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Studies on *Artemisia afra* Jacq.: The Phloem in Stem and Leaf

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Summary

The structure of the phloem was studied in stem and leaf of *Artemisia afra* Jacq., with particular attention being given to the sieve element walls. Both primary and secondary sieve elements of stem and midvein have nacreous walls, which persist in mature cells. Histochemical tests indicated that the sieve element wall layers contained some pectin. Sieve element wall layers lack lignin. Sieve elements of the minor veins (secondary and tertiary veins) lack nacreous thickening, although their walls may be relatively thick. These walls and those of contiguous transfer cells are rich in pectic substances. Transfer cell wall ingrowths are more highly developed in tertiary than in secondary veins.

Keywords: Anatomy; *Artemisia;* Cell wall composition; *Compositae;* Phloem; Sieve element.

1. Introduction

Artemisia afra Jacq. (*Compositae:* tribe *Anthemideae*) forms part of a group of plants that has adapted to and spread throughout most of the dry Eastern Cape region of the Republic of South Africa. In a previous, preliminary report on the anatomy of *A. afra* (Botha and Herman 1980), attention was drawn to the presence of thick-walled sieve elements in the phloem. Thick-walled sieve elements are fairly wide-spread among vascular plants, being found in angiosperms, gymnosperms, and vascular cryptogams (Esau 1969, Deshpande 1976, Evert and Eichhorn 1976, Evert, Eschrich and Heyser 1978, Warmbrodt and Evert 1979, and literature cited therein). The thickened walls of sieve elements have been the subject of considerable discussion and controversy, especially as regards their composition and functional significance. In some sieve elements, the walls become so thick that they almost occlude the lumina of the cells, a condition which would seemingly impede the ready flow of substances through such sieve elements.

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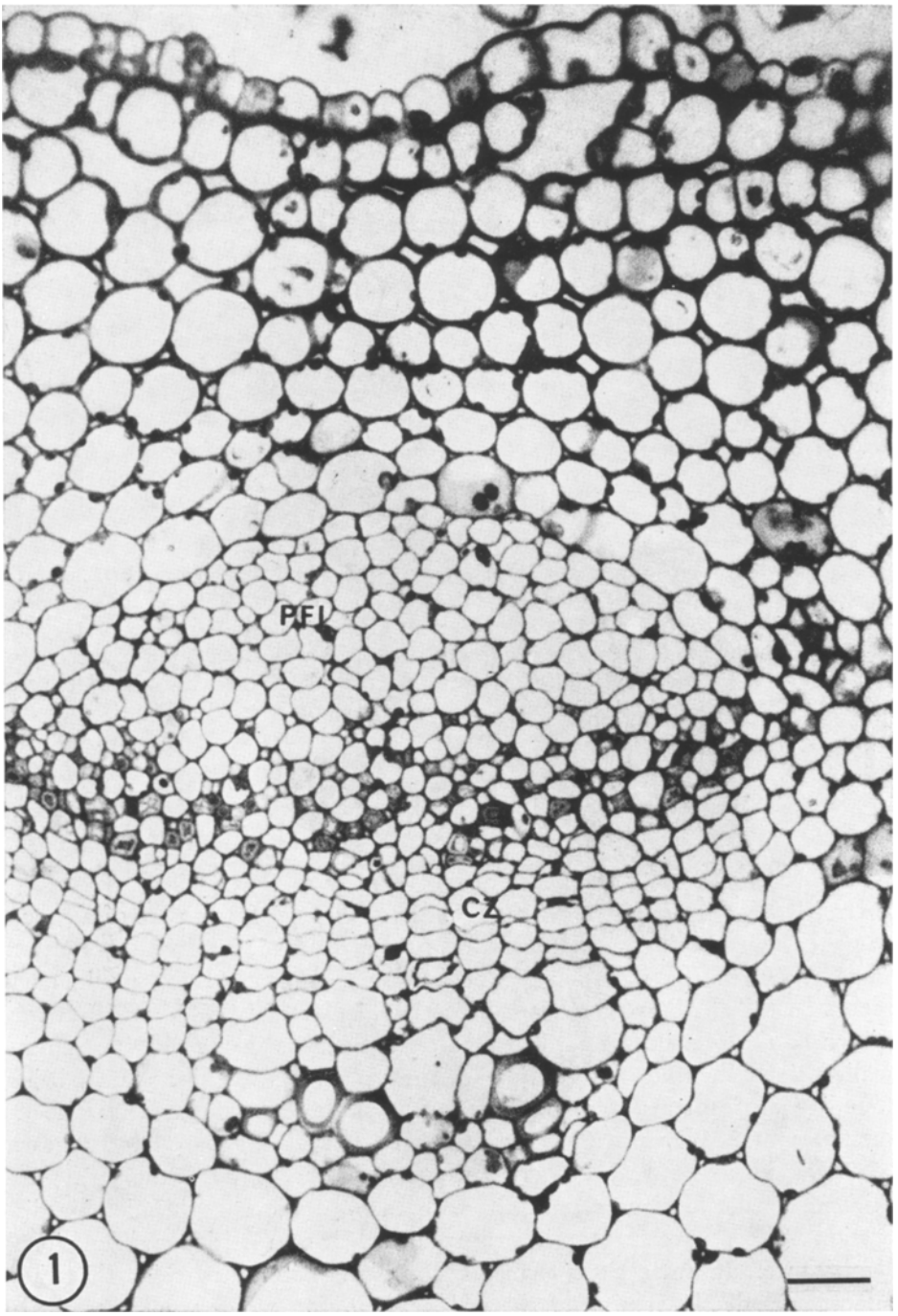

