THE TRANSVERSE VEINS OF THE WHEAT LEAF

By J. Kuo,* T. P. O'Brien,* and S.-Y. Zee†

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Abstract

The histology and fine structure of the transverse veins that connect the longitudinal veins in the leaf of wheat (*Triticum aestivum* L. cv. Heron) are illustrated and discussed. The transverse veins consist of a single sieve tube and vessel, and two files of vascular parenchyma cells. They lack the mestome sheath and parenchyma sheath that is present around longitudinal veins except where they make connection with longitudinal veins. The vascular elements of the transverse veins are connected to metaxylem and metaphloem of the longitudinal veins and these connections are illustrated in sections. The tracheary elements have an unusual pattern of wall thickening. Experiments with ferric chloride solutions reveal that the vessel wall may severely restrict passage of solutes from the transpiration stream to the free space of the mesophyll.

I. Introduction

Recently, O'Brien and Carr (1970) demonstrated the presence of a suberized lamella in the walls of the mestome sheath of wheat leaves and proposed that this layer might provide a barrier to passive flow of solutes and water between the mesophyll and vascular tissues. However, it has been known since the observations of Duval-Jouve (1875) that the longitudinal veins of the leaves of many grass species are interconnected by a series of very small transverse veins. The presence of these transverse veins raises a number of questions. What types of vascular element are present in transverse veins? Do the transverse veins possess a mestome sheath? How are their vascular elements connected to those of the longitudinal veins? What happens to the mestome sheath of a longitudinal vein at junctions with transverse veins? Although minor veins of dicotyledonous leaves have received some attention in recent years (see, for example, Esau 1965, 1967; Esau and Hoefert 1971; Gunning, Pate, and Briarty 1968; Trip and Colvin 1970), the transverse veins of grass leaves have been studied very little. Most workers (Arber 1934; Moreland and Flint 1942; Kaufman 1959) have been concerned only with the origin of these veins, paying little attention to their structure at maturity. In wheat, Percival (1921, p. 58) states that

> "the very fine anastomosing bundles, which cross from one parallel bundle to another, consist of short tracheids about 6μ in diameter, with parallel, thin-walled parenchymatous cells about 4μ across accompanying them."

A study of the vascular system of the wheat leaf by Didehvar (1970) adds no new information on this point but a recent study by Blackman (1971) has elucidated

* Botany Department, Monash University, Clayton, Vic. 3168. (Address for reprints.)

† Botany Department, University of Hong Kong, Hong Kong.

several aspects of the transverse veins in wheat. Working with *Triticum aestivum* L. cv. Thatcher, she has shown in a study devoted chiefly to the development of these veins, that they originate by two tangential divisions from a single layer of unexpanded mesophyll cells. She shows that these veins consist at maturity of single files of sieve elements and tracheary elements, and two files of parenchyma cells, which, despite their vascular origins, she believes may be functionally similar to the parenchyma sheath cells of the longitudinal veins. She points out that the transverse veins lack a mestome sheath.

In this paper, we are concerned chiefly with the manner in which connection is effected with the longitudinal veins, the behaviour of the mestome sheath at the junction, and the structure of the vascular elements. Our findings confirm and extend by electron microscopy most of Blackman's observations but our interpretation of the structure of the tracheary elements and vascular parenchyma cells does not support her views.

II. Materials and Methods

Triticum aestivum L. cv. Heron was grown in the glasshouse. Samples of leaf tissue were taken from the region 10–20 mm from the base of the blade of the third leaf of plants at the three-leaf stage of growth. Tissues were fixed for 2·5 hr in 6% glutaraldehyde in 0·025M phosphate buffer at pH 6·8, rinsed in buffer, post-fixed with 2% OsO_4, dehydrated in ethanol, and embedded in epoxy resin (Spurr 1969). Serial paradermal and transverse sections were cut using glass or diamond knives. Sections 0·5–1 μm thick were stained either with 1% toluidine blue O in 1% sodium borate or by the periodic acid–Schiff's (PAS) reaction, or both. At appropriate intervals, adjacent sections were collected for electron microscopy, stained with uranyl acetate and lead citrate, and examined in a Hitachi HS 8a electron microscope at 50 kV.

All the longitudinal veins, from the midrib to the smallest, are interconnected by transverse veins (see Blackman 1971). Serial transverse and paradermal sections of the various vein junctions revealed minor differences in detail, but no major systematic differences were observed and the description which follows is applicable to all of the vein junctions that were studied.

Similar leaves were fed for 2 hr with 1% ferric chloride solution through a cut in the tip of the leaf. Whole mounts were prepared from these leaves by soaking segments 2–3 cm long in saturated ammonium sulphide solution for 12 hr. The tissues were then transferred to 1% sodium sulphide solution for 2 days and mounted finally in water for examination. This treatment partially cleared the leaves, leaving the tracheary elements sharply outlined by their content of precipitated ferric salt.

Some tissue containing ferric salt precipitated either by sulphide treatment or by soaking in 1% silver nitrate was dehydrated rapidly in ethanol and embedded in Spurr's resin for electron microscopy. Since the precipitate lies within the lumina of the tracheary elements and is trapped in the walls of parenchyma cells where these abut the tracheary elements, the somewhat unusual wall thickenings of these elements appear almost as if they had been negatively stained (see Figs. 2, 23, and 24).

To identify the sieve elements in the whole mounts, 1–2-cm segments of fresh tissue were treated with 2% pectinase in 0·8M mannitol and agitated for 48 hr. Fragments of tissue were mounted in a 1 : 1 mixture of 0·8M mannitol and aniline blue (0·05% in M/15 K_3PO_4) and examined by fluorescence microscopy (see Fig. 3).

III. Observations

(a) *Distribution and General Anatomical Structure of Transverse Veins*

Measurements made on cleared leaves show that, in this cultivar, the transverse veins amount to about 7% of the total vein length and that each pair of adjacent longitudinal veins is cross-connected seven or eight times per 20 mm of leaf length.

In detail, no two transverse veins appear to be the same. Some transverse veins lie at right angles to two adjacent longitudinal veins, connecting them almost in a straight line; others make a steeply angled cross-connection, while others may be S-shaped. Most of the different patterns of transverse veins can be found between any pair of adjacent longitudinal veins at some level in the leaf (see Kaufman 1959; Blackman 1971).

Despite the differences in the size of the longitudinal veins and the manner in which the transverse veins connect them, study of sections shows that all transverse veins consist of a single file of interconnected tracheary elements that lie towards the upper side of the leaf and a single file of sieve elements that lie directly beneath the file of tracheary elements (Figs. 1 and 4). The mestome sheath and the parenchyma sheath which encase all of the longitudinal veins are present only where the transverse veins join the longitudinal veins (Figs. 9, 13–22). However, two files of vascular parenchyma cells are invariably associated with the transverse veins throughout their length (Figs. 1 and 4). One of these files lies adjacent to and above the file of tracheary elements while the other file lies beneath (or sometimes just to one side of) the file of sieve elements, facing the lower side of the leaf. These two files of vascular parenchyma cells are therefore rarely, if ever, in contact with one another but rather lie between their respective vascular elements and the photosynthetic cells of the mesophyll. However, the vascular parenchyma cells do not encase the vascular elements completely and it is easy to demonstrate that the free space of the mesophyll cells is in direct contact with the free space of the vascular elements, a phenomenon that does not occur in veins that have a mestome sheath.

