Planta (Berl.) 126, 45—59 (1975)
© by Springer-Verlag 1975

The Distribution of P-Protein in Mature Sieve Elements of Celery

G. P. Dempsey, S. Bullivant, and R. L. Bieleski

Department of Cell Biology, University of Auckland, and Plant Diseases Division, D.S.I.R., Private Bag, Auckland, New Zealand

Received 16 April; accepted 16 June 1975

Summary. The extent of blocking of sieve-plate pores caused by release of cell turgor was investigated by fixing and processing for electron microscopy a long length of celery (*Apium graveolens* L.) phloem. Differences in distribution of P-protein within the pores were observed between those cells near the two cut ends, and the central cells.

To assess the effect of chemical fixation on the distribution of P-protein, strands of celery phloem (fixed or unfixed, and not treated with cryoprotectants) were frozen in Freon 12 and then freeze-substituted. In sieve elements from unfixed tissue there were a greater number of sieve plates displaying partially open pores.

Direct freezing of unprotected phloem tissue in Freon 12 resulted in the formation of ice crystals within the lumen of the sieve elements. Freezing of tissue at rates fast enough to avoid the formation of damaging ice crystals resulted in sieve-plate pores having an unoccluded central channel with a peripheral lining of P-protein. In the lumen of the sieve elements the P-protein filaments occurred as discrete bundles *ca.* 0.5 μm in diameter, and as a parietal layer varying in thickness from 0.1 to 0.5 μm.

Introduction

The major structure contained within a mature sieve element is a filamentous protein commonly referred to as "phloem protein" or "P-protein". It is sometimes seen as a shapeless mass aggregated at the sieve plate, while sometimes the filaments are loosely oriented and partly disposed through the sieve-element lumen. Electron-microscopical investigations of sieve-element structure are plagued by the extreme sensitivity of the sieve element and its contents to cutting, chemical fixation and conventional embedding procedures. Consequently controversy still exists over the *in-vivo* arrangement of the sieve-element contents, particularly the degree of blocking of the sieve-plate pores by the P-protein filaments.

For example, Mishra and Spanner (1970) and Siddiqui and Spanner (1970) believed that the occurrence of P-protein filaments in the sieve-plate pores was not an artefact of preparation, and that the natural condition of the pores was to be plugged fairly compactly with P-protein. On the other hand, Cronshaw and Anderson (1969) found that some sieve-plate pores in *Nicotiana* were unplugged when plants were frozen in liquid nitrogen before fixation or had been wilted prior to fixing for electron microscopy, and in specimens from plants thinly sliced before fixation (Anderson and Cronshaw, 1970). Evert *et al.* (1973) reported that after treatment of *Cucurbita* seedlings aimed at lowering the hydrostatic pressures within the sieve elements, the P-protein was almost entirely parietal in distribution in both the lumen and sieve-plate pores. Thaine (1962,

1969) proposed from light microscopy that transcellular strands of cytoplasm, between 1 and 7 μm in diameter and bounded by a membrane, passed along the lumen of sieve elements and through the sieve plates. Thus there are at least three current interpretations of the organisation of the sieve-element contents.

Freezing techniques appear to offer the best chance of avoiding fixation artefacts. Johnson (1968) freeze-etched vascular bundles of *Nymphoides peltata* and described structures which he interpreted as filaments in and near the sieve-plate pores. He subsequently showed (Johnson, 1973) the existence of short bundles of P-protein filaments near sieve plates in freeze-etch preparations of vascular bundles which were translocating ^{14}C before they were frozen.

Thaine and his co-workers (Jarvis and Thaine, 1971; Thaine and De Maria, 1973; Jarvis *et al.*, 1973), using cryo-fixation techniques in association with light microscopy, observed structures which they interpreted as longitudinally-oriented transcellular strands with discrete boundaries on both sides of the sieve plate and often extending through the pores.

The experiments described in this paper use electron microscopy of thin-sectioned and freeze-substituted celery phloem to obtain information on the *in-vivo* distribution of P-protein in the lumen and pores of functioning sieve elements. The aim of this investigation is to answer several questions: 1) What effect does sudden release of turgor have on the state of the pores? 2) What effect does chemical fixation with glutaraldehyde have on the state of the pores? 3) What effect do ice crystals have on P-protein distribution? 4) What is the state of P-protein distribution after freezing phloem tissue rapidly enough to avoid the formation of ultrastructurally damaging ice crystals?

Material and Methods

Plant Material. Mature celery plants (*Apium graveolens* L.) grown in the field were transplanted a few days prior to use and allowed to re-establish themselves in pots under greenhouse conditions until no wilting was apparent. The vascular bundles were "stripped" from the petiole so that they were laterally displaced but still attached at both ends to the petiole. Under a dissecting microscope, the xylem and phloem layers were gently separated from each other. In this way, strands of phloem up to 25 cm long could be laterally displaced from the petiole while attached at both ends to it.

Thin-sectioning. Tissue for conventional thin-sectioning was fixed for 1 h at room temperature in 6% glutaraldehyde in 0.1 M phosphate buffer, then washed in several changes of the phosphate buffer (pH 7.2). Postfixation was for 1 h at room temperature in 2% osmium tetroxide in the same phosphate buffer. The tissue was dehydrated in ethanol and embedded in Epon or Spurr's (1969) embedding medium. Sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope operating at 80 kV.

Freeze-substitution. The tissue to be freeze-substituted was not treated with cryoprotectants. Freezing was either in Freon 12 or on a polished copper surface at liquid-nitrogen temperature (Van Harreveld and Crowell, 1964). The first method involved plunging the tissue into melting Freon 12 and then transferring it to liquid nitrogen with a pair of cooled forceps. The second method was used to achieve faster freezing rates. The tissue was pushed gently but firmly onto a polished copper block held at liquid-nitrogen temperature (−196°), then both block and tissue were immediately immersed in liquid nitrogen.

Once frozen, the tissue was freeze-substituted for 14 days at −70° in either dry ethanol or dry acetone containing 2% osmium tetroxide. The tissue was then warmed in stages (0°, 4°, room temperature) over 6 h, infiltrated overnight in Spurr's embedding medium, and finally embedded in the same medium. Sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope operating at 60 kV.

