

SIGNIFICANCE OF CELL DIVISIONS IN DIFFERENTIATING SECONDARY PHLOEM

BY

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INTRODUCTION

The origin and development of the secondary phloem, particularly the sequence and kinds of cell divisions that occur in the cambial derivatives, are relatively unexplored topics. Yet the morphologic and physiologic specialization of the phloem cannot be properly understood without a knowledge of the ontogenetic development of this tissue. We are investigating a wide range of species with regard to such specialization and accordingly have given considerable attention to ontogeny. The present paper deals with certain aspects of cell division in the vertical system of the differentiating secondary phloem of dicotyledons.

Before the main subject matter is considered, the terms to be used in presenting the observations should be defined. Following common usage, the cells in the vascular cambium that by dividing periclinally give derivatives in two directions, that is, toward the phloem and toward the xylem, are considered to be the cambial initials (fusiform cambial initials in the vertical system). Since it is frequently difficult to distinguish these initials from their most recent derivatives, the initials and the youngest derivatives together are referred to as cambial cells. The cambial cells constitute the cambial zone. The cambial cells that are parts of the developing phloem or xylem but have not yet finished dividing are the mother cells of these tissues, that is, phloem mother cells or xylem mother cells. For the sake of simplicity these mother cells are here spoken of sometimes as phloem initials and xylem initials, respectively. A phloem or xylem initial about to undergo one or more divisions that will give rise to two or more cells seemingly grouped together within the confines of the originally fusiform cell (e.g. Fig. 4B and 5B) may be called the mother cell of the derivative cells. Greater refinement of terms may be necessary in dealing with various cambial derivatives in future work, but need not be introduced here.

As long as a cambial initial persists in the initial layer it produces a continuous complex of cells, the outer part extending radially

