Post-phloem transport: principles and problems

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Received 23 September 1995; Accepted 16 January 1996

Abstract

The movement of assimilates from the sieve element/companion cell complex to sites of utilization has been examined in an extensive array of sinks possessing diverse anatomies. This work has been reviewed with respect to the pathways taken, the conductances and driving forces for movement along the pathways, and interaction between the apoplast and symplast. Most investigations to date have been concerned primarily with determining the pathway of assimilate movement. A symplastic pathway is followed in the great majority of cases studied. However, available methods are less suited for demonstrating apoplastic transport in those instances where it occurs. Far less information is available on quantitative aspects of post-phloem transport. Only a very limited number of observations are available on the diffusive or hydraulic conductances of the apoplast or symplast. In some cases, symplastic conductance appears to be enhanced by a larger-than-usual size exclusion limit for cell-to-cell transport. Measurements of the driving forces for post-phloem transport (i.e., gradients in concentration and/or pressure) are also very few in number nor, to date, are they always readily interpretable. Evaluation of solute movement is complicated by interactions between the apoplastic and symplastic pathways, including water relations effects and solute exchange. The presence of apoplastic domains or, simply, high resistance to movement in the apoplast, can lead to steep water relations gradients within sinks, with important implications for transport. To understand how import into sinks is controlled, many more quantitative measurements are needed. This will require considerable experimental ingenuity.

Key words: Apoplast, plasmodesmata, phloem (unloading), symplast, water relations.

Introduction

Almost invariably, assimilates are utilized at some distance from the conducting cells which deliver them to the sink. In contrast to long-distance conduction via the sieve element–companion cell (SE/CC) complex, the intercellular pathways for post-phloem transport are varied and often fairly complex. Pathway characteristics depend on the particular sink involved and the stage of sink development. To some extent, part of the structural diversity of these pathways simply reflects the anatomical diversity of sinks. More importantly, however, there is a considerable range of structure–function relationships in the pathways for post-phloem transport. This includes such aspects as path conductance, apoplastic versus symplastic solute distribution and movement, solute concentrations, partitioning of sink tissues into domains, and the gradients in concentrations and turgor pressure which cause movement to occur. Given the importance of sinks in controlling assimilate distribution (Gifford and Evans, 1981), it seems likely that the diversity of structure–function relations in sinks is an important factor in their relative ability to import assimilates.

Post-phloem transport is potentially a very broad topic. (Precisely speaking, perhaps it should be termed ‘post-sieve element/companion cell transport’, but that phrase is awkward.) This coverage will focus on the transport pathway which immediately follows the unloading of assimilates from the SE/CC complex. The main effect of this limitation will be to exclude the final steps of assimilate movement to and within embryonic tissues. Also, the very interesting question of plant-to-pathogen transport will not be included. For a detailed case study of post-phloem transport the reader is referred to the accompanying article by Patrick and Offer.

Here, some basic theoretical considerations are presented, followed by experimental observations on the pathway of solute movement, the gradients which drive...
transport, and the role of domains (both apoplastic and symplastic) in post-phloem transport.

Some theoretical considerations

SE/CC unloading

Unloading from the SE/CC complex must be viewed as a separate transport step, distinct from subsequent post-phloem movement. (For a consideration of terminology relating to SE/CC unloading and the subsequent steps involved in cell-cell transport, see Oparka and van Bel, 1992.) The interface between the SE/CC complex and surrounding cells often differs structurally from that between cells of the post-phloem pathway and, because of the high osmotic and turgor pressures of the SE/CC complex, the driving forces and possible controls on transport will almost certainly differ. Nonetheless, the pathway of SE/CC unloading, i.e. whether apoplastic or symplastic, must be considered briefly because of its obvious implications for the pathway taken by assimilates in moving away from the SE/CC complex.