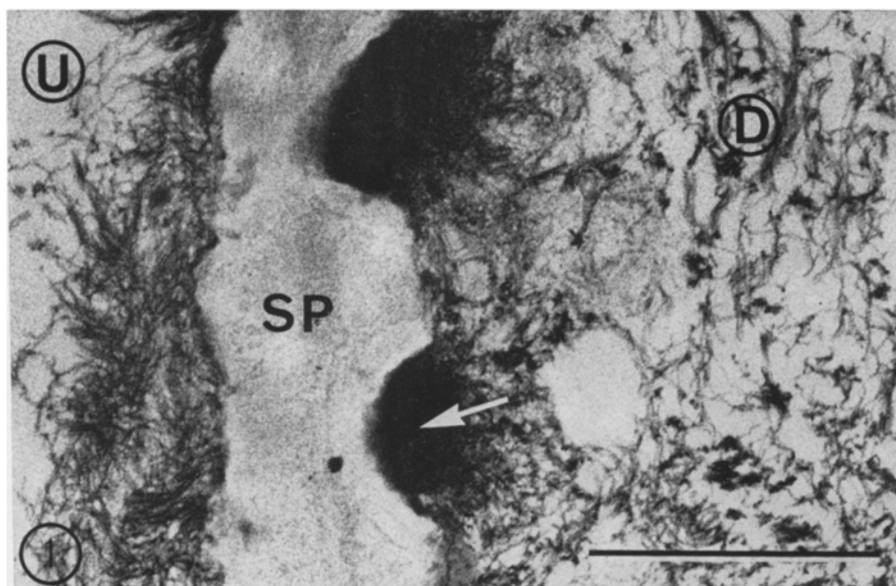
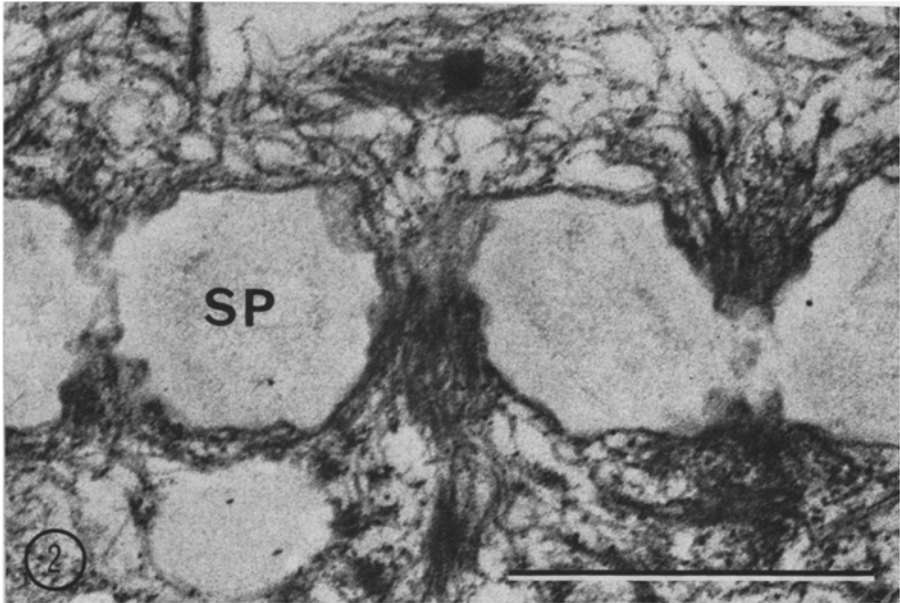


Fig. 1. Thin section of a region of the sieve plate (SP) from a glutaraldehyde-fixed sieve element within 1 cm of the end of a 3.5-cm length of phloem. The sieve-plate pores (arrow) are blocked with P-protein. There is a "piling-up" of P-protein upstream (*U*) from the sieve plate, and a "trailing" condition downstream (*D*). Bar = 1 μm

Results

1. Effect of Turgor Release on P-Protein Distribution

Thin sections of sieve elements from celery petiole fixed and sectioned by conventional procedures displayed sieve plate pores largely blocked with P-protein (similar to those shown in Fig. 1). Since the phloem strands which were fixed and processed for electron microscopy in this part of the study were generally less than 0.5 cm in length, it seemed likely that the release of turgor on cutting the file of sieve elements had caused a movement of the sieve element contents towards the two cut ends prior to fixation. The pattern seen at the sieve plate (the "slime plug") is thought to have resulted from a "piling-up" of P-protein upstream from the sieve plate, and a "trailing" condition downstream. This interpretation was tested in subsequent experiments. The effect of turgor release on the blocking of sieve-plate pores with P-protein was investigated by fixing and processing a phloem strand 3.5 cm long. After embedding, the phloem strand was cut into seven 0.5-cm lengths and thin sections from each segment were examined. Sieve elements within 1 cm of each end displayed sieve plate pores largely blocked with P-protein (Fig. 1). Consistently there was an accumulation of P-protein on the side of the sieve plate away from the adjacent cut end, although a "trailing" condition on the side towards the cut end was not always apparent. Within some sieve-plate pores the P-protein was so closely compacted that individual P-protein filaments could not be distinguished (Fig. 1). P-protein within the lumen of these sieve elements was largely unoriented and fragmented.



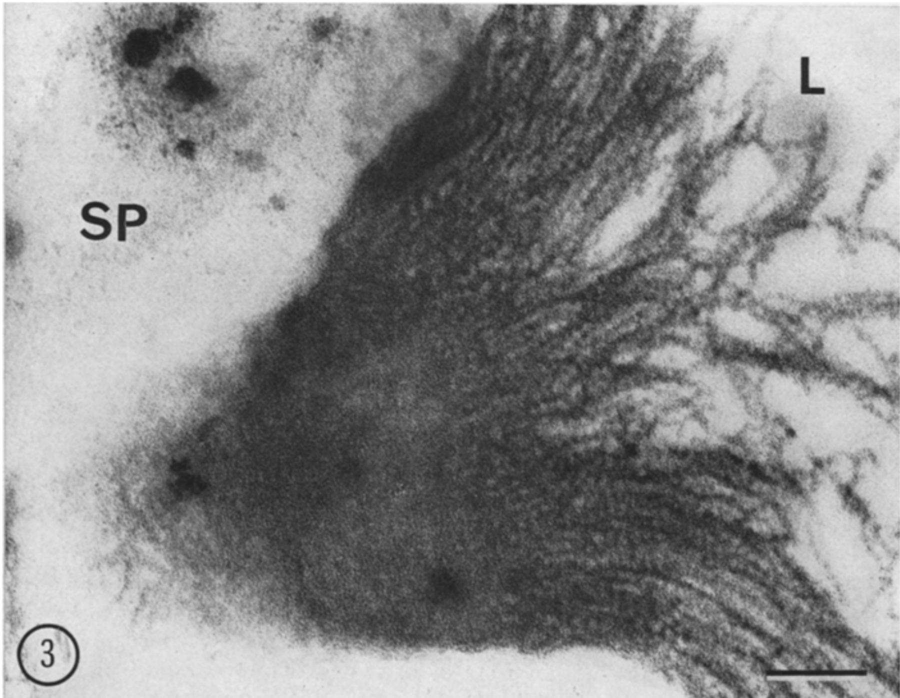


Fig. 2 and 3. Thin sections of regions of the sieve plate (SP) from glutaraldehyde-fixed sieve elements within the central 1 cm of a 3.5-cm length of phloem. Fig. 2. Clear areas exist in the pores between the P-protein filaments. The accumulation of P-protein is approximately equal on both sides of the sieve plate. Bar = 1 μm. Fig. 3. Note the "banded" appearance of the P-protein where it frays evenly from the pore. Bar = 0.1 μm