Fig. 1. Transection of young stem showing vascular bundle. Immature primary phloem fibers (*PFI*) cap the vascular bundle. A distinct cambial zone (CZ) separates remainder of primary phloem (metaphloem) from primary xylem, which is contiguous to parenchymatous pith. Bar = 50 μm

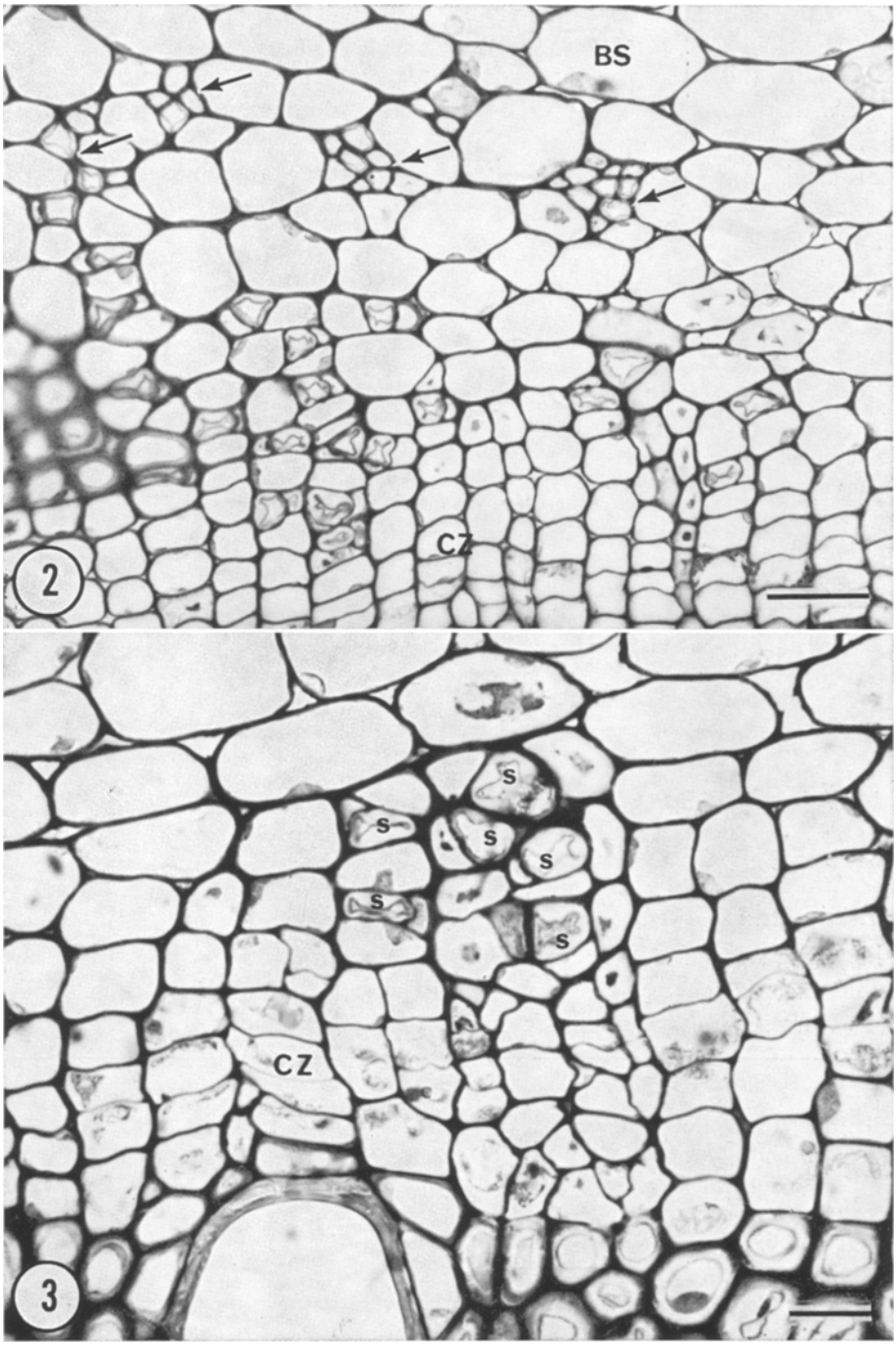

Fig. 2. Transection of interfascicular region of stem. Strands of primary phloem (arrows) are separated from secondary phloem by narrow parenchymatous zone. Both primary and secondary sieve elements have nacreous walls. Bar = 30 μm

Fig. 3. Detail of strand of immature secondary sieve elements (*S*) with nacreous walls. Bar = 15 μm

The aim of this study was twofold: first, to examine the phloem of *Artemisia afra* with the light microscope in an attempt to determine the nature of the sieve element walls; and second, if possible, to relate whatever might be learned about the sive element walls to their probable function.