(*b*) *Histology of the Transverse Veins*

Despite the view expressed by Percival (1921, p. 58) the tracheary elements can be identified readily as vessel members, connected into a single vessel by large perforation plates that commonly lie almost transverse to the vessel axis. The lumina of the vessel elements are about 7–10 μm in diameter, comparable to that of the smaller tracheary elements in the longitudinal veins. The vessel members have thick, lignified walls whose pattern is most unusual. Wherever the vessel member abuts either a sieve element or a mesophyll cell, the wall is free of apertures, but wherever the vessel member abuts a vascular parenchyma cell, the vessel wall is banded (Figs. 1 and 4).

The sieve elements are all connected at transverse sieve plates into a single sieve tube (Figs. 3, 7, 19, and 20). The lateral walls of the sieve elements appear to be uniformly thick throughout the length of the sieve tube, regardless of what type of cell they abut. The wall is distinctly two-layered, with an inner layer that is stained strongly with toluidine blue and by the PAS reaction, while the outer layer is weakly stained with toluidine blue and PAS-negative (Fig. 7, inset). No cytoplamic contents can be detected by light microscopy within the sieve tubes except for the small granules that are the highly modified plastids so characteristic of grass sieve elements (see O'Brien and Thimann 1967; Behnke 1969). Sieve plates stain strongly with the aniline blue reaction for callose (Fig. 3) and show alternating bands of PAS-positive and -negative material when viewed in thin sections (Fig. 7, inset). Individual sieve elements are about 4 μm in diameter and 60 μm long, dimensions somewhat smaller than those of sieve elements in the longitudinal veins.

The vascular parenchyma cells are about 65 μm long and 8 μm in diameter, although the cells close to junctions with longitudinal veins are somewhat shorter. The walls are usually thin and unlignified except close to the junction with longitudinal veins where lignified walls can be detected in the cells associated with the tracheary elements. Nuclei are large, elongated, rich in chromatin aggregates, and the cytoplasm is moderately basophilic. No differences can be detected between the companion cells and the xylem parenchyma cells by light microscopy.

(c) *Fine Structure of the Vascular Elements*

(i) *Vessel Members*

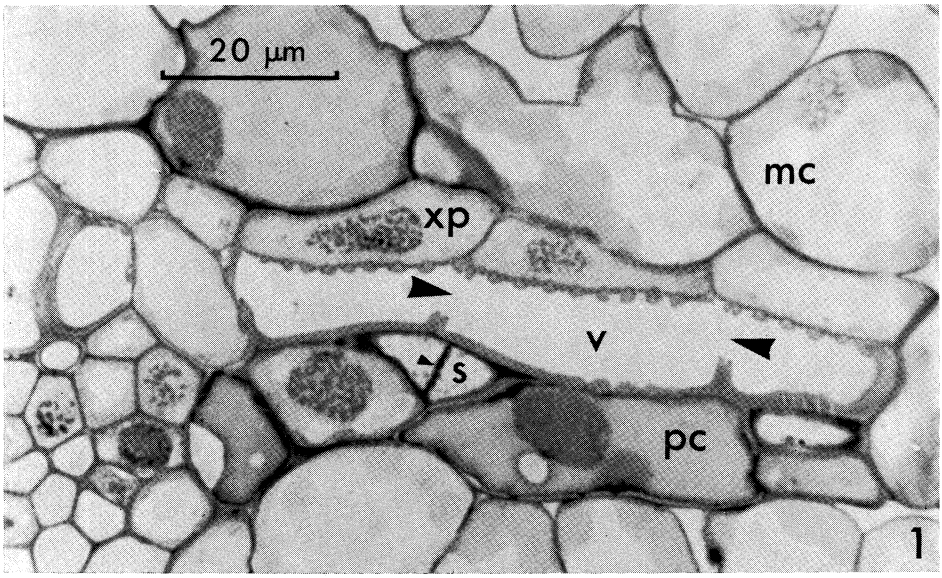
The wall that faces either a sieve element or a mesophyll cell is imperforate and has a uniform electron density throughout its thickness except at the middle lamella which is slightly more electron dense than the remainder of the wall (Figs. 4 and 6). Wherever the vessel abuts a vascular parenchyma cell, the vessel wall is banded but even in the interband regions the primary wall of the original procambial cell cannot be distinguished. Furthermore, in the region between the bands of lignified thickening, no trace of a hydrolysed wall can be detected. Rather, the interband region consists of wall material with a porous texture and high electron density, wall material that we believe has been elaborated by the parenchyma cell (Fig. 5), though this point cannot be established without an ontogenetic study. Thus, these vessel members differ from those described in other situations (O'Brien and Thimann 1967; O'Brien 1970) by the absence of a demonstrable hydrolysed-wall layer and by the absence of a clear-cut primary wall beneath the lignified thickenings. Furthermore, although plasmodesmata were seen occasionally in coleoptile xylem where the tracheary elements abutted a parenchyma cell, in these transverse veins no plasmodesmata have been detected between tracheary elements and any of the three types of living cell (parenchyma cells, sieve elements, and mesophyll cells) with which they share apoplastic boundaries.

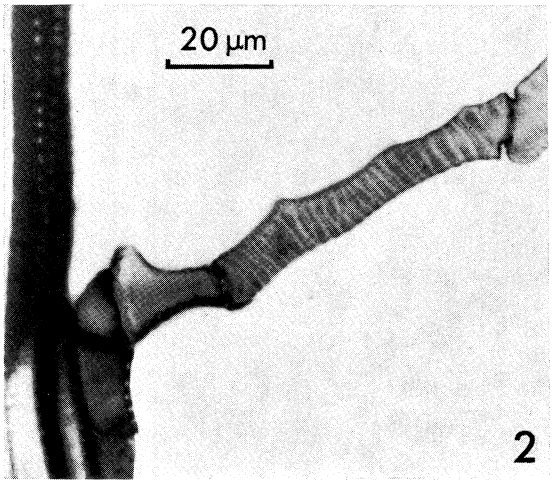
(ii) *Sieve Elements*

Unlike the wall of the vessel, the wall of the sieve elements has a constant appearance and structure regardless of what cell type it abuts. The wall is two-layered, an inner layer of high electron density and an outer layer of more moderate electron density (Figs. 4, 6–8). These two layers correspond clearly to the two layers distinguishable by light microscopy (see Fig. 7, inset).