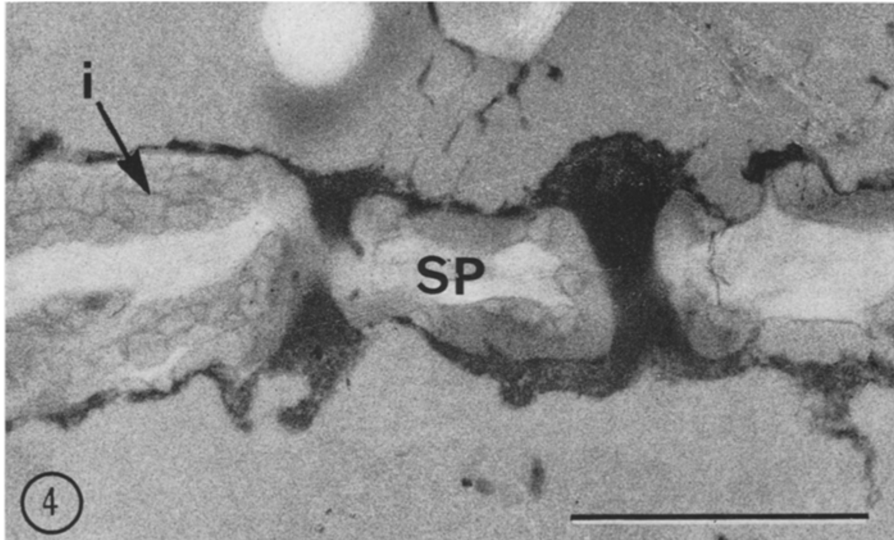


Fig. 4. Freeze-substituted celery phloem fixed in glutaraldehyde prior to freezing in Freon 12. The P-protein occurs as a structureless mass completely blocking the pores and not extending very far into the lumen of the sieve element. Ice crystals (*i*) are visible in the wall of the sieve plate (*SP*). However, no ice crystals are visible in the empty lumen of the sieve element. Bar = 1 μm

Sieve elements in sections taken from the central 1 cm of phloem showed less compaction of the P-protein within the sieve-plate pores, such that clear areas existed in the pores between the P-protein filaments (Fig. 2). In general there was no accumulation of P-protein upstream or downstream. The degree of orientation of the P-protein filaments within the pores and close to the pores was much more marked in this region of the phloem strand. The P-protein frayed evenly from many pores, and the filaments had a "banded" appearance (Fig. 3).

2. Effect of Chemical Fixation on P-Protein Distribution

To assess the effect of chemical fixation on the distribution of P-protein, strands of celery phloem either glutaraldehyde-fixed or unfixed were frozen in Freon 12 and then freeze-substituted. Sieve-plate pores from small pieces of tissue (0.5 cm in length) which were fixed in glutaraldehyde before freezing were invariably blocked with P-protein (Fig. 4). In many cases individual filaments of P-protein could not be identified within either the sieve tube lumen or the pores. Instead, the P-protein was compacted into a structureless mass blocking the sieve-plate pores and not extending any great distance into the lumen of the sieve element.

In sieve elements from tissue frozen in the unfixed state many of the sieve plates displayed partially open pores (Fig. 5). Individual P-protein filaments could be seen extending from the sieve plate into the lumen of the sieve element, often "piling-up" slightly on one side of the sieve plate and "trailing" downstream. Most of the P-protein filaments were oriented longitudinally within the sieve-