Several workers, citing probable limitations to transmembrane water and solute movement, have presented sound theoretical arguments for a symplastic pathway for SE/CC unloading in strong sinks such as meristems, fruits and seeds. Murphy (1989), in a thought-provoking analysis of the water relations of phloem unloading, concludes that a wholly apoplastic (i.e. transmembrane) unloading pathway would lead to unrealistically high water potential and turgor in the sink sieve tubes. Similarly, Offler and coworkers (Offler and Patrick, 1984, 1993; Wang et al., 1995) point to the unrealistically high transmembrane sugar fluxes, in comparison to known values for plants, required for apoplastic unloading. These arguments are appropriate but, as the authors note, they depend on the assumed bounds for membrane transport. The values chosen by Murphy for membrane hydraulic conductivity, though taken from measurements on sieve tubes, are only 1–10% of most values for higher plant cell membranes (Steudle, 1990). (However, it should be noted that the latter figures are actually composite values for symplastic and transmembrane water movement; Steudle, 1990.) The upper value cited by Offler and coworkers for sugar flux are the highest for plants, but more rapid movement is possible (e.g. the flux for facilitated glucose transport into red blood cells is higher by almost two orders of magnitude; Jung, 1975). Also, given the non-specific nature of unloading, it is conceivable that unloading could occur through membrane pores. Thus, apoplastic unloading appears feasible for strong sinks, even if scepticism is warranted. In any event, the observed range of transmembrane fluxes could support lower rates of assimilate demand (e.g. maintenance respiration) or, by maintaining an apoplastic solute pool of non-moving or slowly-moving solutes, could profoundly influence the water relations of symplastic unloading and post-phloem transport.

If the apoplast is at atmospheric pressure, apoplastic solutes will lower cell turgor pressure. Thus the issue of apoplastic versus symplastic unloading from the SE/CC complex has important implications beyond the mere localization of solutes within the phloem or along the post-phloem pathway. These have been noted by several workers, and were elaborated upon by Lang and Thorpe (1986).

Diffusive transport in the apoplast

In the absence of a pressure gradient, solute movement through the apoplast must depend on diffusion along a concentration gradient. Solute diffusion through a tissue, however, will be greatly reduced in comparison to movement through a similar volume of water. Typically, walls represent less than 10% of the tissue cross-section. Diffusion within the walls will be hindered, mostly by collisions with wall components, but potentially by binding and increased viscosity of the aqueous phase within the wall. Together, the effects of reduced cross-sectional wall area and hindered diffusion will slow considerably the apoplastic diffusion of solutes through a tissue, as compared to a similar volume of water.

The number of estimates for diffusion rates within the walls of living cells is disappointingly few, especially for sugars. Richter and Ehwald (1983) estimated the diffusion coefficient for sucrose in the cell walls of sugar beet (c. \(8 \times 10^{-11} \text{m}^2\text{s}^{-1}\)) to be about 1/6th of that in water (4.7 \(\times 10^{-10} \text{m}^2\text{s}^{-1}\)). Since cell walls occupied only 3% of the tissue area, the effective diffusivity of sucrose in the tissue (c. \(2.4 \times 10^{-12} \text{m}^2\text{s}^{-1}\)) was only about 0.5% of that in water. This is comparable to typical values for symplastic movement (see below). Canny (1990), in following the movement of fluorescent tracers, provides estimates for the diffusion of these solutes in cell walls of wheat leaves which are much lower, by 0.01–10%, than Richter and Ehwald’s value for sucrose.

Solute concentrations and concentration gradients in the apoplast have profound implications for conditions within the symplast. If apoplastic transport is supposed to occur by diffusion, there must be a concentration gradient along the pathway. If water potential is presumed to be uniform, there must be either a corresponding osmotic gradient along the symplast or, if not, a turgor gradient in the opposite direction. Either condition will tend to cause movement along the symplast. If the balancing component of water potential is by a symplastic concentration gradient, there will be a diffusion of solutes within the symplast, parallel to the direction of apoplastic transport. If a turgor gradient is present, it should cause bulk flow in the opposite direction to apoplastic transport. Potentially, diffusive and convective transport could...
cancel, given a particular combination of concentration and turgor gradients. These factors must be considered and accounted for in situations where significant concentrations of apoplastic solutes are presumed to occur.