The cytoplasm of the sieve tube consists of a thin parietal layer bounded by a cell membrane. Within this parietal layer one can distinguish mitochondria and stacks of endoplasmic reticulum (Figs. 6–8). The small granules seen in the light microscope are highly modified plastids, bounded by a double membrane and containing numerous electron-dense crystalloids (Figs. 7 and 8). Slime fibrils, and the membranous(?) tubules described in the more badly damaged oat sieve-elements (O'Brien and Thimann 1967, Figs. 20, 23, and 25) are absent, and the cytoplasm appears to be covered in some areas by a remnant of a vacuolar membrane (Fig. 6).

The sieve plates show the familiar callose plugs of low electron density that penetrate through the non-transformed regions of the primary walls of the adjacent





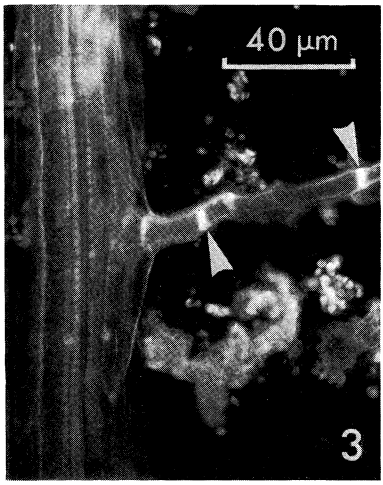
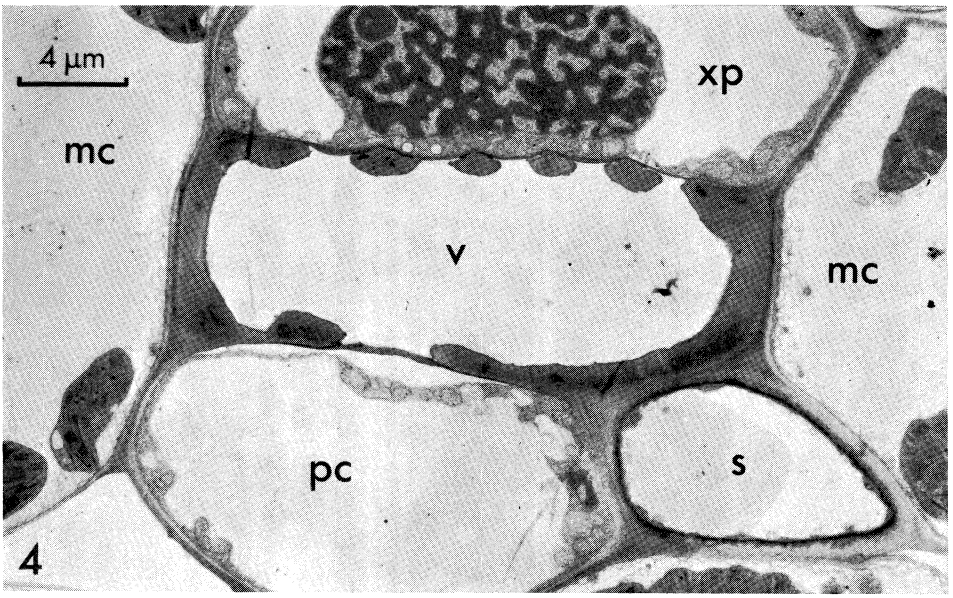
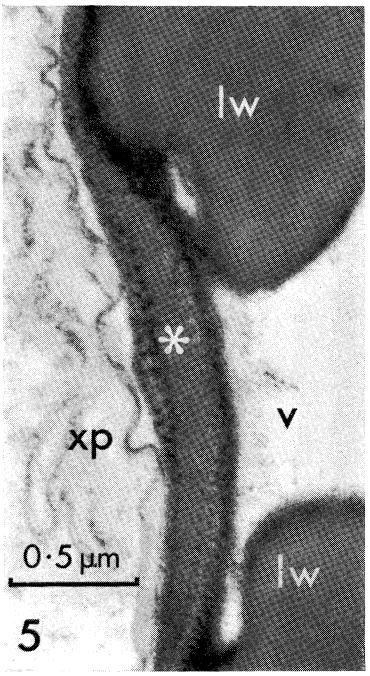


Fig. 1.—Transverse section of wheat leaf showing a transverse vein in longitudinal section. The vein consists of xylem parenchyma cells (*xp*), a vessel (*v*) with two perforation plates (arrows), a sieve tube (*s*) with sieve plate (small arrow), and phloem parenchyma cells (*pc*). Note that the vessel members have banded walls where they abut the xylem parenchyma cells, but a solid wall where they contact the sieve tube. *mc*, mesophyll cell. Epoxy section, stained with toluidine blue at pH 10.

Fig. 2.—Cleared preparation of a leaf fed 1% ferric chloride and treated with sodium sulphide. Note the continuity of the tracheary elements in the transverse vein with those in the longitudinal vein.

Fig. 3.—Fluorescence micrograph of an aniline-blue-stained vein preparation from a pectinase-digested leaf to show the callose fluorescence on the sieve plates of the transverse veins (arrows). Figure courtesy Mr. P. F. Lumley.