2. Materials and Methods

Young and mature stem and leaf material of *Artemisia afra* Jacq. was collected on the University of Fort Hare experimental farm and transported immediately to the laboratory, where suitable pieces of stem and leaf material were excised in 0.1 M phosphate buffer. The material was then transferred to 4% glutaraldehyde in 0.1 M phosphate buffer and fixed at 5 °C overnight. The material was dehydrated in an alcohol series and propylene oxide. Embedment was in Spurr's resin (Spurr 1969).

2.1. Light Microscopy

Serial 0.5 to 2.0 µm sections were cut on an LKB Ultrotome II with glass knives. Sections were routinely stained in 0.005% toluidine blue O (Feder and O'Brien 1968). The following specific cytochemical tests were used to determine the nature and chemical composition of the cell walls: pectic substances, ruthenium red; Weisner test for lignins (phloroglucinol—HCl; Johansen 1940). Enhanced contrast of ruthenium red stained walls was obtained when a Zeiss green filter was placed in the HBO-200 mercury lamp pathway.
Selected sections were viewed and photographed using a Zeiss photomicroscope III fitted with bright-field and phase-contrast optics.

2.2. Electron Microscopy

Thin sections were cut with glass knives, stained in uranyl acetate followed by lead citrate and then examined and viewed using an Hitachi HU-11 B electron microscope.

3. Results

3.1. The Stem

The young stem of *A. afra* consists of a uniseriate epidermis, beneath which is a chlorenchymatous cortex 6 or more cells wide (Fig. 1). The vascular bundles are separated from one another by interfascicular regions of variable width,

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Figs. 4–8. Primary (Fig. 4) and secondary (Figs. 5–8) sieve elements from *Artemisia* stem material

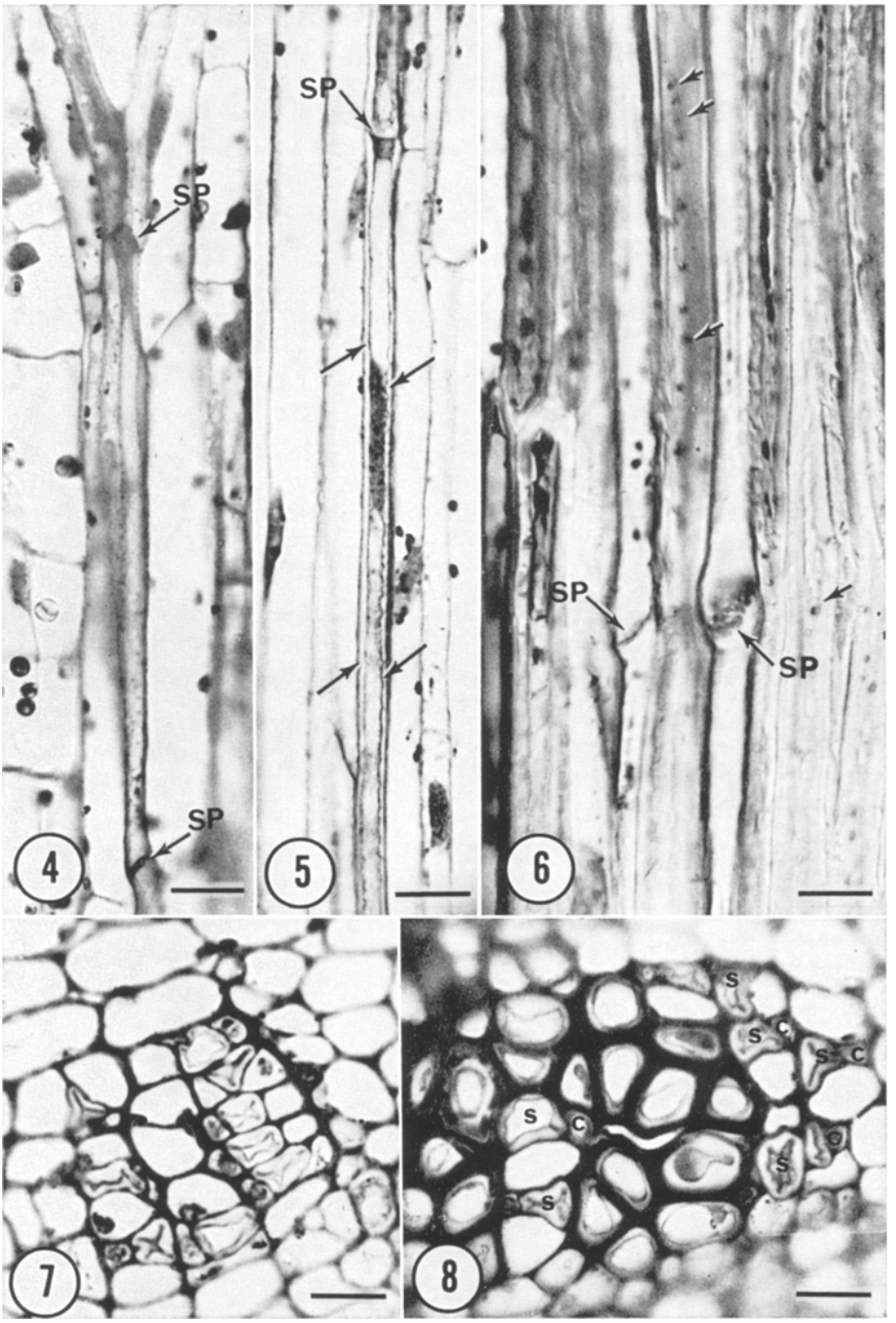
Fig. 4. Longitudinal view of metaphloem sieve-tube member with inclined sieve plates (*SP*)

