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Ultrastructure of Minor Veins in *Cucurbita pepo* Leaves

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With 16 Figures

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Summary

The minor veins of *Cucurbita pepo* leaves were examined as part of a continuing study of leaf development and phloem transport in this species. The minor veins are bicollateral along their entire length. Mature sieve elements are enucleate and lack ribosomes. There is no tonoplast. The sieve elements, which are joined to each other by sieve plates, contain mitochondria, plastids and endoplasmic reticulum as well as fibrillar and tubular (190–195 Å diameter) P-protein. Fibrillar P-protein is dispersed in mature abaxial sieve elements but remains aggregated as discrete bodies in mature adaxial sieve elements. In both abaxial and adaxial mature sieve elements tubular P-protein remains undispersed. Sieve pores in abaxial sieve elements are narrow, lined with callose and are filled with P-protein. In adaxial sieve elements they are wide, contain little callose and are unobstructed. The intermediary cells (companion cells) of the abaxial phloem are large and dwarf the diminutive sieve elements. Intermediary cells are densely filled with ribosomes and contain numerous small vacuoles and many mitochondria which lie close to the plasmalemma. An unusually large number of plasmodesmata traverse the common wall between intermediary cells and bundle sheath cells suggesting that the pathway for the transport of photosynthate from the mesophyll to the sieve elements is at least partially symplastic. Adaxial companion cells are of approximately the same diameter as the adaxial sieve elements. They are densely packed with ribosomes and have a large central vacuole. They are not conspicuously connected by plasmodesmata to the bundle sheath.

1. Introduction

In recent years phloem physiologists have shown considerable interest in the mechanism of vein loading whereby sugars are transported into the leaf vascular system prior to their translocation. Part of this interest has been directed toward the description of minor vein ultrastructure (ESAU 1967, 1972, ESAU and HOEFERT 1971, GEIGER, MALONE, and CATALDO 1971, GUNNING, PATE, and BRIARTY 1968).

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As part of a continuing investigation into leaf development and phloem transport in *Cucurbita pepo* (TURGEON and WEBB 1973) the minor veins of this species have been examined with the electron microscope. The stem phloem of *Cucurbita* has long been a favorite material for the study of phloem structure and physiology. In addition the *Cucurbitaceae* are particularly interesting since it is the only known plant family in which the bicollateral structure of the vascular system extends into the minor veins. The results of a light microscope examination of the minor veins of *Cucurbita pepo* were reported by FISCHER (1885, *cf.*, ESAU 1969, p. 154).

2. Materials and Methods

Cucurbita pepo L. plants were grown in nutrient solution in the greenhouse at Carleton University as previously described (TURGEON and WEBB 1973) or in soil in the greenhouse at the University of Wisconsin. Freshly excised tissue from mature leaves was carefully cut into pieces about 1.5 mm^2 on a glass slide previously wetted with 0.05 M sodium cacodylate buffer pH 6.8. The pieces were immediately transferred to vials containing 6% glutaraldehyde in the same buffer. The samples were fixed, with gentle aspiration, for 12 hours at room temperature, washed in buffer 5 times at 30 minute intervals and post-fixed in 2% osmic acid in the same buffer overnight in the refrigerator. After fixation in osmic acid the tissue was warmed slowly to room temperature, washed in buffer for 10 minutes, dehydrated in a graded ethanol series over a period of 2 hours and embedded either in Epon-Araldite (MOLLENHAUER 1964) or SPURR (1969) resin. Sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Specimens were viewed and photographed with a Siemens Elmiskop 101 or a Hitachi HU-11 C microscope.
For light microscopy the material was embedded in epoxy resin and cut 1 μm thick with glass knives and stained with methylene blue. Views of unsectioned material were obtained from pieces of leaf cleared in 2.5% NaOH and stained with safranin in equal parts absolute ethanol and xylene (PRAY 1955).

3. Observations

3.1. The Minor Veins

The leaves of *Cucurbita pepo* have the reticulate pattern of minor venation characteristic of many dicotyledons (Fig. 1). Areoles are typically delimited by veins which contain a single file of xylem elements (Figs. 1 and 2). These were classified as seventh order veins by FISCHER (1885). It is these veins which are the object of the present study. Occasionally a lower order (larger) vein borders an areole on one side. Areoles are frequently traversed by veinlets which end blindly and may branch.
Minor veins are bounded by a single layer of bundle sheath cells (Fig. 2). There are no intercellular spaces within the vein or between the cells of the vein and bundle sheath (Fig. 2). Only the adaxial companion cell and occasionally the abaxial intermediary cells are exposed to the air space of the leaf. Apart from their direct contact with the minor vein the bundle sheath cells can not be distinguished morphologically from the mesophyll. The

walls of bundle sheath cells which abut the minor vein are usually found free of chloroplasts.

3.2. The Abaxial Phloem

In transverse section the cells of the minor veins are arranged in a highly regular pattern (Fig. 2). The abaxial phloem generally consists of 2 or 3 large specialized parenchyma cells and associated sieve elements. The parenchyma-