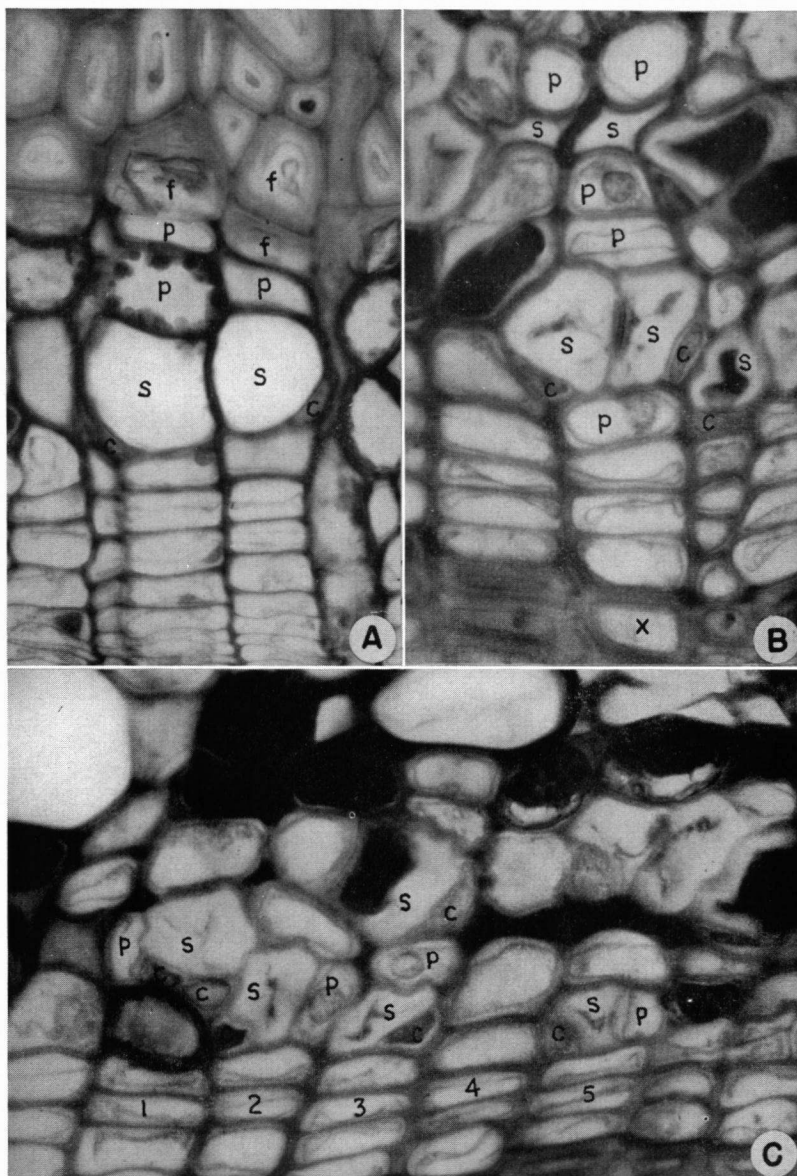


Plate I. Transverse sections of secondary phloem of *Populus grandidentata* Michx. (A) and *Umbellularia californica* Nutt. (B, C). The vascular cambium is below in each figure. In A the two sieve elements (s) belong to two separate tiers and each was derived from one phloem initial. In B the two sieve elements in the center of the figure belong to one tier and were derived from one phloem initial that divided by a longitudinal anticlinal wall. In C the parenchyma cells (p) in tiers 1, 2, and 5 were formed by longitudinal anticlinal divisions from the same phloem initials as the associated sieve elements (s). The parenchyma cell in tier 3 may have been derived from the same initial as the lower sieve element by a tangential division. Details are: c, companion cell; f, fiber; p, parenchyma cell; s, sieve element; x, xylem cell. (All $\times 820$).

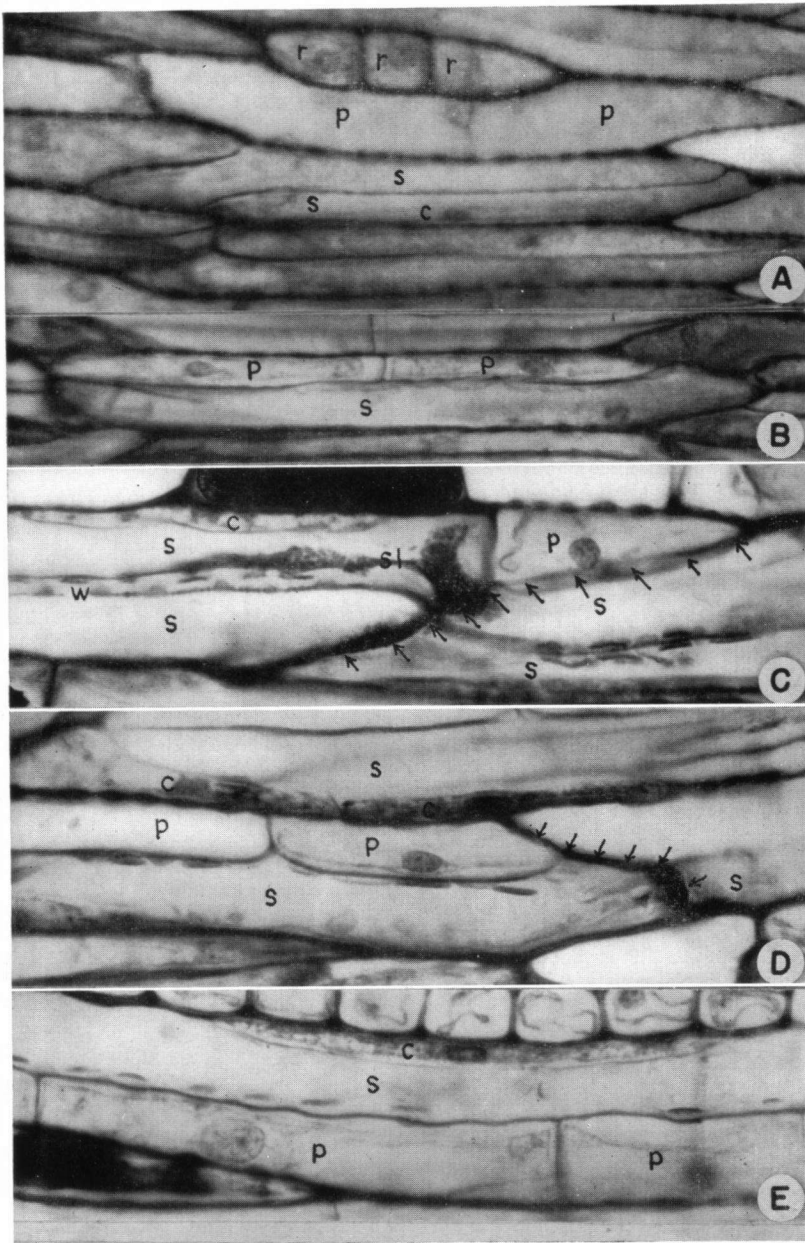


Plate II. Tangential longitudinal sections of secondary phloem of *Asimina triloba* Dunal (A, B) and *Cinnamomum camphora* Nees & Eberm. (C-E). In A a phloem initial divided into two sieve elements (s) by a vertical wall. The two parenchyma cells (p) originated from one initial by a transverse division. In B the sieve element (s) and the parenchyma strand (pp) were derived from one initial by a vertical division (Companion cell nucleus at the right of s.). In C the series of arrows indicate the outline of the former end wall between two overlapping fusiform phloem initials. The initial to the left formed two sieve elements (s) separated by a vertical wall (w), and a parenchyma cell (p) separated by a transverse wall from one of the sieve elements. The initial to the right formed two sieve elements by a vertical division. In D the two parenchyma cells (p) were derived from the same initial as the sieve element below them. The arrows indicate the former end of this initial. E contrasts parenchyma cells (p) with the companion cell (c), all derived from the same initial as the sieve element (s). Details are: c, companion cell; p, parenchyma cell; r, ray cell; s, sieve element; sl, contracted slime in sieve element; w, cell wall. (A, $\times 430$; B, $\times 380$; C-E, $\times 700$).