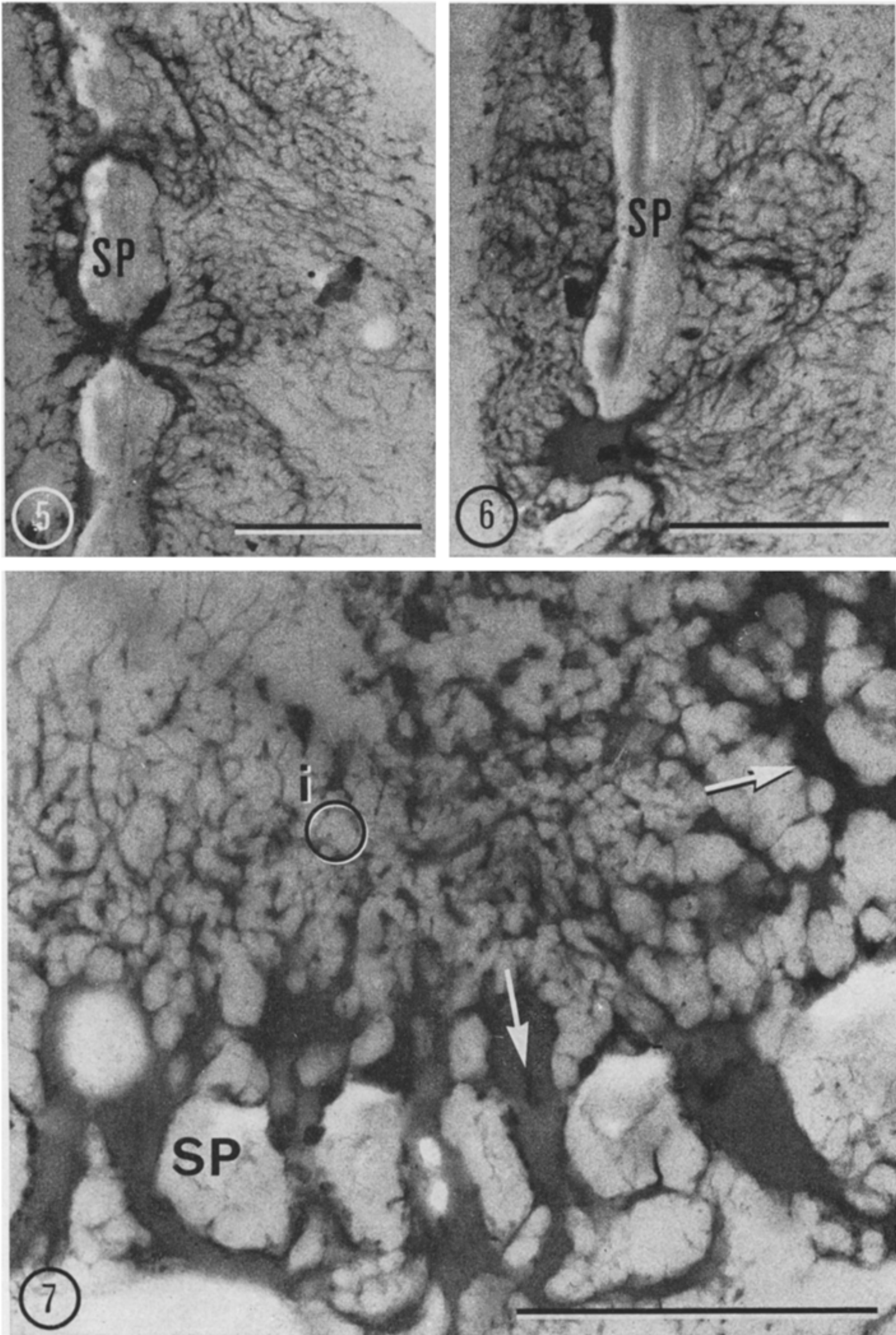


Fig. 5—7. Sieve elements from unfixed celery phloem frozen in Freon 12 then freeze-substituted. Bars = 1 μm. Fig. 5. Sieve plate (SP) from an unfixed phloem strand 1 cm in length. P-protein filaments extend from the sieve plate into the lumen of the sieve element. There is a "piling-up" of P-protein on one side of the sieve plate and a "trailing" condition on the other. Fig. 6. Sieve plate (SP) from a loop of phloem frozen while still attached to the plant at both ends. The effect of turgor release is much less marked. Fig. 7. At higher magnification ice crystals (*i*) can be seen between the P-protein filaments. The ice crystals appear to displace the P-protein (arrows), forcing the filaments into aggregates in the eutectic

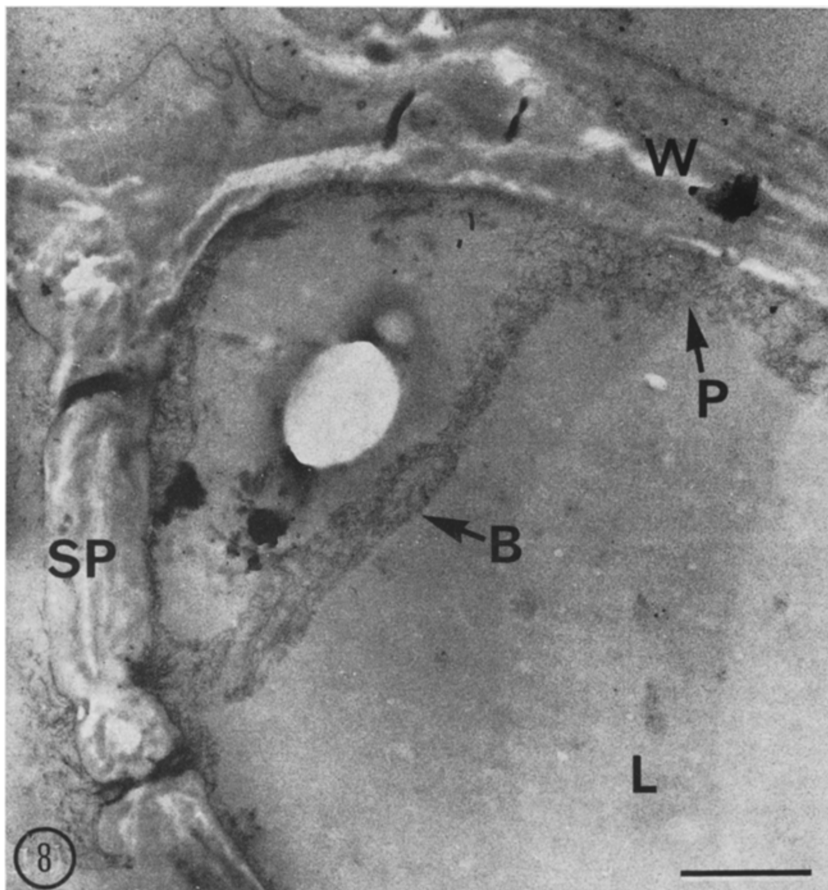


Fig. 8. Freeze-substituted sieve element from the surface 12 μm layer of celery phloem, frozen onto a copper surface at liquid-nitrogen temperature, while still attached to the plant at both ends. P-protein exists as discrete bundles of filaments (*B*) extending from the sieve plate (SP) into the lumen of the sieve element. P-protein also exists as a parietal layer (*P*) lining the sieve plate and the lateral walls (*W*) of the sieve element. Bar = 1 μm

plate pores and also in the sieve-element lumen. The degree of accumulation of P-protein on one side of the sieve plate depended on the length of the phloem strand which had been frozen. Fig. 5 shows a section taken from an unfixed phloem strand 1 cm in length; note the uneven distribution of P-protein on different sides of the sieve plate. However, Fig. 6 shows a section taken from a loop of phloem that was frozen while still attached to the plant at both ends. Such sieve plates displayed only minimum disturbance due to turgor release, although there was often slight disorientation of the P-protein filaments on one side of the sieve plate. They characteristically had discrete filaments of P-protein, oriented along the sieve tube, extending well into the lumen and not completely blocking the sieve-plate pore.