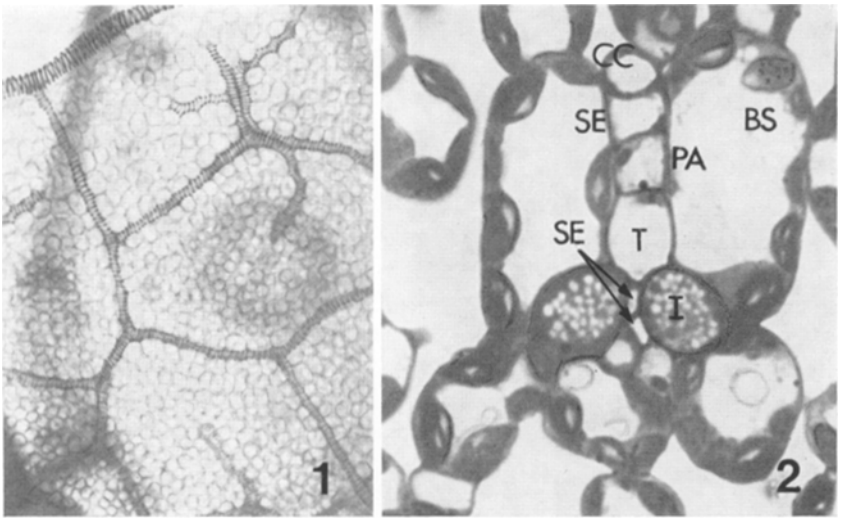

Fig. 1. Paradermal view of cleared leaf stained with safranin. Light micrograph. Minor veins are arranged in a reticulate pattern and contain a single file of tracheary elements. Dark shadows are out-of-focus trichomes. ×110

Fig. 2. Transection of minor vein. Light micrograph. Cells of the vein are arranged in a highly regular pattern. Cell types from the abaxial (lower) surface are: intermediary cells (*I*) and sieve elements (*SE*), tracheid (*T*), parenchyma cell (*PA*), adaxial sieve element (*SE*) and adaxial companion cell (*CC*). Vein is bounded by cells of the bundle sheath (*BS*). ×1,000

tous cells are probably companion cells in the ontogenetic sense and were termed intermediary cells (*Übergangszellen*) by FISCHER (1885) to emphasize their presumptive role in the exchange of photoassimilate between the mesophyll and sieve elements. Intermediary cells are much larger than the associated sieve elements (Fig. 2) in contrast to the companion cell/sieve element relationship in the shoot where the sieve element is of greater diameter. This transition in relative size occurs in the larger veins of the leaf and was quantitated by FISCHER (1885, *cf.*, ESAU 1969, p. 157). Terminal veinlets which end blindly within the areoles may contain only a single intermediary cell near the end. This single intermediary cell may or may not be accompanied by a sieve element.

Sieve elements in the minor vein abaxial phloem closely resemble those in the vascular bundles of the shoot except for their greatly reduced size. The mature sieve elements are enucleate and lack tonoplast and ribosomes (Figs. 3–6). Transversely oriented sieve plates interrupt the sieve tube conduit (Fig. 3). Although it is not possible to resolve the pores of the sieve plate in the light microscope they are plainly visible when viewed in paradermal section with the electron microscope. The sieve plates of sieve elements are so narrow that generally 1 or at most 2 pores are visible in a single paradermal section. The pores are narrow, lined with callose and, in the micrographs obtained in this study, plugged with P-protein (Fig. 3). Lateral sieve plates between sieve elements are common (Figs. 4 and 6).

Mature sieve elements contain fibrillar and tubular P-protein (Figs. 3–6). Most of the P-protein is tubular and is aggregated into discrete bodies. The tubules measure 190–195 Å in diameter. In contrast the fibrillar P-protein is dispersed in mature sieve elements. The distribution of both fibrillar and tubular P-protein is generally entirely parietal (Fig. 13).

The aggregation of the tubular P-protein in the minor vein sieve elements is similar in appearance to the 240 Å tubular P-protein described by CRONSHAW and ESAU (1968 a) and EVERT *et al.* (1973) and termed P 1-protein by the former authors. The presence of a smaller diameter (180 Å) tubular form termed P 4-protein by CRONSHAW and ESAU (1968) could not be demonstrated in the minor veins. In certain cases however some tubular P-protein of the minor veins appears superficially similar, in its less orderly arrangement, to P 4-protein (Fig. 6). However these tubules also measure 190–195 Å in diameter.

The plasmalemma of mature sieve elements is intact and is continuous from cell to cell through the pores (Fig. 3). Numerous membranes, which in section often appear as concentric pairs, occupy the lumen of the sieve tube (Figs. 3–6). Cisternae of endoplasmic reticulum (ER) lie in a single layer closely appressed to the plasmalemma where they form a parietal network (Fig. 4). Occasionally the ER is repeatedly folded to form a stack of membranes.

Plasmodesmata connect sieve elements to intermediary cells (Fig. 5). Where plasmodesmata occur the cell walls of both the sieve element and intermediary