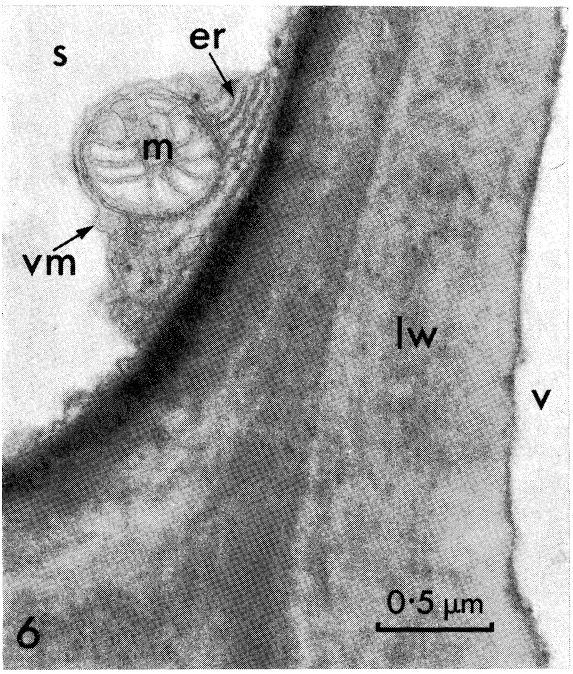
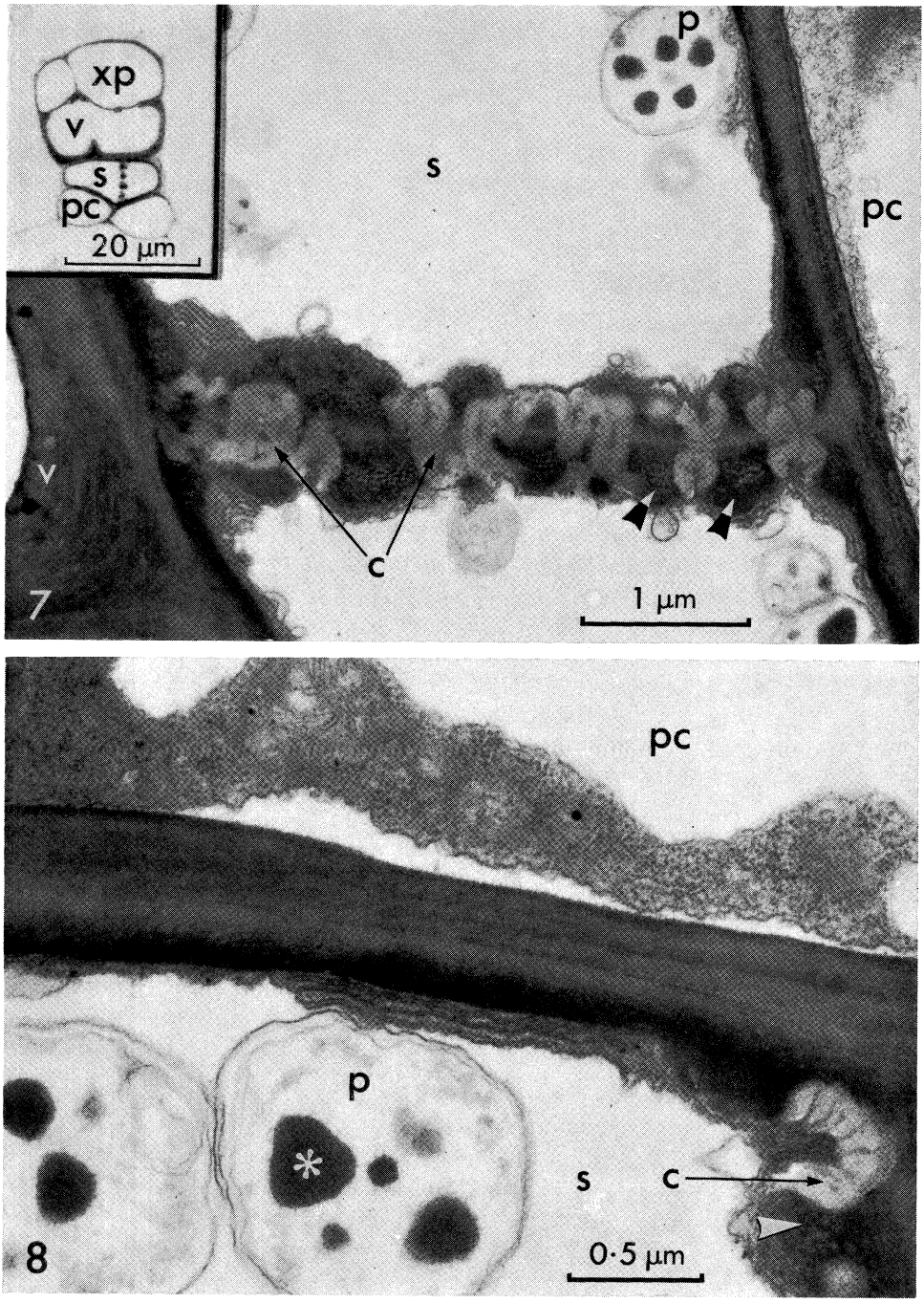


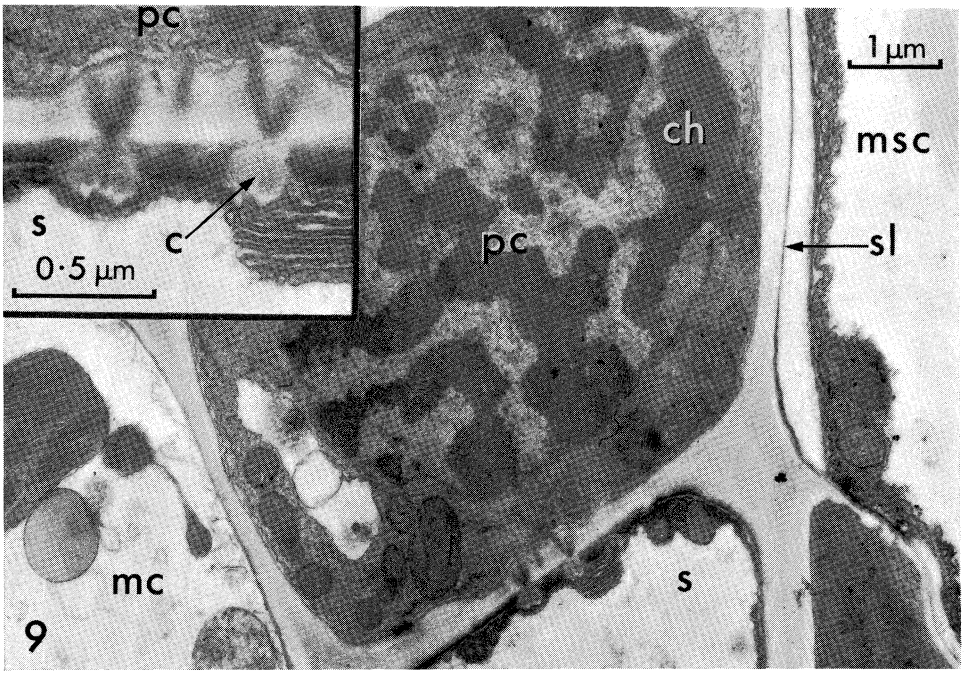
Fig. 4.—Electron micrograph of a transverse vein in transverse section. *mc*, mesophyll cell; *pc*, phloem parenchyma cell; *s*, sieve tube; *v*, vessel member; *xp*, xylem parenchyma cell.

Fig. 5.—Part of the boundary between a vessel (*v*) and a xylem parenchyma cell (*xp*), showing the texture of the unlignified wall (asterisk) that lies between the bands of lignified thickening (*lw*).

Fig. 6.—Part of the boundary between a vessel (*v*) and a sieve element (*s*). The cytoplasm of the sieve element contains a mitochondrion (*m*), endoplasmic reticulum (*er*), and a distinct remnant of a vacuolar membrane (*vm*). Although the wall of the sieve element is clearly two-layered, the transition between the lignified wall (*lw*) of the vessel member and the wall of the sieve element is not very distinct.