Fig. 5. Longitudinal view of apparently immature, late-formed secondary sieve-tube member, with transverse sieve plate (*SP*). Unlabelled arrows point to nacreous thickening. Note P-protein body near upper pair of unlabelled arrows

Fig. 6. Longitudinal view of mature secondary sieve elements with inclined sieve plates (*SP*). Contiguous fibers are thick-walled and contain fairly conspicuous pits (arrowheads)

Fig. 7. Transection of similar-aged stem material as that shown in Fig. 5. The secondary sieve elements shown here have thick nacreous walls, some of which almost occlude the lumina of the cells

Fig. 8. Transection of similar-aged stem material as that shown in Fig. 6. Most sieve elements (*S*) and companion cells (*C*) are in direct contact with phloem fibers. Figs. 4–8, toluidine blue, bar = 15 µm



Figs. 4–8

and the pith is parenchymatous. An individual vascular bundle, capped by immature primary phloem fibers, is shown in Fig. 1. The remainder of the bundle phloem consists of thick-walled sieve elements and parenchymatous cells, including companion cells. A well-defined cambial zone (fascicular cambium) already separates the phloem from the xylem in this bundle.

In addition to the phloem of the vascular bundles, strands of primary phloem occur in the interfascicular regions (above in Fig. 2). As the stem grows older, interfascicular cambia develop between the vascular bundles and produce strands of secondary sieve elements along more or less the same radii as the interfascicular phloem strands. As can be seen in Fig. 2, the secondary sieve elements contain thick walls that are similar in appearance to those of the primary sieve elements.

Fig. 3 shows, at higher magnification than in Fig. 2, a portion of interfascicular cambium and associated vascular tissues. A single strand of thick-walled sieve elements can be seen external to the cambium and some xylem elements, including part of a large vessel, internal to it. Most of the cells external to the cambial zone in this section are immature.

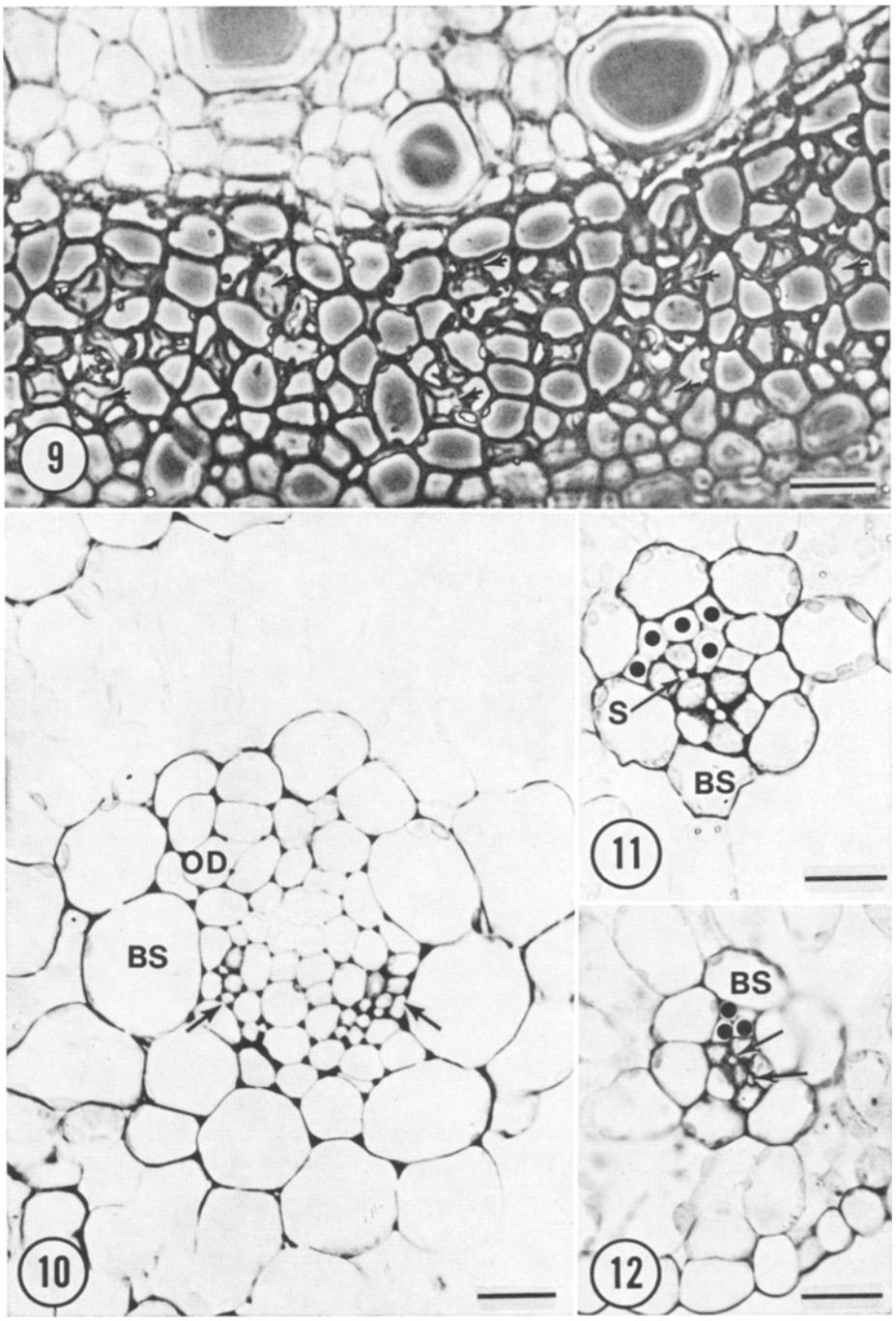
The walls of both primary and secondary sieve elements consist of two distinct parts: a relatively thin outer layer next to the middle lamella, and a more or less thick inner layer next to the cell lumen. Such inner wall thickenings are called nacreous thickenings, or nacreous walls (Esau 1969). In many angiosperms, the nacreous thickening of primary sieve elements is transitory in nature and becomes greatly reduced or disappears entirely as the cell ages. When that happens, the sieve element walls may become as thin as those of neighboring parenchyma cells. In the secondary phloem of dicotyledons, the behaviour of the nacreous thickening is variable, shrinking in some species and persisting in others as the tissue passes into a nonconducting, or non-functional, state (Esau and Cheadle 1958). In *A. afra*, the nacreous layer persists in both primary and secondary sieve elements of the stem, although in some primary sieve elements it undergoes a reduction with age.