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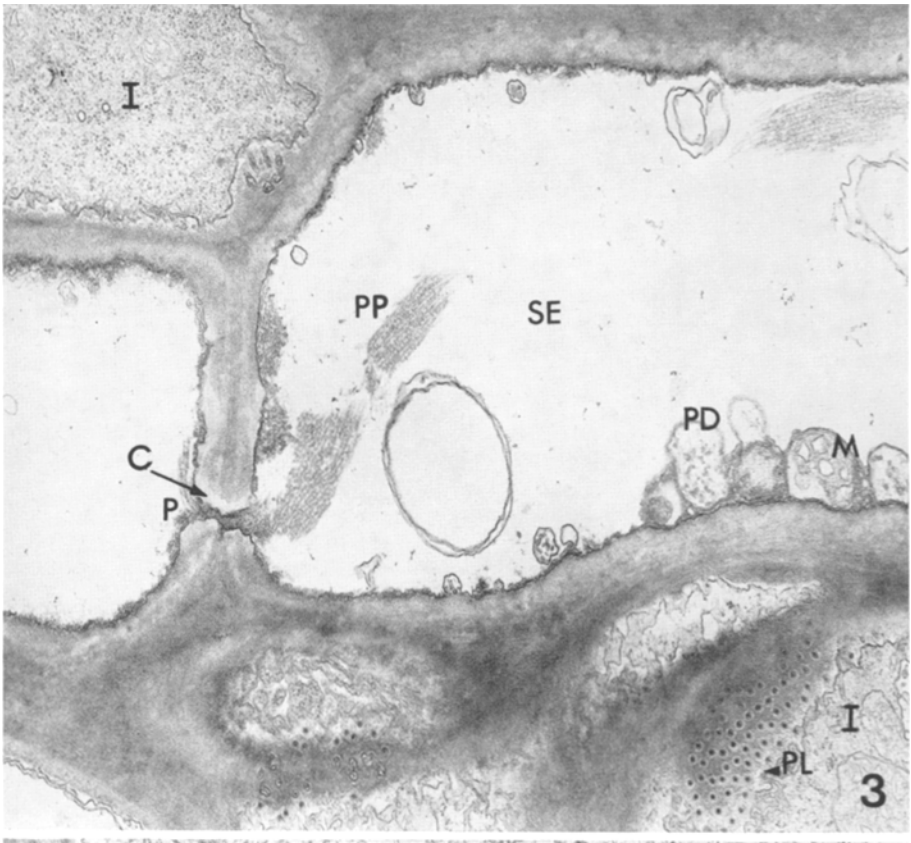
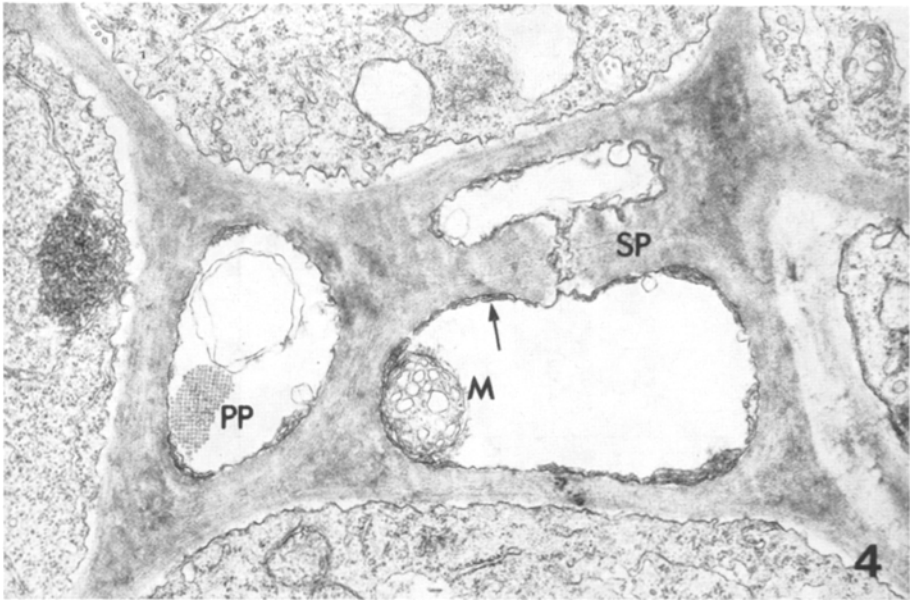
Fig. 3. Paradermal section of minor vein abaxial sieve elements (*SE*) and intermediary cells (*I*). Pore (*P*) in the sieve plate is plugged with P-protein (*PP*). Intermediary cells are connected to bundle sheath cells by numerous plasmodesmata (*PL*). Mitochondrion at *M*; plastid at *PD*; callose at C. ×22,300

Fig. 4. Transection of three abaxial sieve elements and surrounding intermediary cells. A P-protein body (*PP*) composed of tubular P-protein is present in one sieve element. Two sieve elements are connected by a lateral sieve plate (*SP*). Single profiles of endoplasmic reticulum (arrow) lie closely appressed to the plasmalemma. Mitochondrion at *M*. ×20,900





Figs. 3 and 4

cell, especially the intermediary cell, are thickened (Fig. 5). Plasmodesmata are single on the sieve element side and branched on the intermediary cell side.

The mitochondria of mature sieve elements, in contrast to those of other cell types, are always rounded in appearance (Fig. 4). They are slightly smaller than those of associated nucleate cells and the matrix is more electron dense. There is an abundance of internal membranes and the cristae appear swollen. Sieve element plastids are not as numerous as mitochondria. The plastids are bounded by a double membrane, internal membranes are scarce or absent, and the matrix is extremely sparse (Figs. 3 and 5). Plastids are approximately the same size or slightly smaller than mitochondria in the same cell. No starch granules were seen in plastids of mature sieve elements.

The cytoplasm of intermediary cells contains numerous free ribosomes and a dense groundplasma (Figs. 3–5 and 7). Mitochondria are particularly numerous and are situated, for the most part, in a parietal position (Fig. 7) although they are not excluded from the interior of the cell by a large central vacuole. Many small vacuoles are dispersed throughout the cytoplasm (Fig. 7). The contour of the vacuoles is irregular although this may be an artifact of tissue preparation. The degree of interconnection, if any, between vacuoles is unknown. Very few plastids are present in intermediary cells. There are no grana but starch may be formed.

Numerous plasmodesmata traverse the adjacent walls of intermediary and bundle sheath cells (Figs. 7–9). These plasmodesmata occur in clusters at points where the walls are slightly thickened. Over 300 plasmodesmata have been counted in a single cluster.

Intensive symplastic connection to the bundle sheath is characteristic of intermediary cells. In the abaxial phloem of the larger fourth and fifth order veins, where large intermediary cells and typical companion cells are found together, the intermediary cells occupy a position at the periphery of the phloem adjacent to the cells of the bundle sheath (Fig. 9). As in the smaller minor veins intermediary cells and bundle sheath cells are joined by numerous clusters of plasmodesmata (Fig. 9).