through the phloem, the inner through the xylem. In the vertical system of phloem or xylem such complexes of cells may be conveniently called tiers. (In the horizontal system an entire ray may be regarded as a tier.) In transverse section a secondary-phloem tier in the vertical system appears to be composed of one or more (rarely more than two) radial files of cells, depending on the absence or presence of longitudinal anticlinal divisions in the phloem initials of the tier. Sometimes irregular overlapping of members of superimposed tiers renders recognition of a given tier somewhat difficult (e.g. radial file at left in Fig. 1A) and gives an erroneous impression of doubling of a radial file in a tier.

The most familiar examples of divisions in the phloem initials are those producing a parenchyma strand from a single mother cell and those separating a companion cell or cells from a sieve-tube member. Less familiar are the occurrence, duration, and effect of tangential divisions among the phloem initials, although evidence is available that such divisions play an important role in the growth of an annual phloem increment (BANNAN, 1955; RAATZ, 1892). There may be in addition tangential divisions partitioning a mother cell into two or more sieve elements or into sieve elements and parenchyma cells. The wall separating a companion cell from a sieve element may also, of course, be tangential.

The present paper is concerned not with the divisions just mentioned, but with those little explored divisions that obscure the ontogenetic relation between the cells of a tier and the cambial initials originating the tier. These are various anticlinal and approximately anticlinal divisions (including the radial divisions) that may cause a discrepancy in length between the cambial cells and the sieve elements, or may bring about a splitting of a phloem initial into two or more sieve elements (Plate I, B and II, A) or into one or more sieve elements and parenchyma cells (Plate I, C and II, B-E).

The survey for the occurrence of anticlinal divisions included 91 species of 77 genera in 44 families of dicotyledons. The slides were prepared according to a previously described technique (CHEADLE *et al.*, 1953). Some of the material was collected only in the dormant state, the remainder in both the dormant and the active-growth states. In the dormant material the occurrence and kind of anticlinal divisions were determined by analysis of cell patterns in individual tiers. In the active tissue, newly formed walls in the cambial zone furnished supporting evidence.

OBSERVATIONS

To exemplify the behavior of the phloem initials in different species reference is made to the series of drawings in Fig. 1-5. In each of these figures, A shows in cross section a complex of at least four tiers; B illustrates sieve elements, singly or in connected series, with or without other phloem cells; C shows fragments of cambium (initials or immediate derivatives) or individual cambial cells; and D shows vessel elements, singly or in connected series. B-D were drawn from tangential

sections except the vessel element in Fig. 3D which was obtained from a radial section. The cross section in Fig. 3A (*Weigela*) includes two rays; the other cross sections represent tiers from the vertical system only.