Finally, it should not be supposed that apoplastic solutes and concentration gradients are necessarily most important in the context of transport within the apoplast itself. Clearly, given the interactions noted above, they would have a profound influence on symplastic transport even if no movement at all were occurring in the apoplast. In many instances, the apoplast may be of far greater importance as an exchange compartment for the regulation of turgor pressure. Tomos et al. (1992) review the evidence for this over the course of a growing season in beet roots; it is but a step to suppose that turgor might be held constant with distance as well as time, leaving concentration gradients as the driving force for transport, primarily along the symplast. Sucrose uptake is turgor-regulated in potato tubers (Oparka and Wright, 1988), suggesting that it may be a component of a turgor-regulatory system.

**Diffusive transport in the symplast**

Assimilates move along the symplastic pathway by passing from cell to cell via plasmodesmata and across cells via the cytosol. Despite the much longer distance of the transcellular pathway, the overall rate of symplastic transport is limited by cell-to-cell movement through plasmodesmata, but because the channels through plasmodesmata are comparable in size to the solutes involved, the biophysical principles that describe the transport properties of plasmodesmata are quite complex (Finkelstein, 1987). This is compounded by the absence of basic information concerning plasmodesmal structure, such as the site and length of the restricted passage within channels, and the number of channels per plasmodesma and the apparently dynamic nature of plasmodesmal structure (Lucas et al., 1993). Given these circumstances, quantitative descriptions of plasmodesmal transport are largely restricted to extrapolations from measurements made on only a few plant cells, supplemented by speculations based on theory or on the behavior of better characterized model systems.

Quantitatively, the relationship between concentration gradient and diffusive transport along the symplast may be expressed as either a cell-to-cell permeability coefficient \( J = P \Delta C \), where \( \Delta C \) is the concentration difference between cells) or as an overall diffusivity coefficient \( J = D \delta C / \delta x \), where \( \delta C / \delta x \) is the distance-smoothed concentration gradient). \( P \) and \( D \) will be related by \( P = D / \Delta x \), where \( \Delta x \) is the trans-cell distance. Goodwin et al. (1990) measured a permeability coefficient of \( 1.1 \times 10^{-6} \text{ m s}^{-1} \) for intercellular carboxyfluorescein (CF) movement in *Egeria* leaves, and the data of Tucker et al. (1989) provide a similar value of \( 0.8 \times 10^{-6} \text{ m s}^{-1} \) for CF movement in *Setcreasea* stamen hairs. Unfortunately, only the latter workers provided data on plasmodesmatal frequency, required for (tentative) extrapolation of their observations to cell-to-cell junctures with different plasmodesmatal frequencies.

Based on Tucker et al.'s measurements and their own morphometric analysis of the symplastic pathway in corn root tips, Bret-Harte and Silk (1994) calculated expected values for the diffusivity of sucrose in the root tip tissues distal to the protophloem. In general, the most important factors in determining the diffusivity for symplastic transport will be cell size, plasmodesmatal frequency (assuming functionality) and the number and dimensions of the conducting channels in the plasmodesmata. Bret-Harte and Silk's treatment is a very useful synthesis, since it is based on specific, informed, assumptions and allows the calculation of expected solute fluxes from observed or hypothetical concentration gradients. Also, values of diffusivity can be compared for different paths, for example, with apoplastic transport. Their values for sucrose diffusivity range from about \( 10-20 \times 10^{-12} \text{ m}^2 \text{ s}^{-1} \). This is distinctly higher than estimated above for diffusivity in the apoplast. However, plasmodesmatal frequencies in corn root tips are higher than usual. Thus, as a rough comparison, expected diffusivities for the apoplast and symplast appear to be comparable. Obviously, their relative importance in a particular tissue may be quite different.

The dimensions of the conducting channels within plasmodesmata are a crucial factor in determining cell-to-cell conductivity. Almost universally, the channel dimensions in plasmodesmata between healthy plant cells impose a size exclusion limit (SEL) of about 800–1000 Da \( (R_h \text{ approximately } 0.9 \text{ nm}) \), suggesting a channel diameter of about 3 nm (Terry and Robards, 1987). However, the phloem may be an exception. Kempers et al. (1993) showed that 3 kDa fluorescent dextran could move between *Cucurbita* companion cells and sieve elements. More importantly for the present context, Wang and Fisher (1994b) found an SEL of about 3 nm (hydrodynamic radius) for movement along the post-phloem pathway in wheat grains, suggesting a channel diameter within these plasmodesmata of about 7 nm. Oparka and Prior (unpublished observations) have also demonstrated, by microinjection, that 3 kDa probes move freely from cell to cell in the post-phloem pathway of *Arabidopsis* root tips. Evidently, the plasmodesmata in post-phloem pathways may be modified to cope with high solute fluxes.