3.3. The Adaxial Phloem

The adaxial phloem of sixth and seventh order veins contains a single sieve element and companion cell (Fig. 2). The companion cell differs markedly

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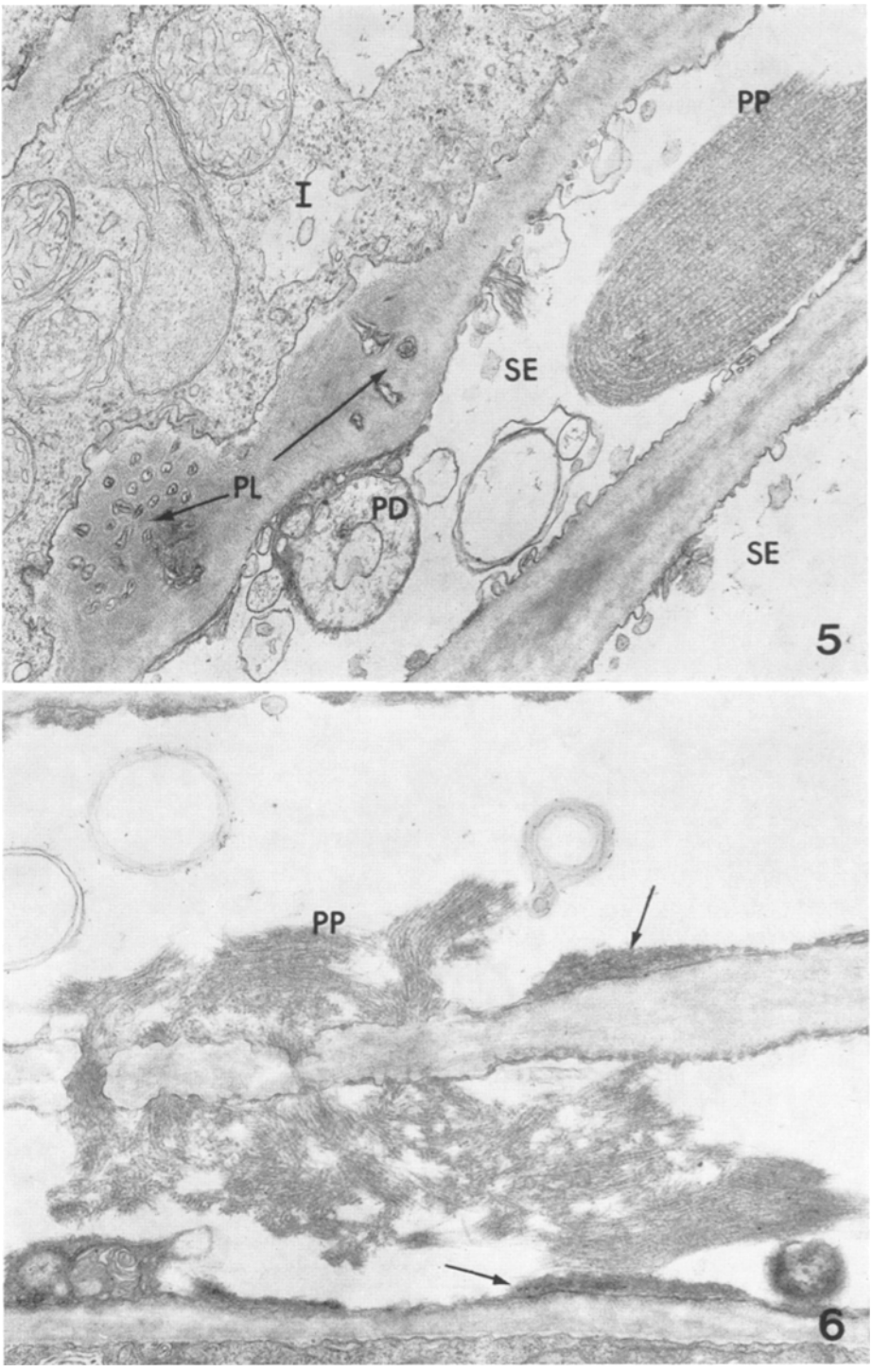
Fig. 5. Paradermal section of two abaxial sieve elements (*SE*) and an intermediary cell (*I*). Tubules of P-protein (*PP*) are aggregated into a discrete body. Branched plasmodesmata (*PL*) connect the intermediary cell and a sieve element. Plastid at *PD*. ×29,400

Fig. 6. Paradermal section of two abaxial sieve elements joined by a lateral sieve plate the pores of which are plugged with tubular P-protein (*PP*). Fibrillar P-protein (arrows) lies closely appressed to the plasmalemma. ×21,800



Figs. 5 and 6

in appearance from the intermediary cells of the abaxial phloem and in certain respects the sieve elements of the adaxial and abaxial phloem also differ. Adaxial sieve elements are much wider in diameter than abaxial sieve elements and are approximately equal in diameter to the adaxial companion cells (Fig. 2).

The sieve pores of adaxial sieve elements are wide enough to be seen in the light microscope and are clearly unobstructed in electron microscope views (Fig. 10). Callose is either absent or forms only a thin boundary layer at the edge of the pores.

P-protein bodies, both tubular and fibrillar, remain undispersed in mature adaxial sieve elements (Figs. 12 and 13). Fibrillar P-protein is more abundant than in sieve elements of the abaxial phloem. Tubules of P-protein measure 190–195 Å in diameter. In general, the P-protein is parietal in position although it is sometimes displaced toward the sieve plates (Fig. 10), probably as a result of pressure release when the sieve tube is cut during tissue preparation.