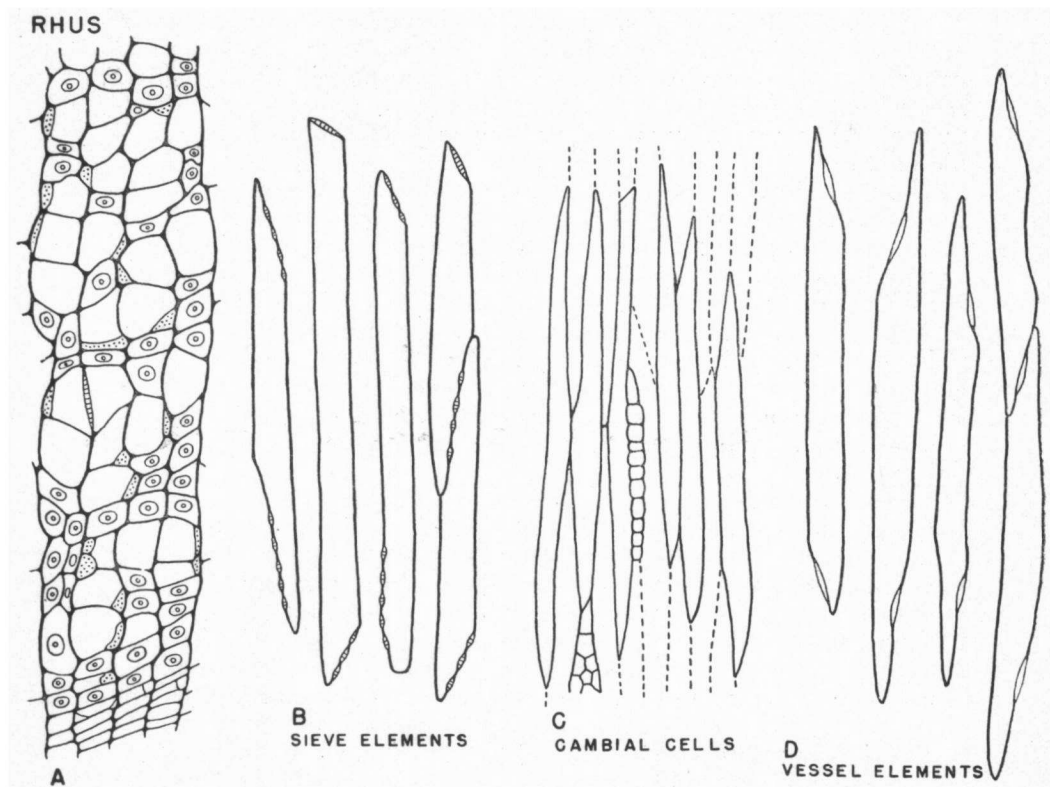


Fig. 1. *Rhus typhina* Torner. Transverse section of secondary phloem (A), and certain elements of secondary vascular tissues from a tangential section (B-D). Sieve elements and vessel elements are similar to cambial cells in length. (A, $\times 225$; B-D, $\times 175$).

Rhus typhina Torner (Fig. 1) exemplifies phloem in which the phloem initials that give rise to sieve elements usually undergo no anticlinal divisions other than those concerned with the formation of companion cells. As a result, the sieve elements are comparable to the cambial initials in length and shape. The sieve elements, however, increase in width during differentiation. In some species of dicotyledons this expansion may be so great that the radial seriation of cells is obscured. Such expansion was not observed in *Rhus*. The orderly arrangement of tiers in the form of easily discernible radial files in transection (Fig. 1A) reflects the simple ontogenetic relation between the phloem and the cambium and the lack of any striking expansion of sieve

elements or any other cells. The apparent doubling in part of the radial file at the left in Fig. 1A is a result of partial overlapping of two superimposed tiers.

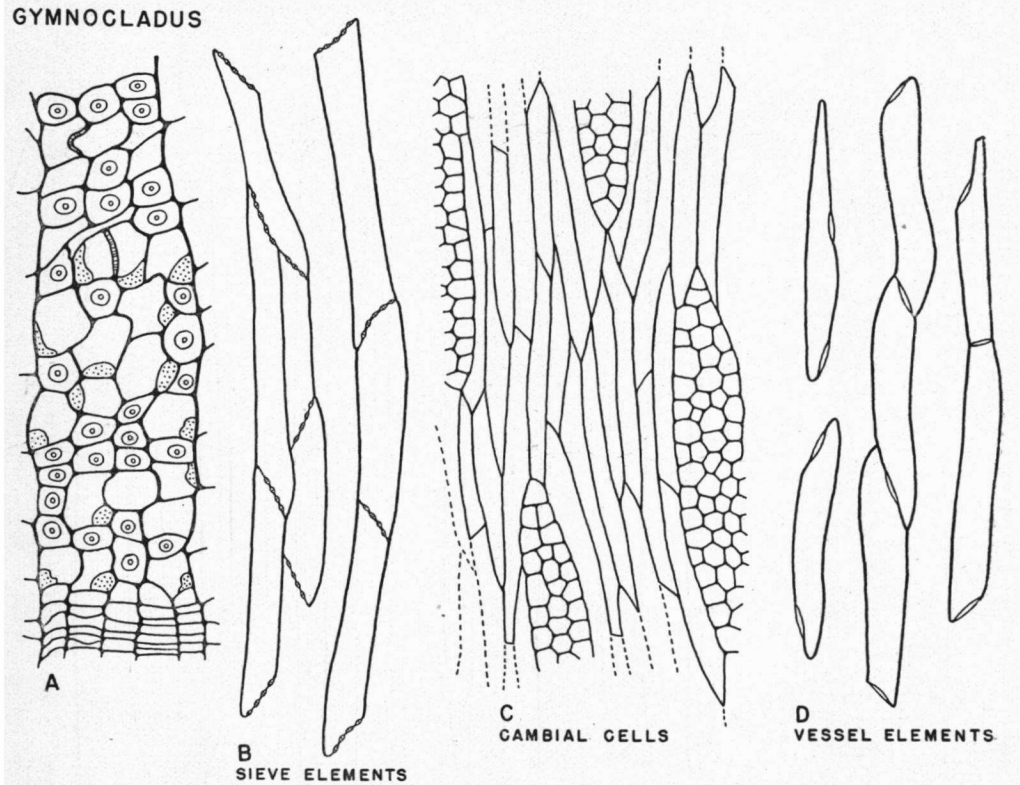


Fig. 2. *Gymnocladus dioica* Koch. Transverse section of secondary phloem (A), and certain elements of secondary vascular tissues from a tangential section (B-D). Sieve elements and vessel elements are similar to cambial cells in length. (A, $\times 225$; B-D, $\times 175$).