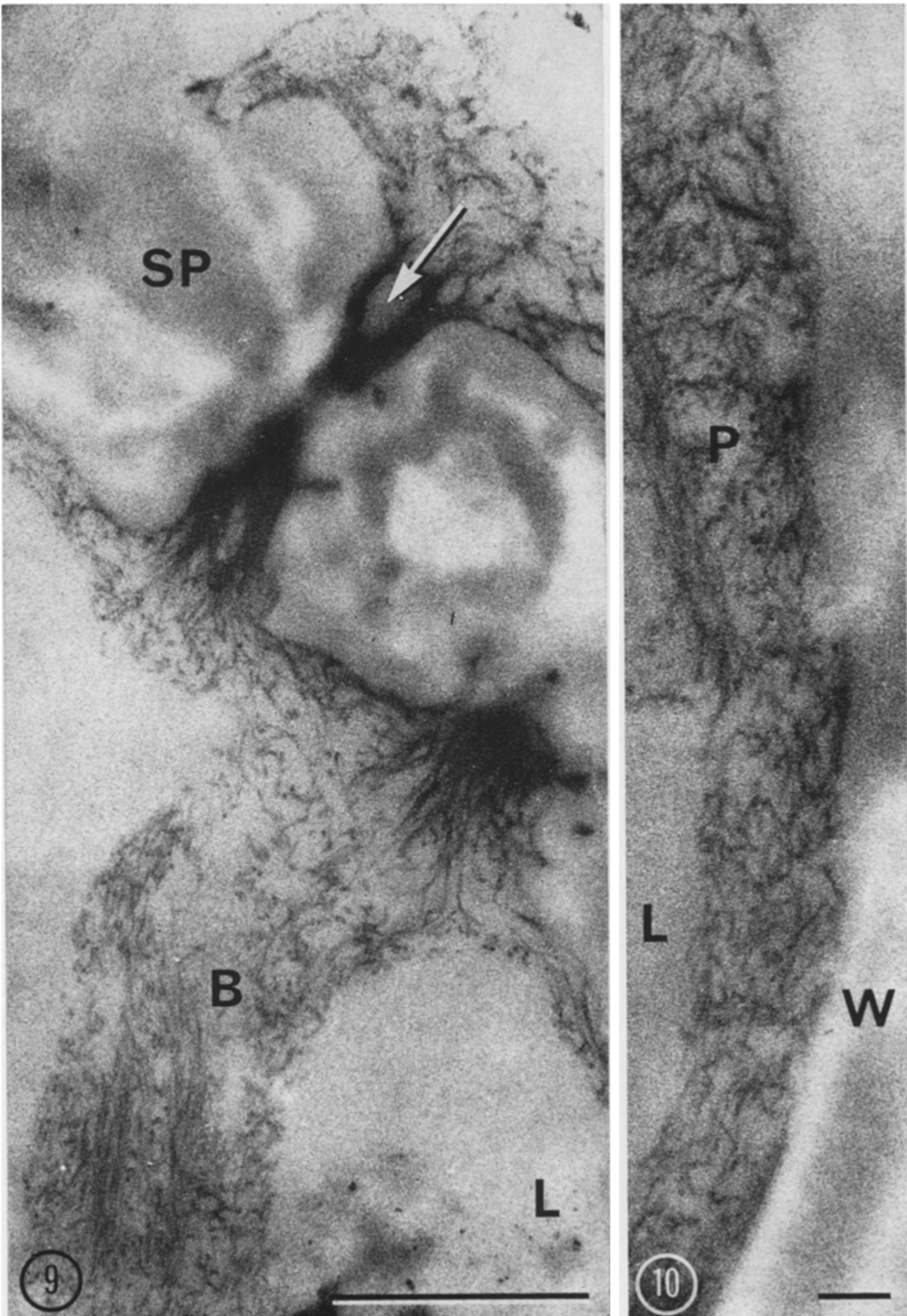


Fig. 9 and 10. Higher-magnification view of regions of the sieve element shown in Fig. 8. Fig. 9. The sieve-plate pore (arrow) shows an unobstructed central channel, with P-protein occurring as a peripheral lining. The P-protein within the lumen (*L*) of the sieve element is confined to a discrete bundle (*B*), with no indication of a limiting membrane. The filaments within the bundle are oriented longitudinally with respect to the lateral walls of the sieve element when the plane of the section is parallel to the longitudinal axis of the bundle. There is no evidence of ice crystals in the wall or lumen of the sieve element. Bar = 0.5 μm. Fig. 10. The parietal layer (P) of P-protein lines the lateral walls (*W*) of the sieve element. Most filaments run parallel to the lateral wall of the sieve element. Bar = 0.1 μm

3. Effect of Ice Crystal Formation on the Arrangement of P-Protein Fibrils

Tissue frozen directly in Freon 12 without the use of a cryoprotectant, invariably displayed ice crystal damage. Ice crystals were particularly noticeable in the cytoplasm and cell wall (Fig. 4), their size being 70–100 nm for both fixed and unfixed tissue. No ice crystals were visible in the empty lumen of the sieve elements (Fig. 4). However, when the standard of preservation was such that P-protein filaments were present in the lumen, ice crystals were visible between P-protein filaments (Fig. 7), and in fact appeared to displace the filaments. One ice crystal has been outlined in Fig. 7. The average diameter of the ice crystals among the P-protein filaments was 50 nm. Thus we believe ice crystals were present in the empty lumen, even though they were not visible in the sections.