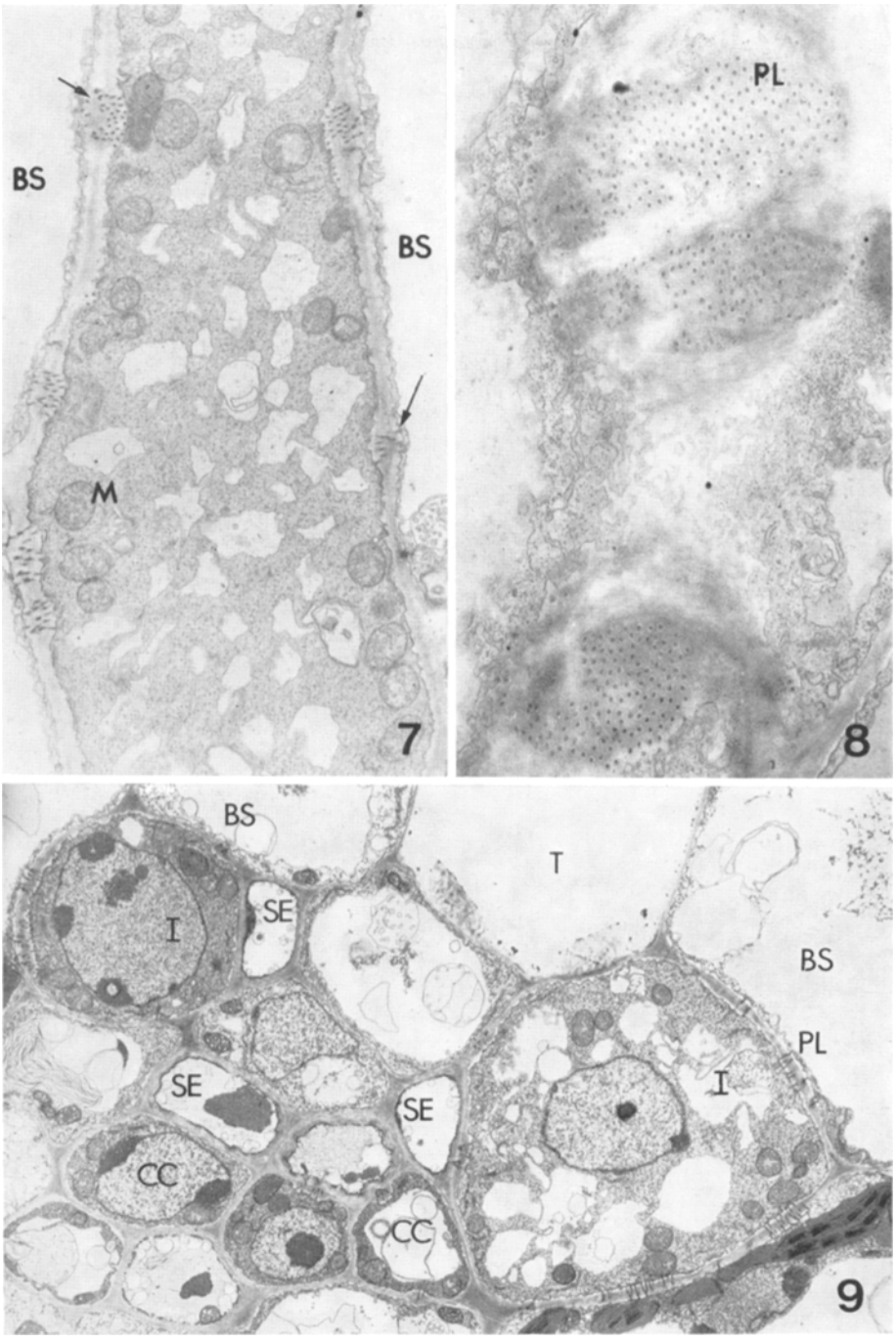
Mitochondria and plastids (Fig. 11) resemble their counterparts in the sieve elements of the abaxial phloem. In mature adaxial sieve elements the plasmalemma is intact and extends through the pores (Fig. 10). Membranes are common in the cell lumen.

Adaxial companion cells resemble in appearance the companion cells found in vascular bundles of the shoot. A large percentage of the cell volume is occupied by a central vacuole and the cytoplasm is extremely dense (Figs. 2 and 13–15). This density is primarily due to closely packed ribosomes (Fig. 15). Mitochondria are numerous but are degenerate in appearance; the matrix is sparse and there are few internal membranes (Fig. 15). Plastids are large and without grana but often produce starch granules. The cytoplasm also contains large osmiophilic bodies (Figs. 14 and 15), dictyosomes and dictyosome derived vesicles (Figs. 13–15). The companion cell and sieve element are connected by plasmodesmata which are branched on the companion cell side (Fig. 13). Plasmodesmata also connect cells of the palisade mesophyll and bundle sheath with the adaxial companion cell. These plasmodesmata are not nearly as numerous as those between bundle sheath cells and intermediary cells in the abaxial phloem.