Gymnocladus dioica Koch. (Fig. 2) has shorter cambial cells than *Rhus*; otherwise it illustrates a similar relation between sieve elements and cambial cells. In this species also the single radial series of cells in transection clearly demarcate the tangential boundaries of the individual tiers. At the right below in Fig. 2A a division in a cambial initial resulted in the appearance of two initials and thus of two tiers in this position. A similar doubling occurred somewhat earlier in the growth of this phloem so that the oldest part of the section shows only three tiers, the younger four, and the cambium five.

Weigela sp. (Fig. 3) deviates from the two preceding species in that transverse or somewhat oblique divisions commonly occur in the sieve element mother cells so that the sieve elements are shorter than the cambial cells. These divisions do not disturb the orderly arrange-

ment of cells of the tiers as evidenced by the simple radial seriation exhibited in transection (Fig. 3 A). The doubling of radial rows in the cambial zone seen at the left probably resulted from division of an initial; immediately to the right of this double cambial row there is evidence of overlapping of two superimposed tiers.

WEIGELA

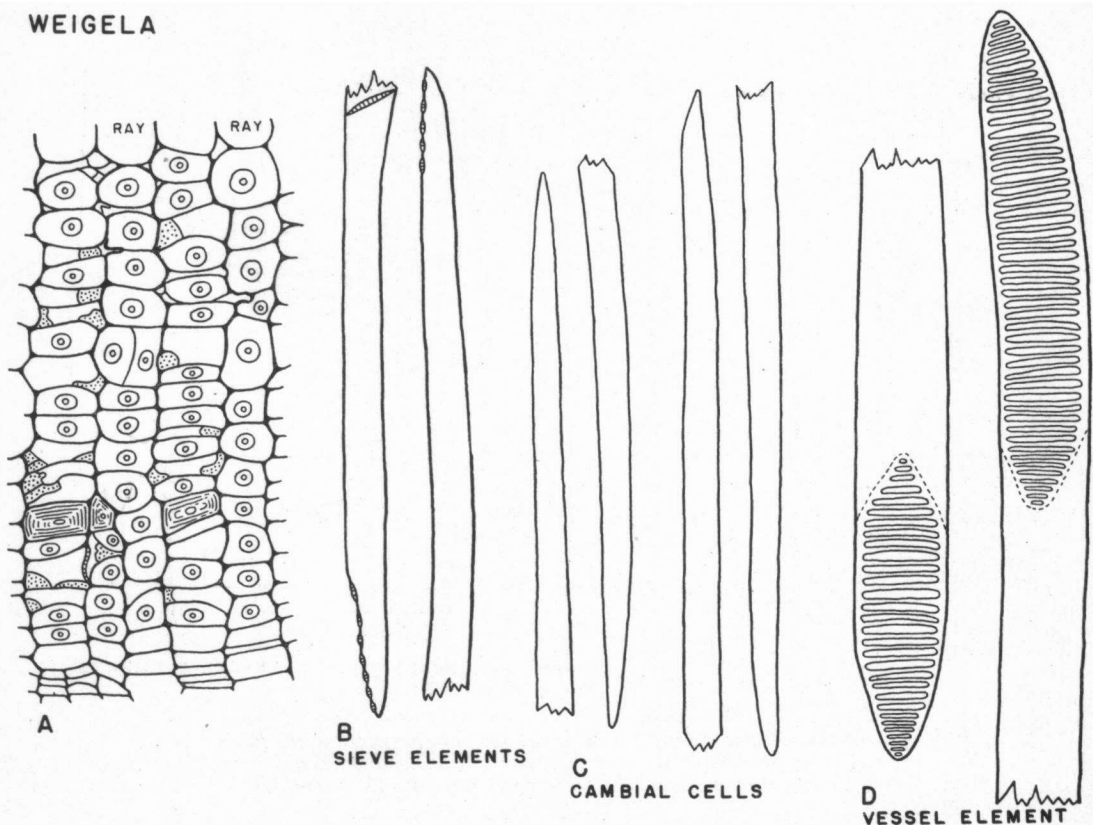


Fig. 3. *Weigela* sp. Transverse section of secondary phloem (A), sieve elements and cambial cells from a tangential section (B, C), and a vessel element from a radial section (D). The sieve elements are shorter than the cambial cells because a transverse division occurred in the sieve-element initial. The vessel element is similar to the cambial cells in length. (A, $\times 225$; B-D, $\times 175$).