The effect of channel dimensions on the per channel conductance for diffusive transport is quite marked. Renkin (1954; his equation 11) provides a quantitative basis for estimating the combined effects of steric exclusion and frictional resistance. For sucrose, the per channel conductance of a 7 nm channel should be \( 12 \times \) that of a
'standard-size' 3 nm channel. Clearly, additional measurements of SEL are required for plasmodesmata in a wider variety of sink regions.

**Convective transport**

Because the transpiration rate of sinks is typically very low, the rate of water movement in sink tissues will also be slow, comparable in many cases (e.g. seeds, fruits and root tips) to the rate of water delivery by the phloem. Even when significant transpiration might occur (e.g. growing stems), it can be essentially eliminated (darkness, high humidity) without slowing growth. Thus there appears to be no requirement for a substantial rate of water flow, above that supplied by phloem transport, to move solutes into sinks. The highest proportion of solute movement during convective transport will occur in the phloem, because the concentration is highest there. Elsewhere in the sink, where concentrations are usually much lower than in the phloem, convective transport will necessarily carry a lower proportion of solutes. Most likely, then, a large proportion of post-phloem solute movement in many sinks occurs by diffusion.

Convective transport must, nevertheless, contribute significantly in some cases to apoplastic and/or symplastic solute movement, especially when concentration is high. However, values for hydraulic conductivity, of either the apoplast or symplast, are notoriously difficult parameters to obtain, and will clearly vary, depending on the site and circumstances. Even for undifferentiated parenchyma cells, estimates for cell wall hydraulic conductivity range over three orders of magnitude (Steudle, 1990). Values for the symplast are even more uncertain (Molz and Ferrier, 1982), although Gunning and Hughes (1976) provide a credible estimate for prenectar movement in the *Abutilon* nectary. Thus, even if the pressure gradients were known, it would be difficult to use them to estimate rates of transport. At present, a more useful approach is to use estimated rates of water movement, based on other information (e.g. growth rate, secretion rate), to calculate possible rates of convective solute transport.

Because the SEL for the post-phloem pathway may be larger than the 'standard' 800 Da, it is instructive to draw a comparison between the expected effect of channel size on the diffusive versus hydraulic conductance of the channel. That there will be a marked difference can be seen from the Hagen–Poiseuille equation, which states that the hydraulic conductance of a cylindrical channel will increase with the fourth power of the radius. Diffusive transport, however, will be proportional to the area of the capillary, that is, to the square of the radius. Thus the hydraulic conductance of a channel will be much more sensitive to channel area than the diffusive conductance. The relative effects on solute transport, however, will depend on the specific conditions, especially concentration. Diffusive solute flux is proportional to the concentration gradient, while convective solute flux is proportional to concentration.

**Domains**

Neither the apoplast nor the symplast is continuous throughout the plant. Within the apoplast, cell wall deposits or, simply, thin cell walls may obstruct solute movement between different parts of the apoplast. In effect, this divides the apoplast into regions, or 'domains', where adjacent regions of the apoplast may differ substantially in their solute concentrations and water potentials. Clearly, this will profoundly affect the driving forces for transport between the regions. As Bradford (1994) notes, blockage in the apoplast may not be complete. If water can move, but solutes can not or are strongly restricted, the barrier would be semi-permeable or nearly so.