Figs. 7 and 8.—Part of a sieve tube, sieve plate, and adjacent phloem parenchyma cell (*pc*), and vessel member (*v*). Each sieve element is lined by an extremely thin layer of parietal cytoplasm within which one may detect profiles of endoplasmic reticulum and the modified plastids (*p*) with their distinctive inclusions (asterisk). Between the callose plugs (*c*), additional wall material with an open texture has been deposited (arrows). It is this material that is stained so strongly by the PAS reaction (Fig. 7, inset). *s*, sieve element; *v*, vessel; *xp*, xylem parenchyma cell.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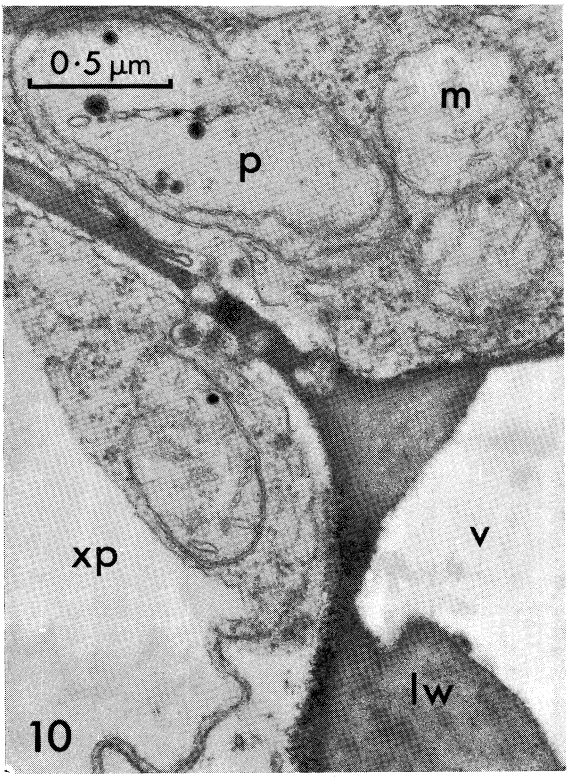
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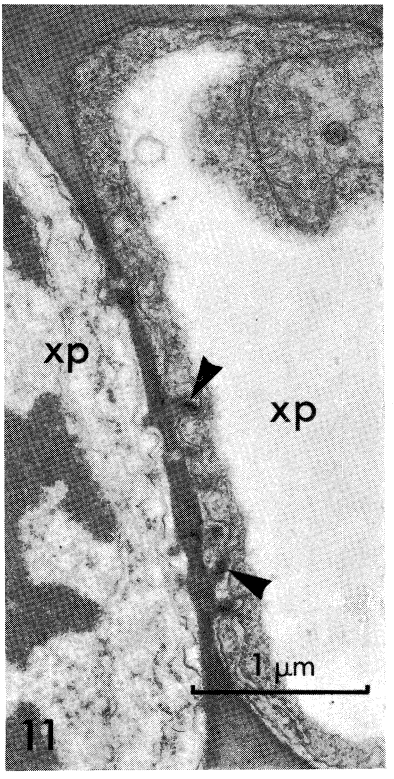
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sieve elements. Additional wall material, with a very porous texture, appears to overlie the non-callosic parts of the wall. It is this additional wall material which is stained so strongly by the PAS-reaction in Figure 7, inset.

Although membranous material can be detected within the callose plugs, its identity is uncertain and it is quite impossible to demonstrate continuity of cell membrane between adjacent sieve elements. Observations with the electron microscope confirm the absence of plasmodesmatal connections between the sieve elements and adjacent mesophyll cells, though specialized plasmodesmatal connections are evident between the sieve elements and adjacent vascular parenchyma cells (Fig. 9 and inset).

(iii) *Vascular Parenchyma Cells*

We have been able to detect only two differences in the fine structure of the companion cells and xylem parenchyma cells; the presence of sieve areas in the companion cells and a "protective wall" where a xylem parenchyma cell abuts a tracheary element. These protective walls have a high electron density and an open texture: they are thick where the cell abuts the lumen of the tracheary element and thinner or absent where the cell abuts lignified walls of the tracheary element (Figs. 4 and 5). Both types of vascular parenchyma cell are highly vacuolate, and contain a large nucleus with interconnected masses of aggregated chromatin (Figs. 4, 9, and 11). The cytoplasm is rich in mitochondria, plastids that lack grana but contain a few fenestrated thylacoids and an abundance of plastoglobuli, and is very rich in ribosomes (Figs. 9, 10, and 11). Plasmodesmata are common between parenchyma cells, and between parenchyma cells and mesophyll cells. Wall ingrowths, characteristic of those found in transfer cells (Gunning, Pate, and Briarty 1968; Gunning and Pate 1969) were also detected between adjacent parenchyma cells (Fig. 11) and occasionally where a pit field of a parenchyma cells abuts a mesophyll cell.

(*d*) *Junction of the Transverse Vein with the Longitudinal Vein*

It was evident from experiments with ferric chloride solutions introduced into the leaf by transpiration that the tracheary elements of the transverse vein were connected to those in the longitudinal vein (Fig. 2). Furthermore, experiments in which ferric chloride solution was administered to one-half of a leaf slit longitudinally for 5 cm while water was administered to the other half established clearly that the tracheary elements provided a continuous luminal pathway from one longitudinal vein to the other (Fig. 12). However, the precise manner in which these elements, and

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Fig. 9.—A phloem parenchyma cell (*pc*) that abuts a mestome sheath cell (*msc*) close to the junction with a longitudinal vein. Note the highly condensed chromatin aggregates (*ch*) in the nucleus of the phloem parenchyma cell. The inset (from part of Fig. 9) shows the characteristic branched pit field, filled with callose (*c*) on the sieve element side, where these cells abut a sieve element (*s*). *mc*, mesophyll cell; *sl*, suberized lamella.

Figs. 10 and 11.—Xylem parenchyma cells (*xp*) showing simple plastids (*p*), numerous mitochondria (*m*), and plasmodesmatal connections between adjacent cells. Note the tiny wall protuberances (arrows) that accompany the plasmodesmata. *lw*, lignified wall; *v*, vessel member.

the sieve elements, make connection with corresponding elements of the longitudinal veins required a detailed analysis of serial sections, both transverse and paradermal.

The mode of connection illustrated in Figures 13–18 is quite typical. As the transverse vein approaches the longitudinal vein the cells of the parenchyma sheath of the longitudinal vein encase it. At this region, the mestome sheath of the longitudinal vein contains two cells (numbered 1 and 2 respectively in Figs. 13–15) that are much wider tangentially than the other cells of the mestome sheath. These modified mestome sheath cells come into close contact with vascular parenchyma cells of the transverse vein and share pit connections with them (Figs. 14 and 15). A "gap" opens between these two modified mestome sheath cells (1 and 2 of Figs. 14 and 15),