Figs. 9–12. Transections of leaf material. Fig. 9, portion of midvein near base of lamina; Fig. 10, second order vein; Figs. 11 and 12, third order veins

Fig. 9. Phase contrast photomicrograph showing part of xylem and abaxially situated phloem. As in the stem, the sieve elements have nacreous walls (arrowheads). Toluidine blue, bar = 15 μm

Fig. 10. Photomicrograph of secondary vein with two phloem strands (arrows). This section and those of Figs. 11 and 12 were stained with ruthenium red to identify and localize pectic substances. *BS* bundle sheath, *OD* oil duct. Bar = 12.5 μm

Figs. 11 and 12. Photomicrographs of a tertiary vein near its junction with a secondary vein (Fig. 11) and closer to its terminal ending (Fig. 12). The walls of the tracheary elements (closed circles), with exception of the middle lamellas, showed little or no affinity for ruthenium red. The arrowheads in Fig. 12 point to sieve elements which are associated with transfer cells. *BS* bundle sheath. Bars = 12.5 μm



Figs. 9–12

Figs. 4–6 show primary (Fig. 4) and secondary (Figs. 5 and 6) sieve elements in longitudinal view. The sieve plates of both primary and secondary sieve elements (or more specifically, sieve-tube members) are simple, and either inclined (Figs. 4 and 6) or transverse (Fig. 5) in orientation. Metaphloem sieve elements—both fascicular and interfascicular in origin—ranged from 100 to 150 μm in length and 7 to 10 μm in width, whilst secondary sieve elements ranged from 180 to 220 μm in length and 10–15 μm in width.

The secondary sieve element of Fig. 5 and those shown in transection in Fig. 7 are from similar-aged portions of the stem. Unlabelled arrows point to the thickened sieve element walls in Fig. 5. Note the undispersed P-protein body near the upper pair of arrows in this apparently immature cell. Most of the cells bordering the sieve elements and companion cells in Figs. 5 and 7 are fiber primordia.

Figs. 6 and 8 show mature or nearly mature secondary phloem in longitudinal and transverse section, respectively. Secondary wall formation is underway in the fibers in these sections. In mature portions of the stem most primary and secondary sieve tubes appear as small groups surrounded by fibers. The spatial relationship of the sieve tubes and fibers can be seen in Figs. 6 and 8. Many sieve tubes are in direct contact with fibers.

3.2. The Leaf

The leaves of *A. afra* are pinnatisect. Each leaflet contains three order of veins more or less completely embedded in mesophyll tissue (ESAU 1977). In addition, the minor veins are enclosed by a layer of compactly arranged chloroplast-containing parenchymatous cells forming the bundle sheath.

Fig. 9 shows part of a primary vein, or midvein, which comprises the first vein order. The phloem of the midvein contains thick-walled sieve elements similar in appearance to those encountered in the stem. Examples of secondary and tertiary veins are shown in Fig. 10 and Figs. 11 and 12, respectively. Both secondary and tertiary veins may contain either one or two phloem strands on the abaxial side of the leaf. The secondary vein shown in Fig. 10 has two phloem strands (arrows) separated from one another by relatively large parenchyma cells, whilst the tertiary vein in Figs. 11 and 12 exhibits a single phloem strand. Both secondary and tertiary veins contain transfer cells (BOTHA and HERMAN 1980).