Cryptocarya sp. (Fig. 4) and *Asimina triloba* Dunal (Fig. 5), the former with longer, the latter with shorter cambial initials, furnish good examples of phloem in which various anticlinal divisions occur before the differentiation of sieve elements. As may be seen from Fig. 4 B and 5 B, longitudinal and transverse divisions may occur in the same phloem initial and may give rise to two or more sieve elements or to combinations of sieve elements and parenchyma cells. Many of the longitudinal divisions in these species are truly vertical extending from end

wall to end wall (Plate II, A, B), others are more or less oblique (Fig. 4B, cells marked x). Transverse divisions are less common than the longitudinal and are found most frequently near the rays. The occurrence of longitudinal anticlinal divisions causes an increase in the

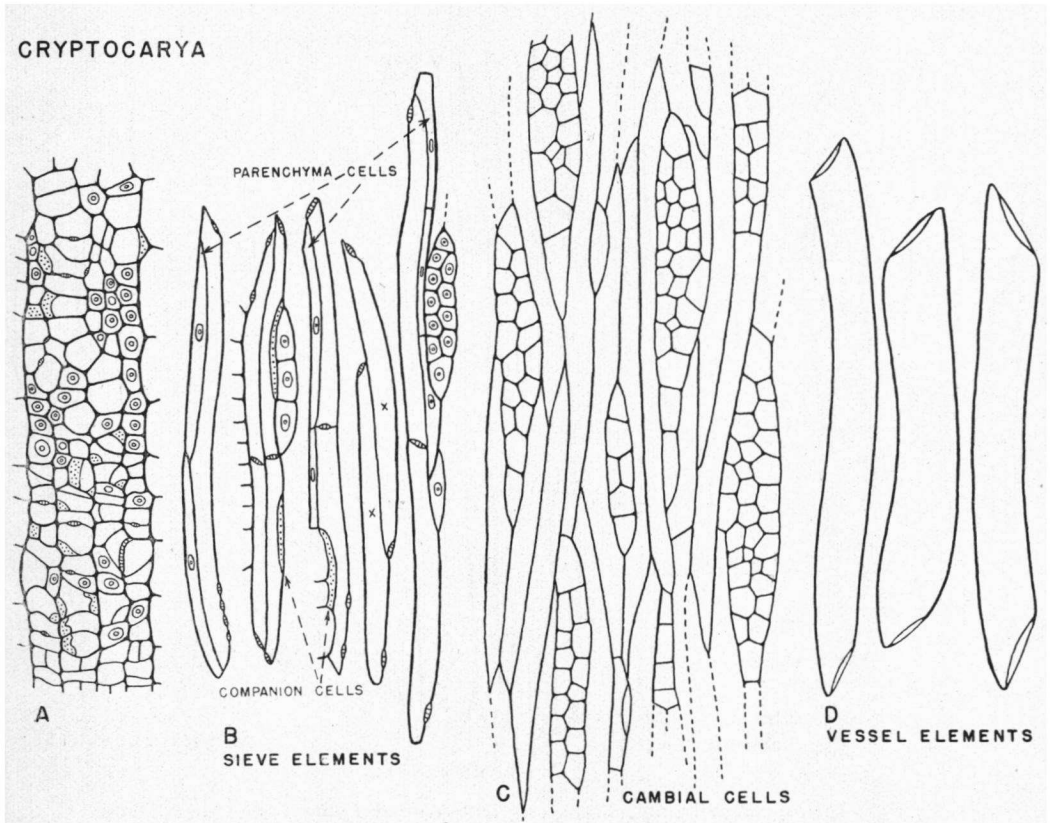


Fig. 4. *Cryptocarya* sp. Transverse section of secondary phloem (A), and certain elements of secondary vascular tissues from a tangential section (B-D). The sieve elements arose from phloem initials that divided along various anticlinal planes. Some of the sieve elements are, therefore, shorter than the cambial cells. The formation of short sieve elements may result from either transverse or longitudinal divisions (cells marked x in B). The vessel elements are similar to cambial cells in length. (A, $\times 225$; B-D, $\times 175$).

number of cells in a tier in the tangential direction. Since the successive tangential derivatives from a cambial cell may show different numbers and orientations of these longitudinal anticlinal divisions, the orderly radial seriation of cells as seen in transection is disturbed and the tangential limits of the tiers are obscured (compare Fig. 4A and 5A with 1A, 2A, and 3A). The above description may be complemented by reference to two other species, *Populus grandidentata* Michx. (Plate I, A) in which no anticlinal divisions occur before the differentiation

of sieve elements, and to *Umbellularia californica* Nutt. in which phloem mother cells divide by anticlinal walls into more than one sieve element (Plate I, B) or into sieve elements and parenchyma cells (Plate I, C).