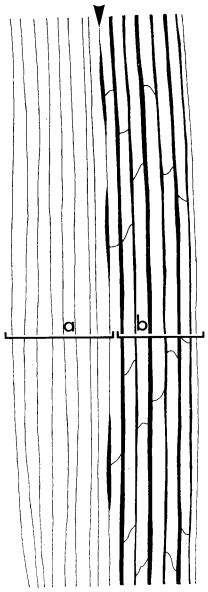
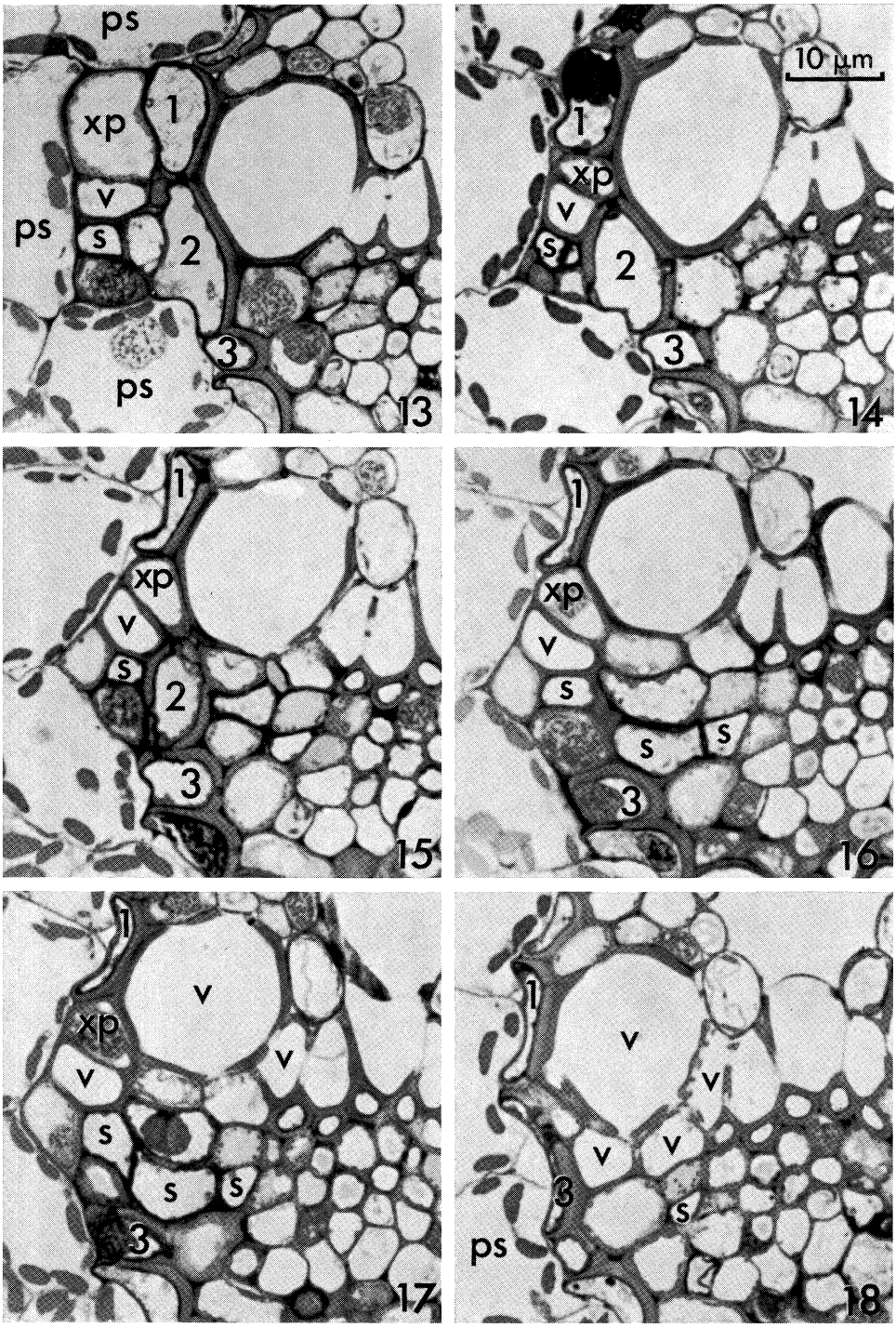


Fig. 12.—Diagram of a portion of a leaf that was split at its base and fed 1% ferric chloride to the half marked *b*. The half marked *a* was fed water. The leaf was cleared in sodium sulphide and the distribution of ferric salt in the veins is shown by the dense lines. The incompletely filled vein (arrow) that lies in the water-fed half demonstrates conclusively that ferric salt has crossed through the vessels of the transverse veins.

bringing the xylem parenchyma cell of the transverse vein into contact with both of the modified mestome sheath cells and with one of the large metaxylem vessels in the longitudinal vein. At this level, both the sieve element and the tracheary element of the transverse vein are "excluded" from the longitudinal vein by cell 2 of the mestome sheath (Fig. 15). Sieve element contact occurs as soon as cell 2 disappears (Figs. 16 and 17). Continuity between the tracheary elements is effected at a somewhat lower level, in this case both with the large vessel and with another metaxylem element that contains some cytoplasmic remnants (Fig. 18).

These observations can be confirmed in paradermal sections which also give a better idea of the shape of the joining cells. Sieve element continuity is effected