Fig. 7. Paradermal section of an intermediary cell and two bundle sheath cells (*BS*) in the abaxial phloem. Numerous clusters of plasmodesmata (arrows) join these two cell types. The mitochondria (*M*) in the intermediary cell are distributed parietally. ×9,100

Fig. 8. Longitudinal section through the common walls of an intermediary cell and a bundle sheath cell. The wall is traversed by clusters of plasmodesmata (*PL*). ×14,400

Fig. 9. Transection of the abaxial phloem of a fifth order vein. Companion cells (*CC*) and larger intermediary cells (*I*) are both present. Clusters of plasmodesmata (*PL*) join the intermediary cells to the bundle sheath cells (*BS*). Tracheary element at *T*; sieve elements at *SE*. ×3,800



Figs. 7–9

3.4. The Xylem

Elements of the minor vein xylem appear, in paradermal section, to be tracheids although wall perforations may have gone undetected. Secondary wall thickenings are deposited in a helical pattern (Fig. 1). Tracheids are devoid of cytoplasm at maturity although terminal tracheids in blind endings are often filled with a dense substance which has an amorphous appearance in electron micrographs (Fig. 16). The primary wall of mature tracheids is hydrolyzed except where protected by secondary wall (Fig. 16).

3.5. Parenchyma

A single, highly vacuolate, parenchyma cell is situated between the adaxial phloem and xylem of minor veins while the abaxial phloem abuts directly into the xylem (Fig. 2). In larger minor veins one or more parenchyma cells may be present in the xylem or between the xylem and the abaxial phloem. Parenchyma cells contain mitochondria which are of similar size and appearance to those of the mesophyll cells. Chloroplasts in the parenchyma are slightly smaller than those of the mesophyll but grana and starch are present. Occasional plasmodesmata connect the parenchyma cell(s) to bundle sheath cells.

4. Discussion

Minor veins constitute the interface between the photosynthetic and vascular systems of the leaf, a specialization which is reflected in the ultrastructure of the component cells. The large, densely staining parenchymatous cells of the abaxial phloem, which FISCHER (1885) named intermediary cells, could be engaged in the active uptake of photosynthate from the mesophyll. This function is suggested by a number of structural and ultrastructural features. The intermediary cells are much larger than associated sieve elements and they contain numerous mitochondria which lie close to the plasmalemma perhaps cooperating in an active membrane process. The common wall

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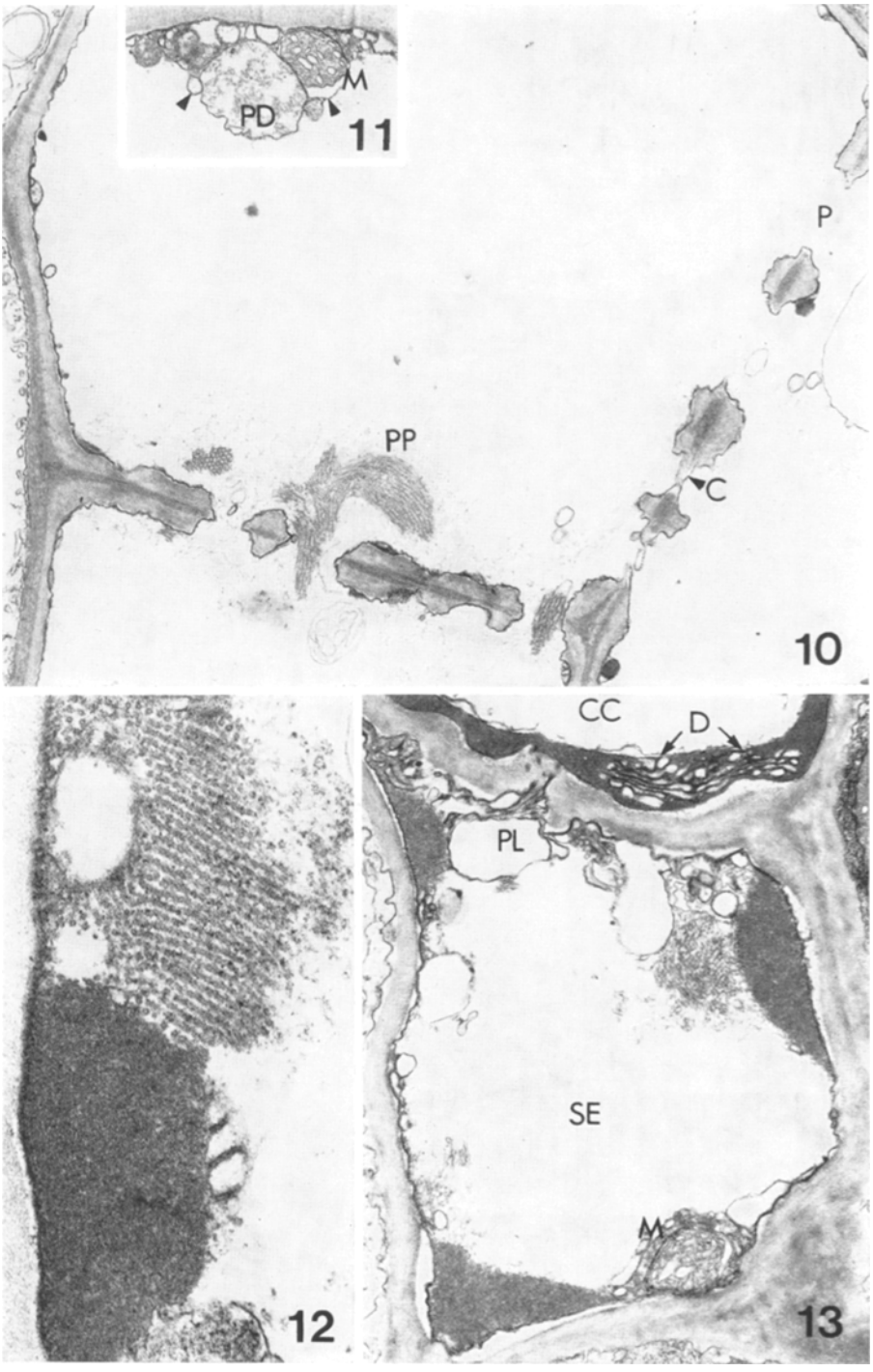
Fig. 10. Paradermal section of adaxial sieve elements. P-protein (*PP*) extends through some pores (*P*); the rest are open, Callose at *C*. ×16,100