4. P-Protein Distribution after Rapid Freezing on a Copper Surface

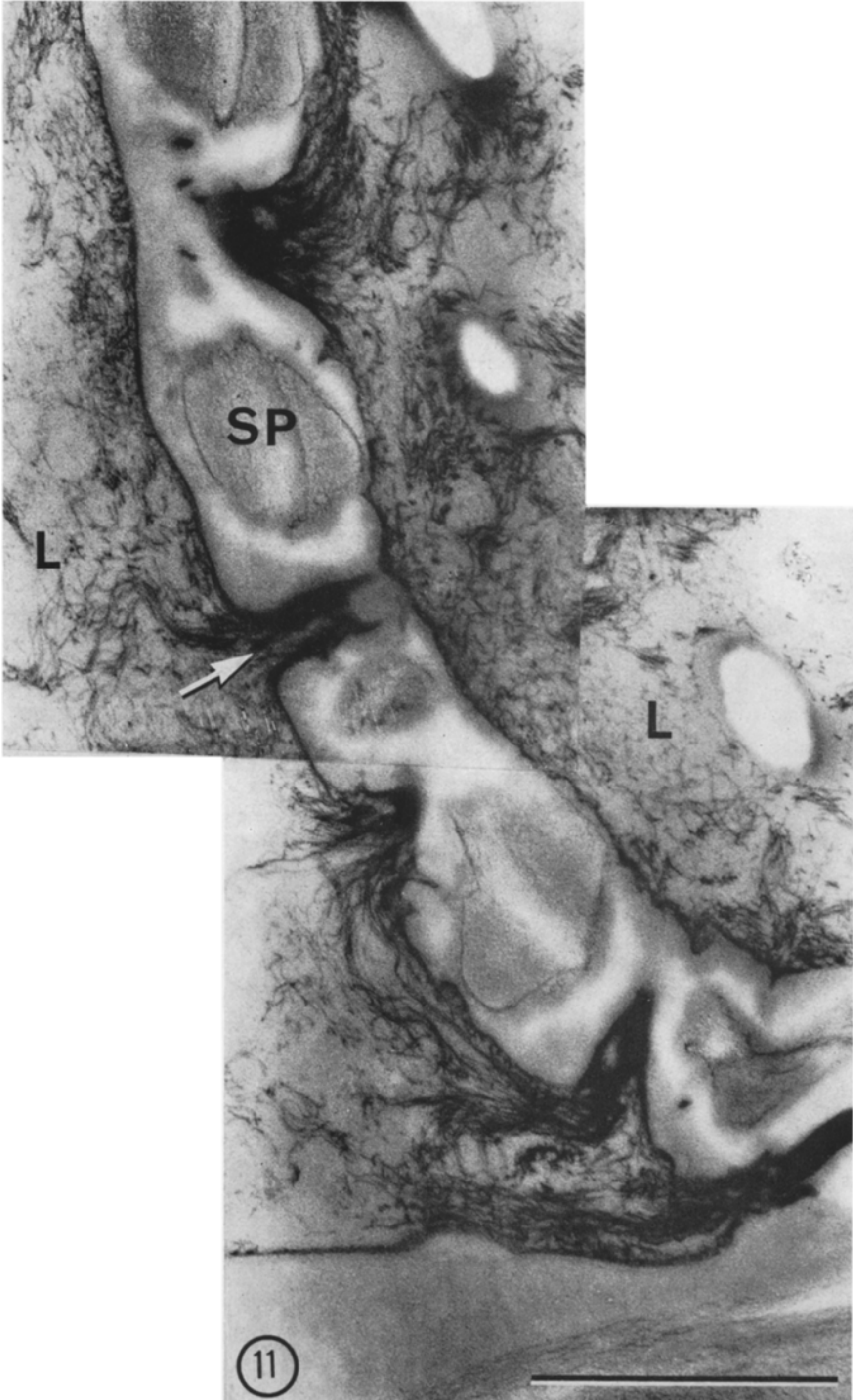
To obtain faster freezing rates and hence avoid the formation of damaging ice crystals, use was made of the good heat conducting properties of copper. The depth of the surface layer of tissue that was free of electron-microscopically visible ice crystals was found to be 12 μm for pea root tip (Dempsey, 1974). Within this layer in sieve elements from celery petioles, the cell walls and cytoplasm were very well-preserved, and the P-protein filaments were not displaced by the formation of ice crystals within the sieve element lumen. The sieve-plate pores were lined with callose and generally showed unobstructed central channels, although variable amounts of P-protein lined the pores and frayed out into the sieve-element lumen (Fig. 8).

The most remarkable feature of sieve elements in this region was the distribution of P-protein within the lumen of the sieve element. P-protein commonly existed as discrete bundles of parallel filaments, *ca.* 0.5 μm in diameter, oriented longitudinally within the sieve element (Figs. 8, 9). There was no evidence of any membrane enclosing the bundles. Bundles of filaments in the lumen of adjacent sieve elements did not appear to be continuous through the sieve-plate pores. In fact in only one sieve plate was a bundle seen to meet the sieve plate exactly opposite a pore. Similarly oriented P-protein also existed as a parietal layer, varying in thickness from 0.1 μm to 0.5 μm (Fig. 10). Of ten sieve plates examined in this well-frozen region, only one displayed randomly oriented P-protein filaments within the sieve-element lumen.

At a distance slightly greater than 12 μm from the tissue surface which had made contact with the copper, very small ice crystals (*ca.* 25 nm) were seen within the sieve-plate wall. In this region there were no bundles of P-protein filaments and the parietal layer of P-protein, if it existed at all, was very thin and disrupted. Most pores still displayed largely open channels but the P-protein radiating from the pores was often disoriented in the region close to the sieve plate (Fig. 11). There was neither a "piling-up" of P-protein above the sieve plate nor a "trailing" condition downstream.

Discussion

By minimising turgor release and avoiding the use of chemical fixatives, we have produced electron micrographs of sieve elements which we believe are the


SP
L
L
11

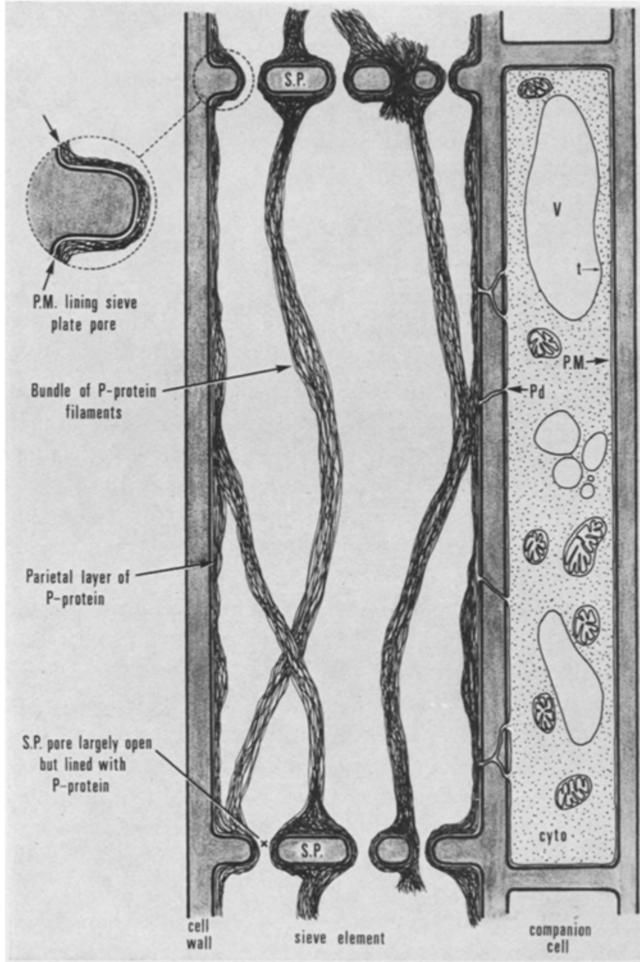


Fig. 12

Fig. 11. Freeze-substituted sieve element from a distance slightly greater than 12 μm from the tissue surface which made contact with the copper block. Small ice crystals (*ca.* 25 nm) exist in the sieve plate wall (*SP*). Note the absence of the bundles of filaments and the disrupted parietal layer of P-protein. The sieve plate pores (arrow) display an open central channel with a peripheral lining of P-protein. The P-protein within the lumen (*L*) of the sieve element is largely disoriented. Bar = 1 μm