Figs. 11 and 12 are transections of the same tertiary vein. Fig. 11 depicts a portion of the vein near its junction with a secondary vein, and Fig. 12 a portion closer to its terminal ending. Tertiary veins terminate in tracheary elements completely enclosed by bundle-sheath cells. At the level shown in Fig. 11 the vein contains three sieve elements and six transfer cells. The cell to the left of the upper sieve element (S) is a transfer cell and the one above it, a vascular parenchyma cell. The two sieve elements below are surrounded by five transfer cells. At the level shown in Fig. 12 the vein contains two

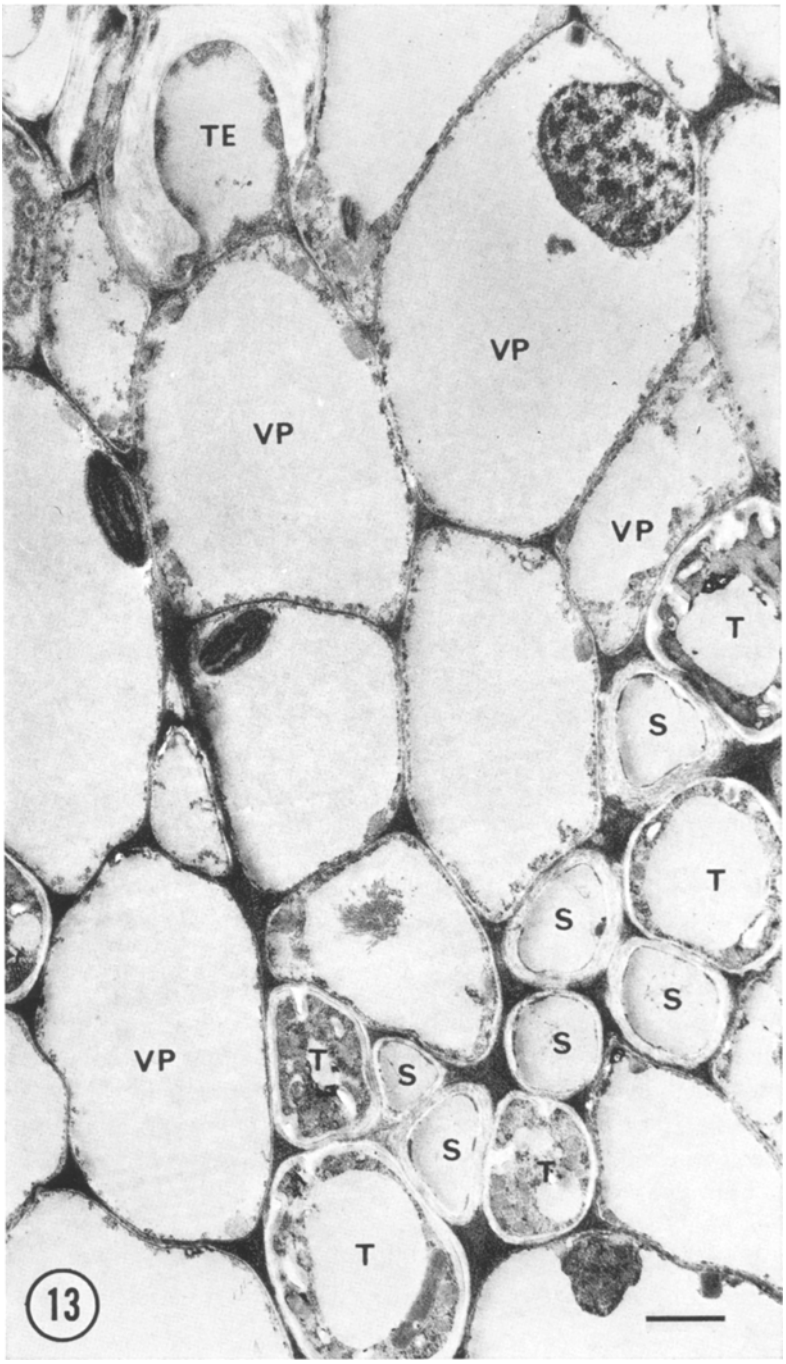


Fig. 13. Electron micrograph of part of secondary vein similar to that shown in Fig. 10. The sieve elements (*S*), which lack nacreous thickenings, are associated with transfer cells (*T*) and separated from the tracheary elements (*TE*) by vascular parenchyma cells (*VP*). Bar = 2.0 μm

sieve elements surrounded by five transfer cells, one of which abuts a tracheary element (arrowhead). In Fig. 11, three of the transfer cells abut tracheary elements.

Fig. 13 is an electron micrograph of part of a second secondary vein from the same leaflet as the one illustrated in Fig. 10. Four of the six sieve elements in Fig. 13 have relatively thick walls, but there is no distinction between outer and inner portions of the walls; that is, these sieve elements lack nacreous thickenings. Each of the sieve elements is associated with one or more transfer cells, which in the secondary veins have poorly developed wall ingrowths. Where the cells have rounded off, an electron dense substance occupies the regions that might otherwise have become intercellular spaces.

Fig. 14 is an electron micrograph of a minor vein similar to the tertiary order vein shown in Fig. 15. Each of the sieve elements shown here is in contact with at least two transfer cells. Most of the transfer cells in tertiary veins had prominent wall ingrowths. Some transfer cells had wall ingrowths on all walls. Such transfer cells, called A-type transfer cells, are regarded as companion cells (PATE and GUNNING 1969, 1972). Others—called B-type transfer cells, or phloem parenchyma cells—had wall ingrowths best developed opposite or nearest the sieve elements and their companion cells such as the lower right transfer cell in Fig. 14. One of the A-type transfer cells shown here (right, above) abuts both sieve element and tracheary element (arrows point to wall ingrowths opposite the tracheary element).