Fig. 11. Plastid (*PD*) and mitochondrion (*M*) in adaxial sieve element. The outer membrane of the plastid has been broken and folded back (arrow heads). ×12,700

Fig. 12. Paradermal section through 2 protein bodies, one composed of fibrillar the other of tubular P-protein. ×42,400

Fig. 13. Transection of adaxial sieve element (*SE*) and companion cell (*CC*) showing the parietal distribution of P-protein and cell organelles in the sieve element. The plasmodesmata (*PL*) which connect the two cells is branched on the companion cell side. Mitochondrion at *M*; dictyosomes at *D*. ×16,900



Figs. 10–13

between intermediary cells and bundle sheath cells is traversed by an unusually large number of plasmodesmata which suggests that transport takes place along a symplastic pathway between these two cell types.

In larger veins where intermediary cells and more typical companion cells are found together, the intermediary cells are still observed adjacent to bundle sheath cells and connected to them by characteristic clusters of plasmodesmata. If these intermediary cells are actively involved in the accumulation of sugar from the mesophyll tissue then they may be used as indicators of where enhanced vein loading occurs. On this assumption the most functional region in terms of vein loading within the minor veins of *C. pepo* would be the abaxial phloem. Clearly, it would be of interest to pursue these possibilities. Reports on the ultrastructure of minor veins have not adequately described the connections between companion cells and bundle sheath cells (ESAU 1967, ESAU and HOEFERT 1971, GEIGER and CATALDO 1969). In those species in which phloem parenchyma is specialized by extensive cell wall ingrowths, *i.e.*, the transfer cells (GUNNING, PATE, and BRIARTY 1968), there are no symplastic connections between parenchyma and bundle sheath cells in the minor veins. This contrasts with our observations of *C. pepo* and suggests that both apoplastic and symplastic pathways exist for sugar transport across the bundle sheath/phloem parenchyma boundary the relative importance of either depending on the species. GEIGER, MALONE, and CATALDO (1971) proposed a partially apoplastic route of solute movement in *Beta vulgaris* on the basis of ultrastructural evidence. In *C. pepo* a complete symplastic route is available from the cells of the mesophyll to the sieve elements. This finding does not of course exclude the possibility of apoplastic movement through the matrix of the cell walls during movement of sugar from the mesophyll to the sieve elements. This type of microscopic examination can only resolve the pathways in a qualitative way. A quantitative study of function can only be approached through some form of kinetic experimentation. This is an important but elusive part of our understanding of translocation and warrants further attention.

Conducting cells of the minor veins appear to be normal sieve elements except for their greatly reduced size. This confirms an established pattern for those species which have been critically examined (ESAU 1967, ESAU 1972,

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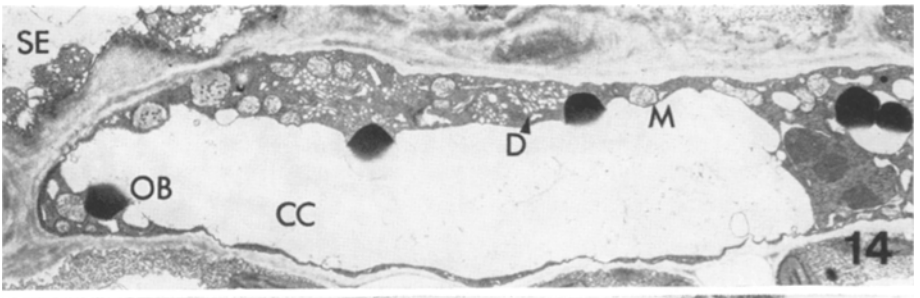
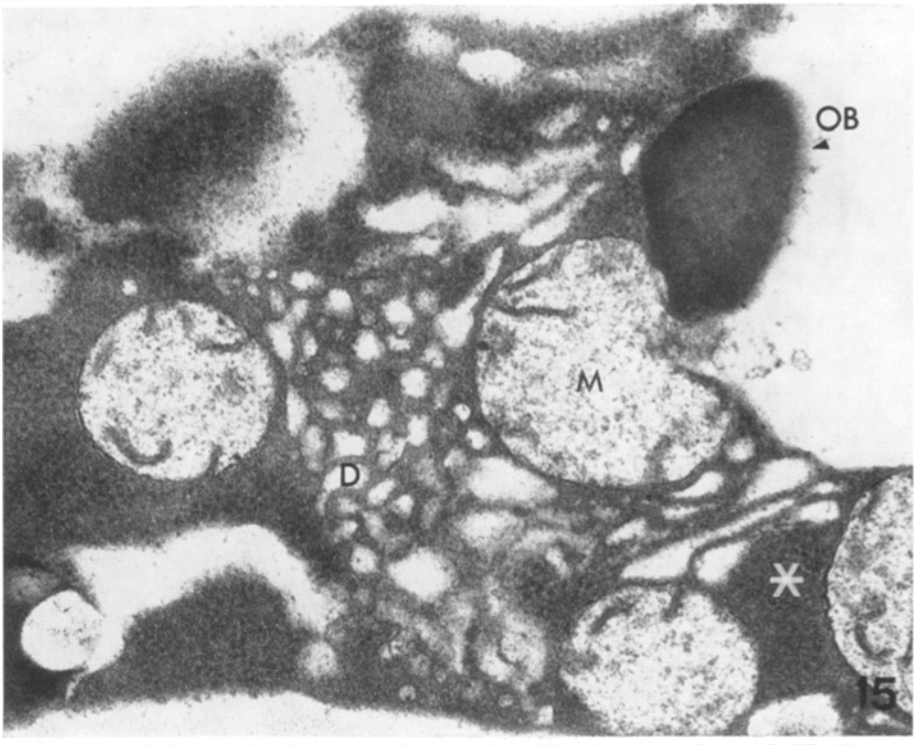
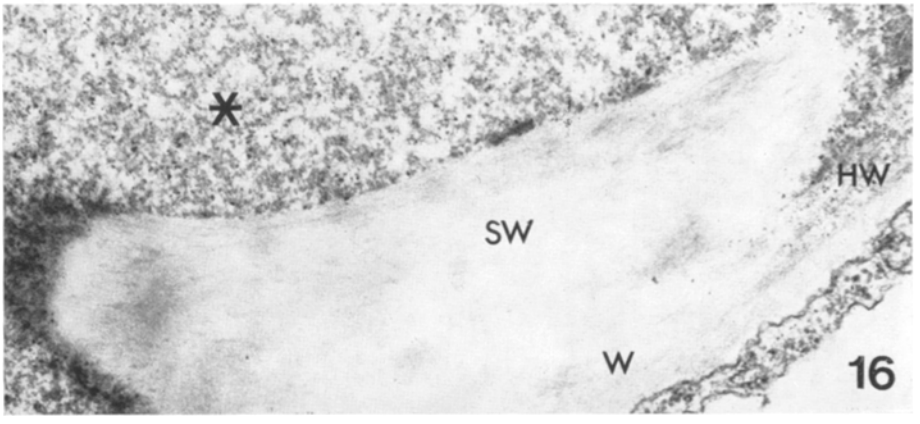
Fig. 14. Paradermal section of adaxial companion cell (*CC*) and sieve element (*SE*). Mitochondrion at *M*; dictyosome at *D*; osmiophilic body at *OB*. ×5,200

