 1975. Ecological strategies of xylem evolution. University of California Press, Berkeley and London.

CHALK, L., AND M. M. CHATTAWAY. 1937. Identification of woods with included phloem. Trop. Woods 50: 1–31.

JAMES, S. H. 1979. Chromosome numbers and genetic systems in the trigger plants of Western Australia (*Stylidium*; Stylidiaceae). Aust. J. Bot. 27: 17–25.

METCALFE, C. R., AND L. CHALK. 1950. Anatomy of the dicotyledons. Clarendon Press, Oxford.

MIKESELL, J. E. 1979. Anomalous secondary thickening in *Phytolacca americana* L. (Phytolaccaceae). Amer. J. Bot. 66: 997–1005.

MILDBRAED, J. 1907. Stylidiaceae. *In* A. Engler [ed.], Das Pflanzenreich IV, no. 278 (vol. 35), 98 pp. Verlag H. R. Engelmann, Weinheim, Germany,

PFEIFFER, H. 1926. Das abnorme Dickenwachstum. *In* K. Linsbauer [ed.], Handbuch der Pflanzenanatomie. Band 9, Lief 15: 1–273.

SCHONLAND, S. 1889. Candolleaceae (Stylidiaceae). *In* A. Engler & K. Prantl [eds.], Die natürlichen Pflanzenfamilien IV(5): 79–84.

SOLEREDER, H. 1908. Systematic anatomy of the dicotyledons (trans. by L. A. Boodle and F. E. Fritsch). Oxford University Press, Oxford.

STEVENSON, D. W., AND R. A. POPHAM.. 1973. Ontogeny of the primary thickening meristem in seedlings of *Bougainvillea spectabilis.* Amer. J. Bot. 60: 1–9.

VAN TIEGHEM, P., AND L. MOROT. 1883. Sur l'anomalie de structure de la tige des *Stylidium* à feuilles espacées. Bull. Soc. Bot. France 30: 308–310.

———, AND ———. 1884a. Anatomie des Stylidiées. Ann. Sci. Nat. Bot., Ser. 6, 19: 281–286.

———, AND———. 1884b. Sur l'anatomie des Stylidiées. Bull. Soc. Bot. France 31: 164–165.

VESQUE, J. 1878. Note sur l'anatomie des *Stylidium.* Ann. Sci. Nat. Bot., Ser. 6, 7: 204–208.