Although not encountered frequently, plasmodesmata were observed in the common walls between transfer cells and sieve elements with walls of variable thickness in secondary veins. No plasmodesmata were observed between transfer cells and sieve elements in tertiary veins, although this does not mean that such connections are lacking entirely.

3.3. Cell Wall Histochemistry

As mentioned previously, tissue sections were stained routinely, with toluidine blue O, a polychromatic stain used by many workers today to stain plastic embedded monitor sections used in conjunction with thin sections during investigations of plant cell ultrastructure. The value of this dye as a polychromatic stain for the investigation of plant cell walls was emphasized by O'BRIEN, FEDER, and MCCULLY (1964), who noted an excellent but not invariable correlation between the green or bluish green color developed by cell walls after staining with toluidine blue O and the presence of lignin.

Staining of *A. afra* stem and leaf sections with toluidine blue O yielded results similar to those reported by O'BRIEN *et al.* (1964): typically lignified cell walls—those of phloem fibers and xylem elements—stained a bluish green color. Moreover, the cell walls of similar cell types gave a positive reaction for the presence of lignin after staining with phloroglucinol-HCl. The walls of all other cell types, including sieve elements, companion cells, and transfer

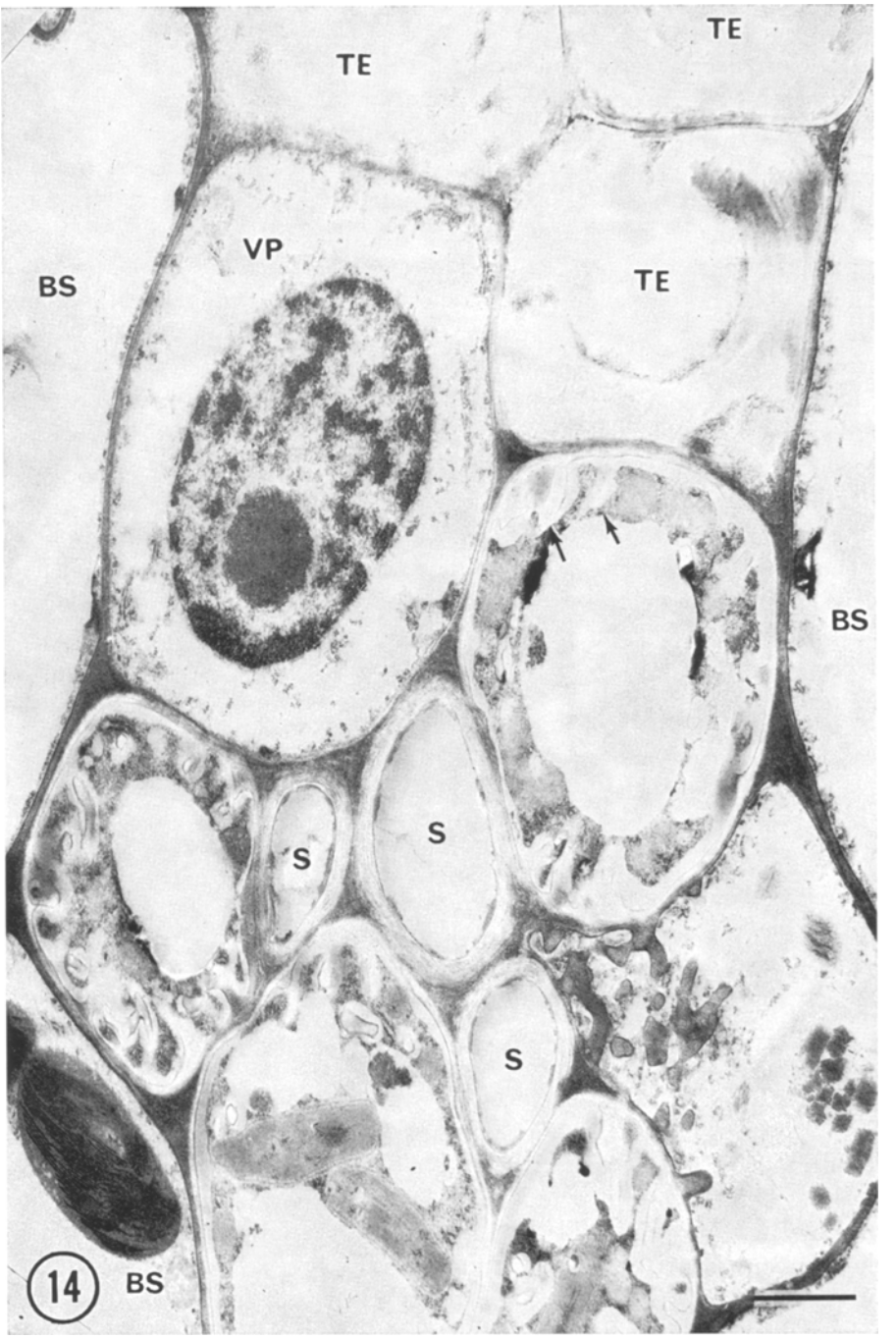


Fig. 14. Electron micrograph showing three sieve elements surrounded by a group of five transfer cells in tertiary vein. The sieve elements are separated from the tracheary elements (*TE*) by a vascular parenchyma cell (*VP*) on left, and a transfer cell on right. Arrows point to wall ingrowths in transfer cell adjacent to tracheary element. *BS* bundle sheath. Bar = 1.0 μm

cells, gave negative reactions for lignin. With the exception of the inner, or nacreous, thickening of the sieve elements in stems and midveins, the walls of all other phloem cells stained a red-purple color with toluidine blue O. The latter included the walls of minor vein sieve elements, of companion cells, transfer cells, and other parencymatous cells, as well as the outer wall layer of stem and midvein sieve elements. The nacreous thickening stained a pale pink-purple.