Figs. 13–18.—Light micrographs of a series of transverse sections showing the manner in which the vascular elements of the transverse vein become continuous with those in the longitudinal vein. The mestome sheath cells that "part" to admit the transverse vein are numbered for ease of orientation. *ps*, parenchyma sheath cell; *s*, sieve element; *v*, vessel member; *xp*, xylem parenchyma cell. In Figures 13–15, the cells labelled *xp*, *v*, and *s* lie within the transverse vein. All epoxy sections at same magnification, stained by toluidine blue at pH 10.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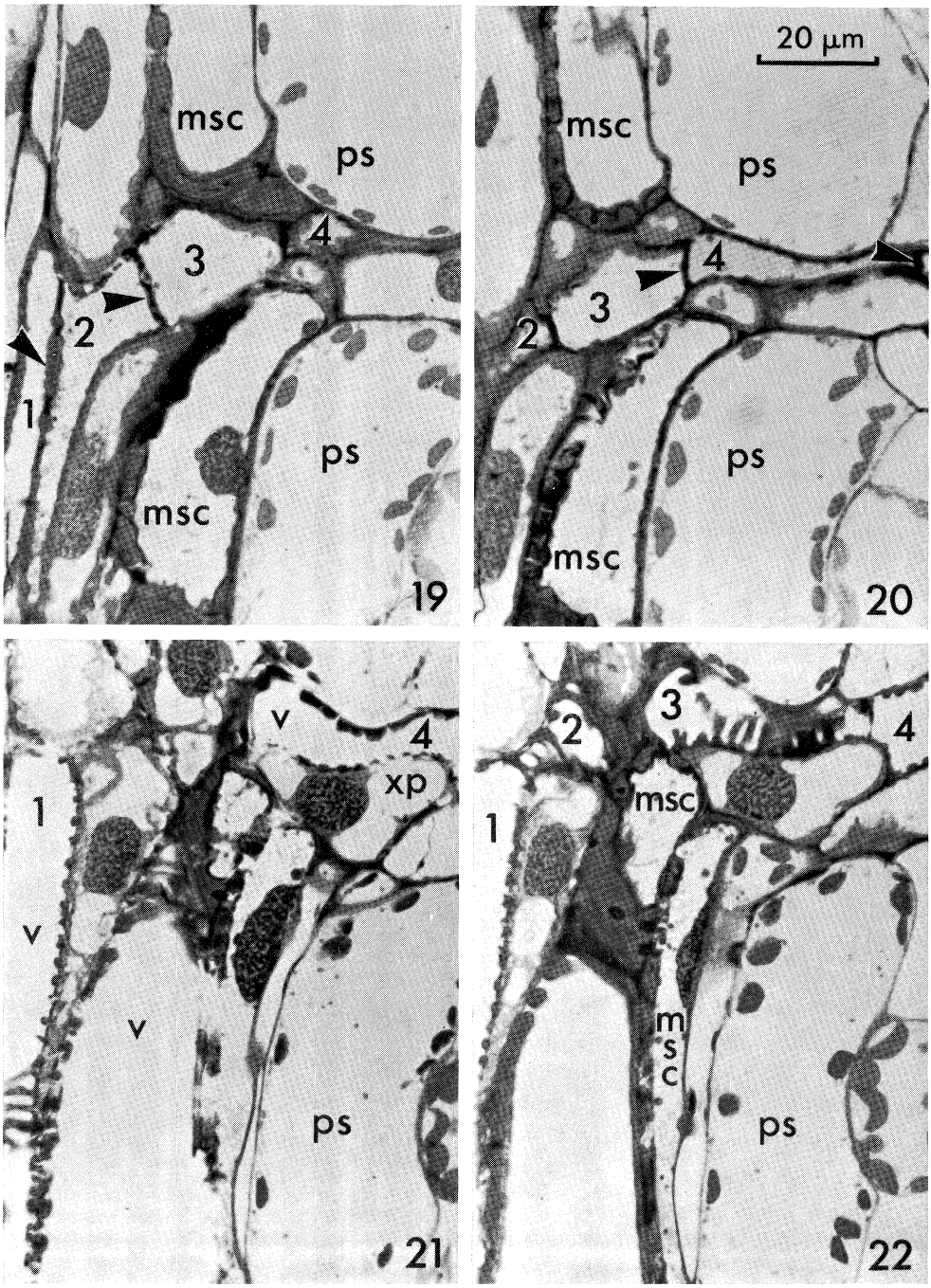
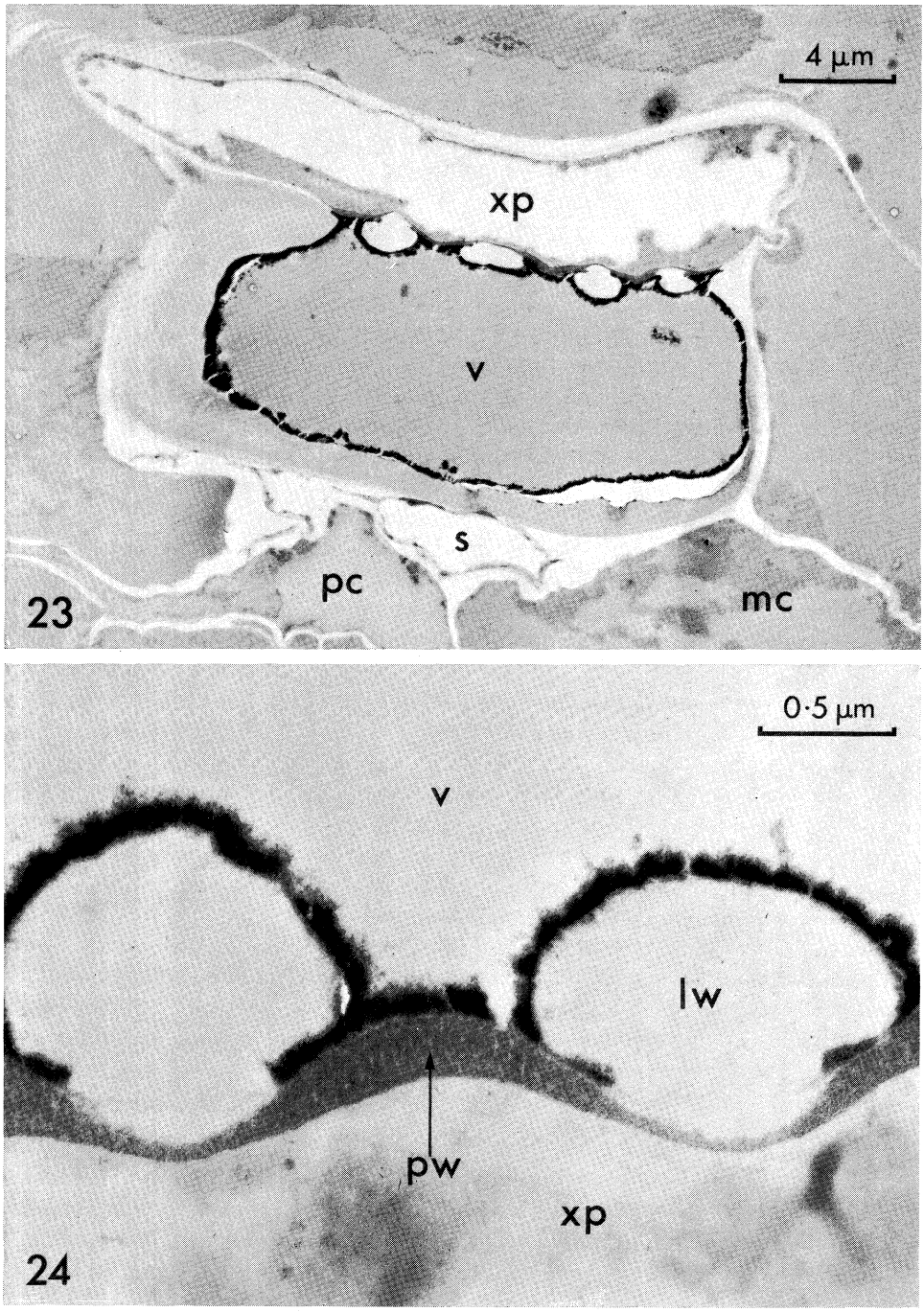


Figs. 19–22.—Light micrographs of paradermal sections of the leaf showing continuity between sieve elements (Figs. 19 and 20) and tracheary elements (Figs. 21 and 22) in the transverse vein and corresponding elements in the longitudinal vein. Elements are numbered for ease of orientation. *msc*, mestome sheath cell; *ps*, parenchyma sheath cell; *v*, vessel member; *xp*, xylem parenchyma cell. Arrows denote sieve plates. All epoxy sections at same magnification, stained by toluidine blue at pH 10.



Figs. 23 and 24.—Electron micrographs of part of a transverse vein fed 1% ferric chloride solution and treated either with silver nitrate (Fig. 23) or ammonium sulphide (Fig. 24), dehydrated, and embedded in epoxy resin. The sections are otherwise unstained. Note the electron-dense precipitate that lines, but fails to penetrate, the lignified walls of the vessel member. However, the unlignified wall that lies between the lignified bands is considerably more electron-dense than walls at greater distances from the vein. *lw*, lignified wall; *mc*, mesophyll cell; *pc*, phloem parenchyma cell; *pw*, primary wall; *s*, sieve element; *v*, vessel member; *xp*, xylem parenchyma cell.

across a large sieve plate that connects cell 1 to an element of unusual shape, cell 2 (Fig. 19). These elements (numbered 1–4 in Figs. 19 and 20) are all interconnected by sieve plates and can be traced into the transverse vein. The demonstration of tracheary element continuity is more difficult from paradermal sections since the connecting cells do not lie in a single plane that is easily intersected with a few sections. Nonetheless, Figures 21 and 22 do illustrate in section the continuity that can be deduced to exist from the experiments with ferric chloride (see Figs. 2 and 12).