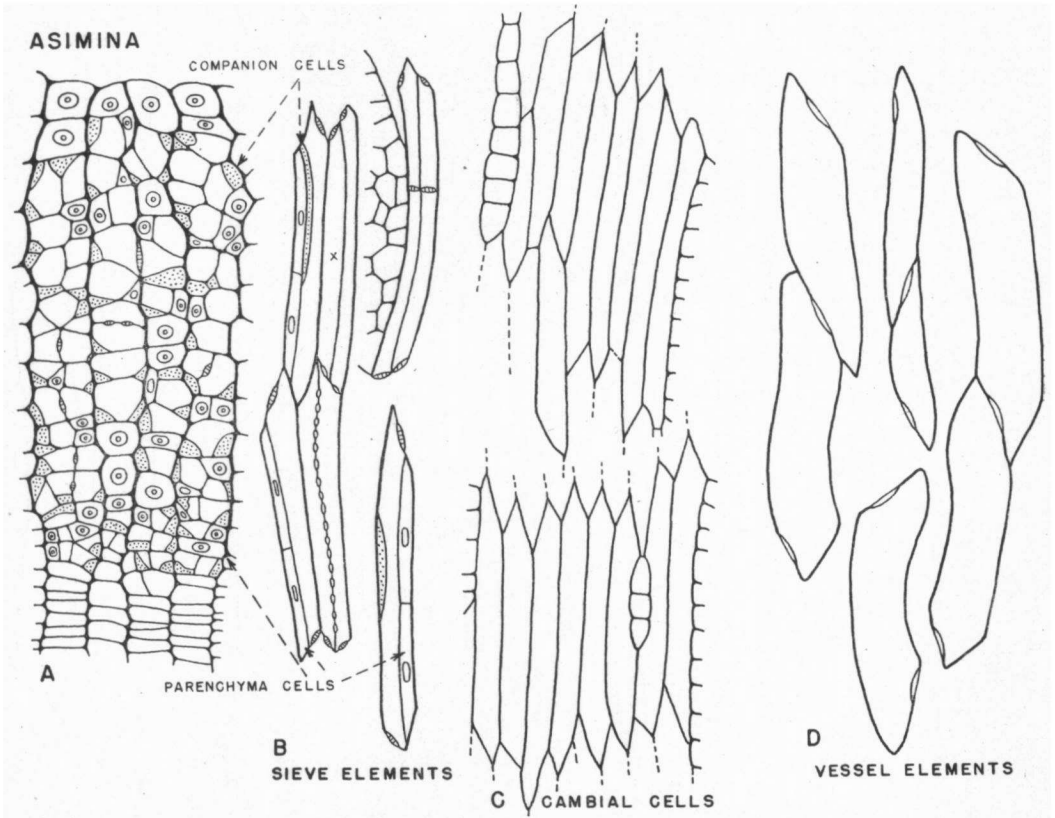


Fig. 5. *Asimina triloba* Dunal. Transverse section of secondary phloem (A), and certain elements of secondary vascular tissues from a tangential section (B-D). The sieve elements arose from phloem initials that divided along various anticlinal planes. Some of the sieve elements are, therefore, shorter than the cambial cells. The vessel elements are generally similar to the cambial cells in length. (A. $\times 225$; B-D, $\times 175$).

Turning our attention to the aspects of the vessel elements shown in Fig. 1-5 we find, typically, a close resemblance in shape and length between the vessel elements and the cambial cells. This observation supports the common concept that cambial cells differentiate into vessel members without further division. The principal change in their form results from an increase in width. Some vessel members, however, appear rather short when compared with the cambial cells. The cause of this appearance, especially as related to very wide vessel elements, needs further investigation.

Using the comparison of phloem in the 91 species of 77 genera

examined in the present study, one can group the possibilities with regard to anticlinal divisions as follows.

A. Anticlinal divisions none or infrequent

Examples:

Species examined in *Acer*, *Rhus*, *Berberis*, *Celastrus*, *Alnus*, *Quercus*, *Aesculus*, *Juglans*, *Lardizabala*, *Gymnocladus*, *Fraxinus*, *Pyrus*, *Prunus*, *Populus*, *Tilia*. The infrequent divisions are mostly transverse or slightly oblique and usually occur near rays. Such divisions were not seen in the observed species of a few genera (*Rhus*, *Cotinus*, *Laburnum*, *Grevillea*, *Cephalanthus*), but it is possible that further search would reveal them in these species also. Occasional vertical divisions occurred in species grouped under A.

B. Anticlinal divisions common or predominating

(1) Divisions mainly transverse or somewhat oblique. Examples: Species examined in *Buxus*, *Viburnum*, *Weigela*, *Cercidiphyllum*, *Clethra*, *Cornus*, *Rhododendron*, *Azara*, *Hypericum*, *Paulownia*, *Ailanthus*. The derivatives are usually sieve elements; combinations of sieve elements and parenchyma cells are infrequent.