Fig. 12. Diagram to show the organisation of structures within a celery sieve element from evidence obtained by freezing the phloem tissue while still attached to the plant and at a rate fast enough to prevent the disrupting effect of intracellular and extracellular ice crystals. (Not drawn to scale.) The salient features of this model are: 1) P-protein exists in discrete bundles within the lumen of the sieve element, and as a parietal layer lining completely the lateral walls and the sieve plate; 2) the sieve plate pores are largely open, but have their effective diameters reduced by about one-third because of the peripheral lining of P-protein; 3) the bundles of P-protein filaments do not appear to be continuous as discrete identities from one sieve element to the next through the sieve plate pores, although the individual filaments may be continuous through the pore; 4) the plasma membrane of the sieve element is continuous through the sieve plate pores. *S.P.* sieve plate, *P.M.* plasma membrane, *V* vacuole, *t* tonoplast, *Pd* plasmodesma, *cyto* cytoplasm

most unmodified from the *in-vivo* condition yet achieved. They show an internal organisation of the sieve element as represented diagrammatically in Fig. 12. Our results indicate that *in vivo* the P-protein of phloem sieve elements from celery petiole occurs as discrete bundles within the sieve-element lumen, and as a parietal layer lining the lateral walls of the sieve element. In general, the sieve-plate pores have a central unobstructed channel, but are lined with a peripheral layer of P-protein filaments, which reduce the effective diameter of the pore by about one-third.

Interpretation of micrographs such as Figs. 1–3 requires that turgor release causes a movement of the liquid in the file of sieve elements towards the two cut ends. Movement would be greatest near the ends, and least in the middle of the phloem strip. Consequently the sieve-plate pores in sieve elements from near the two cut ends are plugged with P-protein, much of which presumably flowed into the pores and lodged there during the sudden release of turgor. The movement of the sieve-element contents during turgor release would be much less at the centre of the file of sieve elements, and this is reflected in the less dense packing of the P-protein within the pores in this region. Disturbances to the contents of the sieve element, such as those caused by turgor release, are likely to disorganise the *in-vivo* distribution of P-protein. Extremely small movements of the sieve-element liquid, coupled with the chemical action of the fixative, would be capable of destroying any fine-structural network of P-protein that may extend between adjacent sieve plates in the lumen of the sieve elements.

Attempts by Jarvis and Thaine (1971) and Jarvis *et al.* (1973) to avoid chemical fixation gave results which they believe reinforced their concept of transcellular strands with discrete boundaries which are continuous through the sieve-plate pores, which they fill. These authors froze vascular bundles which were still attached to the stem at each end, and then warmed the frozen tissue to $-28°$ in a cryostat cabinet just prior to sectioning. The sections were thawed rapidly and viewed in 1% glutaraldehyde in the light microscope. Comparison of the sieve-element contents following different rates of freezing led them to conclude that rapid freezing of phloem tissue in Freon 12 accurately preserved the *in-vivo* structure of the sieve element. Johnson (1973) froze intact vascular bundles in a similar manner, after first showing that they were translocating ^{14}C prior to freezing. The freeze-etch replicas he prepared of this tissue displayed P-protein filaments within the sieve plate pores and lumen, although much of the sieve element was obscured by etched ice crystals.

We have found, however, that the rate of cooling obtained by freezing unprotected tissue in Freon 12 is not sufficient to avoid the formation of damaging intracellular ice crystals. For tissue fixed in glutaraldehyde prior to freezing and freeze-substitution, ice crystals *ca.* 100 nm in diameter are visible in the sieve plate and sieve-element wall (Fig. 4). Since ice crystals were not obvious within the sieve-element lumen, it was at first assumed that the cryoprotective effect of the sucrose in the lumen (which may approach 30%) prevented their formation. However, micrographs of unfixed tissue, in which the P-protein retained its filamentous distribution and did not become a structureless mass, demonstrated that ice crystals *ca.* 50 nm in diameter do exist in the lumen of sieve elements frozen in Freon 12 (see Figs. 5, 6, 7). Since during freeze-substitution all water

or ice is replaced by an organic solvent, it follows that ice crystals that are not outlined by some structure (such as P-protein filaments) would not appear as discrete structures after freeze-substitution. Hereward and Northcote (1972) have also examined celery phloem after freezing the unprotected tissue in Freon 22 and substituting for only 3 days at $-78°$. These authors believed that a surface layer of cells 100 μm thick was frozen rapidly enough to prevent ice-crystal formation. However, our results do not support this opinion. We believe that a substitution time of only 3 days at $-78°$ may not be sufficient, and that ice crystals which were formed on freezing were not properly substituted, so that on warming the tissue the ice crystals melted and were only then replaced by the substitution fluid. Structures initially displaced by the formation of ice crystals on freezing could thus resume their former positions when the ice melts (as envisaged by Baker, 1962). The contention of Jarvis *et al.* (1973) that their light-microscope sections of phloem tissue frozen in Freon 12 represent the *in-vivo* structure of the sieve elements may also be questioned on similar grounds. Ice crystals which formed on freezing would thaw rapidly when sections from the cryostat were immersed in glutaraldehyde on a glass slide; and displaced structures may or may not resume their *in-vivo* positions. The fact that the 50–100-nm ice crystals found in sieve elements following Freon 12 freezing are not visible in the light microscope does not exclude the possibility that ice crystals of such dimensions could cause gross ultrastructural changes which are visible in the light microscope. The formation of ice crystals in the lumen of the sieve element may cause movements of the sieve-element fluid such that blocking of pores and apparent turgor effects occur.

The differences in structure observed with the different fixation procedures fit a logical pattern in which the least modified structure is that observed by freeze substitution after freezing an attached phloem strand on a copper block at liquid-nitrogen temperature. This method shows clear channels in the sieve-plate pores, and the absence of any "piling-up" or "trailing" of P-protein above or below the sieve plate. In addition, the P-protein filaments are arranged into discrete bundles, a condition not apparent in sieve elements prepared for electron microscopy by any other method. Johnson (1973) also described short bundles of filaments near sieve plates in freeze-etched sieve elements from *Nymphoides peltata*. However, the extent and orientation of such bundles be viewed with caution because of the damaging effects of the ice crystals within the lumen. A similar criticism may be levelled at his calculations of the spaces between P-protein filaments within the sieve-plate pores: 200–500-nm ice crystals would cause gross disarrangement of the 10–25-nm filaments within the pores. In light-microscope sections of *Cucurbita pepo* phloem frozen in Freon 12, Thaine and De Maria (1973) and Jarvis *et al*, (1973) describe discrete longitudinally-oriented membrane-bound transcellular strands with diameters approximately the same as those of the sieve-plate pores and which appear to pass through the pores. There are indications that the strands are composed of longitudinally-oriented filaments. We have found no evidence of a membrane surrounding the bundles of P-protein filaments; even though the plasma membrane lining the sieve plate and sieve-plate pores appears to be well-preserved. This observation coupled with the absence of any membrane surrounding bundles of filaments in freeze-etch preparations (Johnson,

1973), appears to argue against the existence of membrane-bound transcellular strands.