Fig. 15. Higher magnification of an adaxial companion cell showing densely compacted ribosomes (*). Mitochondrion at *M*; osmiophilic body at *OB*; dictyosome vesicles at *D*. ×49,600

Fig. 16. Transection of a mature minor vein tracheid filled with amorphous, electron dense material (*). The primary wall (*W*) of the tracheid has been hydrolyzed (*HW*) except where protected by secondary wall (*SW*). ×104,300







Figs. 14–16

ESAU and HOEFERT 1971, GUNNING, PATE, and BRIARTY 1968, GEIGER, MALONE, and CATALDO 1971). The appearance in electron micrographs of the sieve plates of *Cucurbita* minor veins may provide a further clue to the nature of the pores in their functional state. The sieve pores of the abaxial sieve elements observed in this study were plugged with P-protein. However the pores of the adaxial sieve elements were clearly free of obstruction. Since no special precautions were taken to prevent pressure release upon cutting it is most likely that the very narrow pores of abaxial sieve elements were rapidly plugged with dispersed P-protein during tissue preparation while the wider pores of the adaxial sieve elements remained open. Results of a recent study on P-protein distribution in sieve elements of the hypocotyl in *C. maxima* support this view (EVERT *et al.* 1973). If this interpretation is correct it does not lend support to the hypothesis that sieve-plate pores are normally plugged and that material in the pores aids an electroosmotic mechanism of phloem transport as postulated by SIDDIQUI and SPANNER (1970). However the problem of whether the adaxial or abaxial phloem or both are major routes of sugar transport in the minor veins has still to be resolved. Evidence for an equal facility of sugar transport by both the internal and external phloem of petiole and stem tissue of *C. pepo* has already been clearly established (WEBB and GORHAM 1965).

The distribution of P-protein in sieve elements is essentially parietal and the lumen of the cells appears empty except for occasional membranes. It is difficult to reconcile the presence of membranes with a mass flow of solute unless the membranes are attached to a solid structure. The presence of concentric pairs of membranes suggests, in fact, that they are extensions of the ER which normally lies closely appressed to the plasmalemma. It is not clear whether these membrane extensions are present in functional sieve elements or are dislodged during tissue preparation.

As mentioned above the adaxial phloem does not appear to be specialized for active uptake of photosynthate. On the other hand the adaxial sieve elements demonstrate wide open sieve pores that are not occluded with definitive callose and the internal structure of the elements show some evidence of displacement characteristic of pressure release upon cutting. It has been reported that excised phloem of stem tissue is capable of taking up sucrose from the free space (BIELESKI 1966). Perhaps the adaxial phloem of the minor veins of *Cucurbita* functions in a similar capacity while the bulk of the photosynthate is loaded into the abaxial phloem via the intermediate cells. The degenerate appearance of mitochondria in the adaxial companion cells suggests that the function of these cells is impaired. However this degeneration might also be due to the release into the cytoplasm of hydrolytic substances during fixation. O'BRIEN and THIMANN (1967) reported a similar disorganized structure of the mitochondria in the companion cells of the *Avena* coleoptile.

Sieve elements of the adaxial phloem show a marked resemblance to extrafasicular sieve elements of the stem. It is unlikely that there is a physical connection between these cells. More likely is the suggestion of CRONSHAW and ESAU (1968) that "dispersal or non-dispersal of the P-protein bodies can be related to the positions of the sieve elements relative to the vascular bundles". Perhaps the abaxial phloem and the xylem of the minor veins should be regarded as a collateral vascular bundle and the adaxial phloem to be extrafasicular vascular tissue separated from the vascular bundle by a parenchyma cell. This would explain the apparent dissimilarity between the *Cucurbitaceae* and other plant families in which the bicollateral bundles of the shoot and major veins become collateral in the minor veins.

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