(2) Divisions mainly vertical or strongly oblique. Examples: Species observed in *Annona*, *Asimina*, *Michelia*, *Paeonia*, and all 9 genera of Lauraceae used in this study. The derivatives may be sieve elements only or sieve elements and parenchyma cells; usually combinations of the two possibilities occur in the same species. Many species show transverse or slightly oblique divisions also. Some species (e.g. those seen in *Hedycarya* and *Peumus*) appear to have divisions in categories 1 and 2 with about equal frequency.

Among the 91 species studied 35 showed anticlinal divisions. Transverse to slightly oblique divisions occur in species with relatively short cambial initials (e.g. those in *Rhododendron*, *Hypericum*, *Paulownia*, all genera of Lauraceae) and in those with relatively long initials (e.g. those in *Cercidiphyllum*, *Clethra*, *Cornus*, *Hedycarya*, *Peumus*, *Hydrangea*). Similarly, longitudinal divisions occur in species with initials of various lengths. The transverse to somewhat oblique divisions always result in a sieve element shorter than its mother cell. The longitudinal divisions may have similar effects (cells marked x in Fig. 4B and 5B).

DISCUSSION

Our studies on the divisions in differentiating phloem were not extensive enough to reveal the full significance of these divisions, but they have been carried sufficiently far to consider the possible bearing of these divisions upon the problem of specialization in the phloem. There is, first, the question of the length of sieve elements with regard to the phylogenetic specialization of these elements. As is well known, the length of vessel members of the xylem has proved to be the most reliable and consistent measure of the degree of specialization in the secondary xylem (BAILEY, 1944; TIPPO, 1946; CHEADLE, 1953). The

progressive evolutionary shortening of the vessel members is correlated with the shortening of the fusiform cambial initials. One might assume that, since the sieve elements are ultimately derived from the same fusiform initials as the vessel members, the sieve elements would show a progressive evolutionary shortening similar to that found in the vessel members. Such an assumption has, of course, been made in the literature (see review by ESAU, 1950). The occurrence of divisions resulting in an ontogenetic shortening of sieve elements observed by us indicates that variations in the length of sieve elements cannot have the same relatively precise phylogenetic significance as do variations in the length of vessel elements.

The second aspect that might be considered with reference to the divisions described in this paper is that of the developmental and physiologic relation between the sieve elements and the various parenchyma cells in the phloem. The occurrence of divisions forming sieve elements and parenchyma cells from the same mother cell suggests that there are degrees of closeness of ontogenetic relation between the sieve element and the associated parenchyma cells. If the timing of the divisions is of any significance in this respect, one might assume that the companion cells, which are separated from the sieve element in the last stages of division in the phloem before it becomes functioning, are most closely related to the sieve elements. Then follow the parenchyma cells that are derived from the same mother cells as the sieve element. They appear to be more intimately associated with the sieve elements than the cells of the parenchyma strands derived from separate mother cells. (Compare parenchyma cells in A and B in Plate II). Frequently the parenchyma cells associated with the sieve elements are relatively small (Plate II, C and D) and sometimes can hardly be distinguished from companion cells. RESCH (1954) found in the primary phloem of *Vicia faba* that the sister cells of a sieve element vary in degree of development of characteristics usually associated with the companion cells in angiosperms. He suggests that there are degrees of specialization of cells as companion cells. Perhaps one might go further in suggesting that companion cells intergrade with phloem parenchyma cells in degree of physiologic specialization, whatever this specialization may be. Such a concept may be useful in contemplating the significance of the apparent absence of typical companion cells in *Austrobaileya* (BAILEY and SWAMY, 1949).

The third and last aspect that merits brief comment at this time is that of the relation between the structure and the function of the sieve element. Without referring specifically to any of the various theories of translocation we simply wish to point out that formulation of any concept of movement of materials in the phloem must take into account the fact that the ontogeny of the sieve element may involve changes, even to the point of introduction of new features, that specifically reduce the size of the conduit. We do not know with certainty whether, with reference to translocation, the sieve plates normally impede, expedite, or have any definitive effect on the movement through the

sieve elements. Whatever may be the role of the sieve plates, their presence, and particularly the tendency of some species to multiply their number during ontogeny, must be fully recognized by physiologists studying translocation. The morphologist, on the other hand, should be aware of the need for further critical study of structure of the sieve plates.

SUMMARY

Among 91 species in 77 genera of 44 families of dicotyledons surveyed in the present study 35 species showed evidence that various anticlinal divisions occur in the secondary phloem mother cells that produce sieve elements. These divisions range in orientation from transverse through oblique to vertical. The derivatives of these divisions consist of two or more sieve elements with their companion cells or of combinations of sieve elements, companion cells, and parenchyma cells. Frequently the divisions result in sieve elements shorter than their mother cells. The occurrence of these anticlinal divisions has a bearing upon the problem of phylogenetic and physiologic specialization of sieve elements.

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