The pink to purple color shown by cell walls when stained with toluidine blue O is believed to be indicative of free carboxyl groups, and is usually ascribed to pectic substances (O'Brien *et al.* 1964, Smart and O'Brien 1979). The toluidine blue thus indicated the possible presence of a fairly high proportion of pectic substances in the walls of the phloem. The presence of pectic substances was confirmed with ruthenium red staining. The weakest staining reaction—a pale red-pink color—was encountered with both the outer and inner (nacreous) wall layers of stem sieve elements, whilst the most intensive reactions were found in the phloem of the minor veins, including sieve elements, vascular parenchyma cell, and transfer cell walls. The reactions of such walls were comparable in intensity to that obtained with stem collenchyma cell walls and the intercellular substance between phloem cells in stem and leaf.

4. Discussion

4.1. The Nacreous Thickenings

The histochemical tests carried out with bright-field and fluorescence microscopy indicated that the sieve element walls in the stem and leaf midveins of *A. afra* consisted of two distinct layers: an outer cellulosic, pectin-rich layer and an inner less cellulosic, pectin-poor layer, the nacreous layer. Neither layer contained lignin.

During their study of the nacreous walls in the secondary phloem of dicotyledons, Esau and Cheadle (1958) found it difficult sometimes to make this layer clearly visible in stained preparations. They noted that such results stood in sharp contrast with those obtained on the nacreous walls of young primary sieve elements, which often exhibited deep staining. (During the present study no perceptible differences were observed in the stainability of the nacreous thickening of primary and secondary sieve elements.) Esau and Cheadle (1958) reported positive reactions in tests for cellulose and pectin in both wall layers, with the nacreous layer giving a weaker pectin reaction than the outer layer. No difference was noted, however, in the degree of reactions for cellulose in the two layers. Neither layer contained lignin.

In addition to those of angiospermous sieve elements, the term nacreous wall has been applied to the wall thickenings in the sieve elements of *Pinus strobus*

(CHAFE and DOOHAN 1972) and to those of a variety of lower vascular plants (HÉBANT 1969, EVERT 1976, EVERT and EICHHORN 1976). Comparison of the wall thickenings in these diverse plant groups reveals that walls designated as nacreous are not alike in all plants (ESAU 1969, EVERT and EICHHORN 1976). For example, in *Cucurbita maxima* (DESHPANDE 1976) the nacreous wall has a distinct polylamellate structure, whereas in *Pinus strobus* (CHAFE and DOOHAN 1972) the walls are distinctly crossed polylamellate. In the polypodiaceous (leptosporangiate) ferns *Phlebodium aureum, Platycerium bifurcatum, Polypodium schraderi,* and *Microgramma lycopodioides* the nacreous thickening consists of a coarse fibrillar material arranged in a loosely woven network (EVERT and EICHHORN 1976), whilst in *Botrychium virginianum,* a eusporangiate fern, it has a polylamellate structure (EVERT 1976) not dissimilar to that in *Cucurbita.* In their study of the nacreous thickening in sieve elements of some polypodiaceous ferns, EVERT and EICHHORN (1976) found evidence which indicated that the nacreous thickening in these elements may serve as a lysosomal compartment or site of autophagic activity. In contrast, no such evidence was encountered during the study of nacreous thickenings in *Botrychium* (EVERT 1976).

HÉBANT (1969) has reported that amongst tropical ferns, nacreous wall development is dependent upon the type of organ, age of the sieve element, and the habitat. He found that ferns which go through periods of drought have well-developed nacreous walls, whilst aquatic types and those growing in regions of constant humidity lack such wall layers. Whilst it is realized that the nacreous walls in ferns may not be equivalent to those in higher vascular plants, HÉBANT's (1969) observations may have some bearing on the results reported herein in that *A. afra* also grows in areas which are subject to drought and it too contains sieve elements with nacreous thickenings. Experimental studies involving the growth of *A. afra* under different levels of relative humidity and of available water might shed some light on this problem.

4.2. The Walls in Minor Veins

Unlike the sieve elements of stem bundles and midveins, those of the minor veins in *A. afra* lacked nacreous walls. The thicker-walled sieve elements in the minor veins are reminiscent of the thick-walled sieve elements in small and intermediate bundles of certain grass leaves (EVERT 1980).

Considerable evidence has been amassed in support of the view that sucrose is loaded from the apoplast into the sieve tube-companion cell complex of minor veins (GEIGER 1975, 1976, GIAQUINTA 1976, EVERT, ESCHRICH, and HEYSER 1978). In the minor veins of *A. afra* both sieve element and transfer cell walls are rich in pectin or pectin-like substances. Perhaps the high pectin content of the sieve element walls and of those of contiguous cells facilitates solute transport in the phloem apoplast of the *A. afra* leaf.

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