At depths slightly greater than 12 μm from the well-frozen surface, where small (25 nm) ice crystals occur in the sieve-element walls and in the adjacent cells, bundles of P-protein filaments are not found near the sieve plate. Although ice crystals are not obvious between individual P-protein filaments at this depth they probably do occur, but are smaller than 25 nm because of the cryoprotective effect of the sucrose in the sieve-element lumen. It thus appears that even very small ice crystals are sufficient to disrupt the fine-structural organisation of the P-protein filaments in the sieve-element lumen. This would explain the finding by Johnson (1973) of only short bundles of filaments with separate filaments nearby, for ice crystals were visible in his preparations. The extreme susceptibility of the bundles of P-protein to physical and chemical manipulation would also explain their absence in glutaraldehyde-fixed thin sections.

In the past it has been difficult to reconcile the established physiological characteristics of sieve-element translocation with structures observed by electron microscopy. Weatherley (1972) has calculated on theoretical grounds that, for the simple Münch pressure-flow hypothesis to apply, the P-protein filaments within the sieve-plate pore need to be at least 100 nm apart if they are uniformly distributed. He also calculated that if P-protein filaments were uniformly dispersed through the sieve-element lumen, there would be a high viscous drag on moving sieve-element solution. If the P-protein filaments were organised into bundles, or into a peripheral layer, the viscous drag would be far less. The distribution of P-protein which we have seen conforms very well to Weatherley's prescriptions for mass flow. Since the P-protein in the lumen of the sieve element is confined to discrete bundles and to a peripheral layer, the major resistance to flow would reside in the sieve-plate pores. We propose that there are open channels through the pores, but with an effective diameter less than that of an unobstructed pore because of the thin layer of P-protein lining the pore. Recalculations of pressure gradients in terms of a reduced pore diameter will be necessary once exact measurements of sieve-pore size, total pore area, frequency of sieve plates, and concentration of sieve-element contents have been made. Preliminary calculations we have made along the lines of those discussed by Weatherley (1972), and using data from a number of different sources and for a number of different species, indicate that a Münch pressure-flow hypothesis of phloem translocation would be feasible. However, recent theoretical considerations by Allen (1974) and Fensom and Williams (1974) suggest that mass flow could be activated by the swishing of microfilament material, occurring as bundles of filaments extending from the sieve plate to the lateral walls of the sieve element, or as microfilaments attached to axial fibrils. We have established the existence of bundles of filaments (but not fibrils with filamentous branches) in the lumen of the sieve elements, and these may be involved in active propulsion of the sucrose solution. The occurrence of pores largely free from filamentous material would greatly reduce the obstruction to flow through the sieve-plate pores.

We thank R. E. F. Matthews and E. G. Bollard for discussion of the manuscript.

References

Allen, N. S.: Endoplasmic filaments generate the motive force for rotational streaming in *Nitella*. J. Cell Biol. **63**, 270–287 (1974)

Anderson, R., Cronshaw, J.: Sieve-plate pores in tobacco and bean. Planta (Berl.) **91**, 173–180 (1970)

Baker, R. F.: Freeze-thawing as a preparatory technique for electron microscopy. J. Ultrastruct. Res. **7**, 173–184 (1962)

Cronshaw, J., Anderson, R.: Sieve plate pores of *Nicotiana*. J. Ultrastruct. Res. **27**, 134–148 (1969)

Dempsey, G. P.: Ultrastructural preservation related to rapidity of freezing. Proc. VIII Int. Congr. Electron Microscopy, Canberra, Australia, vol. II, p. 42–43 1974

Evert, R. F., Eschrich, W., Eichhorn, S. E.: P-protein distribution in mature sieve elements of *Cucurbita maxima*. Planta (Berl.) **109**, 193–210 (1973)

Fensom, D. S., Williams, E. J.: On Allen's suggestion for long-distance translocation in phloem of plants. Nature (Lond.) **250**, 490–492 (1974)

Hereward, F. V., Northcote, D. H.: A simple freeze-substitution method for the study of ultrastructure of plant tissues. Exp. Cell Res. **70**, 73–80 (1972)

Jarvis, P., Thaine, R.: Strands in sections of sieve elements cut in a cryostat. Nature (Lond.) New Biol. **232**, 236–237 (1971)

Jarvis, P., Thaine, R., Leonard, J. W.: Structures in sieve elements cut with a cryostat following different rates of freezing. J. exp. Bot. **24**, 905–919 (1973)

Johnson, R. P. C.: Microfilaments in pores between frozen-etched sieve elements. Planta (Berl.) **81**, 314–332 (1968)

Johnson, R. P. C.: Filaments but no membranous transcellular strands in sieve pores in freeze-etched, translocating phloem. Nature (Lond.) **244**, 464–466 (1973)

Mishra, U., Spanner, D. C.: The fine structure of the sieve tubes of *Salix caprea* (L.) and its relation to the electro-osmotic theory. Planta (Berl.) **90**, 43–56 (1970)

Siddiqui, A. W., Spanner, D. C.: The state of the pores in functioning sieve plates. Planta (Berl.) **91**, 181–189 (1970)

Spurr, A. R.: A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43 (1969)

Thaine, R.: A translocation hypothesis based on the structure of plant cytoplasm. J. exp. Bot. **13**, 152–160 (1962)

Thaine, R.: Movement of sugars through plants by cytoplasmic pumping. Nature (Lond.) **222**, 873–875 (1969)

Thaine, R., De Maria, M. E.: Transcellular strands of cytoplasm in sieve tubes of Squash. Nature (Lond.) **245**, 161–163 (1973)

Van Harreveld, A., Crowell, J.: Electron microscopy after rapid freezing on a metal surface and substitution fixation. Anat. Rec. **149**, 381–385 (1964)

Weatherley, P.: Translocation in sieve tubes. Some thoughts on structure and mechanism. Physiol. vég. **10**, 731–742 (1972)