(*e*) *Retention of Ferric Salt by the Vessels of the Transverse Vein*

Since the transverse veins lack a mestome sheath, we expected that iron, transpired into a leaf as ferric chloride solution, would pass freely into the free space of the adjacent mesophyll from the lumina of the vessels in the transverse veins. Figure 2 shows that this does not happen; almost all of the iron detectable by this method is retained within the vessel and in the walls of the adjacent xylem parenchyma cells. Figures 23 and 24 are electron micrographs of elements similar to those shown in Figure 2.

The element shown in Figure 24 is from tissue treated sequentially with ammonium and sodium sulphide after ferric chloride treatment while that shown in Figure 23 is from tissue soaked in silver nitrate after ferric chloride treatment. In both cases, the tissue was dehydrated rapidly in ethanol after treatment, embedded, and sectioned. The sections were not treated with heavy metal salts and the tissue has not been treated with OsO_4. In both cases, an electron-dense precipitate lines the lignified wall of the vessel but none can be detected within the lignified wall itself. An electron-opaque deposit is present in the protective wall of the xylem parenchyma cell where it abuts the lumen of the vessel but free passage of ferric salt through this wall is obviously impeded since the electron opacity falls off sharply with distance from the vessel lumen. That these deposits are due to treatment with the ferric salt was confirmed by their absence in controls treated with sulphide or silver nitrate that had received no ferric chloride in the transpiration experiment.

These observations suggest, but unfortunately do not prove, that the lignified wall may be very impermeable to both anionic and cationic species present in the ferric chloride solution, and that the layers rich in pectic acid which line the unlignified parts of the vessel wall, although permeable to ferric salt, severely impede its diffusion. The net result is a strong retention of ferric salt within the lumen of the vessel and within the unlignified regions of wall that abut it.

IV. Discussion

The questions posed in the introduction may now be answered. In this cultivar of wheat, the transverse veins consist of a single sieve tube and a single vessel, each of which is accompanied by one or more files of parenchyma cells. The vessel and sieve tube are connected to the metaxylem and metaphloem elements of the longitudinal veins. According to Blackman (1971) the phloem-associated parenchyma cells are ontogenetically companion cells, and the structure of the sieve areas where these cells have pits in contact with the sieve element supports this view. The transverse veins lack both a mestome sheath and a parenchyma sheath, except very

close to their junction with the longitudinal veins where they become encased in parenchyma sheath cells. In the region of continuity, the mestome sheath of the longitudinal vein is modified, consisting of shorter and wider cells arranged around a "gap" through which the transverse vein penetrates. Vascular parenchyma cells that are symplastically connected with those of the transverse vein replace the mestome sheath cells in the gap region, and there is a strong tendency for the xylem parenchyma cells of the gap to be lignified. We conclude that in the lamina of this cultivar, the striate venation is anatomically reticulate, all veins being interconnected both via the xylem and the phloem.

The extraordinary wall pattern of the vessel members deserves further study. Bierhorst and Zamora (1965) believe that all primary xylem tracheary elements are ontogenetically derived from the annular or reticulate state, pitted elements being produced by the addition of more wall material between the gyres of thickening. If their theory is a general one, then these vessel members are most unusual in that the additional wall material is added asymmetrically to produce an imperforate wall where the vessel contacts sieve elements or mesophyll cells, but an annular or reticulate wall where it contacts vascular parenchyma cells. The fine structure of these cells during differentiation needs to be studied to determine just how this unusual pattern of wall thickening is formed, and what is the distribution of microtubules during wall deposition.

Our interest in the transverse veins stemmed initially from attempts to understand the role of the suberized lamella in the mestome sheath of the longitudinal veins (O'Brien and Carr 1970). These studies demonstrate that such sheaths and suberized layers are absent from the transverse veins. We expected, therefore, that the transpiration stream would pass readily into the free space of the mesophyll at these veins and were astonished at the strong retention of ferric salt by these tracheary elements. The lignified wall of the vessel is clearly very impermeable to the ferric salt which enters the free space only at the protective wall layers of the parenchyma cells where these abut tracheary elements. It has been pointed out (O'Brien 1970) that walls of this kind are rich in polyuronides with free carboxyl groups which presumably may react with and bind many of the cationic species present in a 1% ferric chloride solution. However, we did not expect to obtain evidence, as Figure 23 suggests, that the passage of chloride ions is also impeded. Ferric chloride solutions are a poor choice for this kind of study, since such solutions may be colloidal and may contain a variety of ionic species and more work is necessary before these observations are accepted. Nonetheless, the results obtained demonstrate clearly that nutrients in the transpiration stream that is passing into the mesophyll from the transverse veins *may* encounter major barriers to their free diffusion, either in the lignified wall of the tracheary element or in the acidic gel of protective polyuronide that lines all of the unlignified exits from the vessel.

Crowdy and Tanton (1970) used the EDTA chelate of lead as a "free-space marker" in wheat leaves. They demonstrate that the lignified walls of the tracheary elements are also relatively impermeable to this tracer. Unfortunately they do not mention the distribution of lead in the transverse veins.

The absence of any kind of bundle sheath in these transverse veins is an important distinction between them and the minor veins of dicotyledonous leaves.

It was somewhat surprising to discover that this arrangement, which brings the mesophyll into direct contact with the sieve elements, may not be exploited since there is no symplastic connection between the sieve elements and the mesophyll cells. The presence of wall ingrowths where vascular parenchyma cells abut one another is a further puzzle for there is little reason to expect intense solute fluxes between adjacent parenchyma cells. Blackman (1971) suggests that the vascular parenchyma cells of the transverse veins may be carrying out functions similar to those carried out by the parenchyma sheath cells of the longitudinal veins. In the sense that any solute exchange between mesophyll and conducting elements must involve these cells, there is an obvious parallel but the absence of chloroplasts from the vascular parenchyma cells of the transverse vein suggests that they must lack all of the functions dependent upon photosynthesis that the cells of the parenchyma sheath can carry out.

If, as O'Brien and Carr (1970) suggested, the suberized lamellae tend to restrict water movement across the free space from the longitudinal veins to the mesophyll, it can be argued that the transverse veins may provide important loci for water escape to the free space of the mesophyll for these veins are not bounded by a mestome sheath. The vascular parenchyma cells, and especially the xylem parenchyma cells, are likely to be the major sites of solute accumulation from the water that discharges into the mesophyll from the transverse veins. It was shown above that these vascular parenchyma cells are all connected symplastically both to similar cells in the longitudinal veins and to the modified mestome sheath cells of the longitudinal veins. Can it be that the development of wall ingrowths where the vascular parenchyma cells of the transverse vein abut one another reflects a mild solute flux between these cells, a flux directed at returning to the phloem of the longitudinal veins organic matter that escapes via the transpiration stream into the transverse veins?

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