In the symplast, if plasmodesmata are absent altogether, as between maternal and embryonic tissues, the limit of a symplastic domain is clearly marked. When plasmodesmata are present between cells, as they usually are, their frequency (number of plasmodesmata per area of cell wall) is often taken as a quantitative indicator of symplastic continuity and conductance. In the absence of other information, plasmodesmatal frequencies, cleverly summarized in the concept of 'plasmodesmograms' (van Bel et al., 1988), provide a valuable first impression of the potential for symplastic transport within an organ or tissue. However, there are distinct limitations to this representation. These are addressed by van Bel and Oparka (1995), who summarize recent evidence on the variations reported in the SEL for plasmodesmata (from non-conducting to 10 kDa), and for regulation of the SEL. These include instances where plasmodesmata are present but are apparently impermeable (Erwee and Goodwin, 1985; Duckett et al., 1994), the effect of applied pressure differentials between cells (Oparka and Prior, 1992) and responsiveness to intracellular Ca$^{2+}$ levels (Tucker, 1990). Yet other studies indicate that the SEL may be held at a particular value by an ATP-dependent process (Cleland et al., 1994) and that mild water stress may increase the functional diameter of the cytoplasmic sleeve of plasmodesmata (Schulz, 1995). Clearly, these observations mandate caution in interpreting the visual impression provided by plasmodesmograms. As van Bel and Oparka conclude, they are valuable representations of the possibilities for symplastic transport, but must be supplemented by more direct physiological measurements.

**The path taken: apoplast or symplast?**

**Summary of observations**

Post-phloem transport has been investigated in an increasing number of sinks, using a variety of methods. Table 1
Table 1. Summary of evidence concerning the post-phloem transport pathway for assimilates, and the experimental approaches taken to identifying the pathway

<table>
<thead>
<tr>
<th>Organ, species</th>
<th>Path</th>
<th>Evidence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
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<tr>
<td>Pea (tip)</td>
<td>S</td>
<td>Asymmetric succ; apoplastic sugars did not reduce phloem import</td>
<td>Dick and ap Rees (1975)</td>
</tr>
<tr>
<td>Pea (tip)</td>
<td>S</td>
<td>Plasmolysis, no sugar loss to medium</td>
<td>Schulz (1994)</td>
</tr>
<tr>
<td>Corn (tip)</td>
<td>S</td>
<td>Asymmetric succ; EM</td>
<td>Giaquinta et al. (1983)</td>
</tr>
<tr>
<td>Corn (mature?)</td>
<td>S?</td>
<td>EM/morphometric</td>
<td>Warmbrodt (1985)</td>
</tr>
<tr>
<td>Arabidopsis (tip)</td>
<td>S</td>
<td>Dye coupling (CFDA loading)</td>
<td>Oparka et al. (1994)</td>
</tr>
<tr>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>S</td>
<td>Dye coupling (apoplastically-loaded fluorescein)</td>
<td>Cook and Oparka (1983)</td>
</tr>
<tr>
<td>Wheat</td>
<td>S</td>
<td>Dye coupling (CFDA loading); plasmolysis; microautoradiography</td>
<td>Wang and Fisher (1994a, b)</td>
</tr>
<tr>
<td>Wheat</td>
<td>S</td>
<td>Dye coupling (CFDA loading); plasmolysis</td>
<td>Wang et al. (1994)</td>
</tr>
<tr>
<td>Wheat</td>
<td>S</td>
<td>EM/morphometric/flux analysis</td>
<td>Wang et al. (1995)</td>
</tr>
<tr>
<td>Rice</td>
<td>S</td>
<td>EM; microautoradiography; dye coupling (apoplastically-loaded fluorescein)</td>
<td>Oparka and Gates (1981, 1982)</td>
</tr>
<tr>
<td>Corn</td>
<td>S</td>
<td>EM; microautoradiography</td>
<td>Felker and Shannon (1980)</td>
</tr>
<tr>
<td>Legumes</td>
<td>S</td>
<td>EM/morphometric/flux analysis; PCMBS; dye coupling (CFDA loading); plasmolysis; microautoradiography</td>
<td>Offer and Patrick (1984, 1993)</td>
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<tr>
<td>Leaf</td>
<td></td>
<td></td>
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<tr>
<td>Sugar beet</td>
<td>S</td>
<td>PCMBs, whole-leaf autoradiography</td>
<td>Schmalsstig and Geiger (1985)</td>
</tr>
<tr>
<td>Tobacco</td>
<td>S</td>
<td>Anaerobiosis, whole-leaf autoradiography; EM/morphometric</td>
<td>Turgeon (1987)</td>
</tr>
<tr>
<td>Corn</td>
<td>A</td>
<td>EM</td>
<td>Evert and Russin (1993)</td>
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<td>Stem</td>
<td></td>
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<tr>
<td>Elongating</td>
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<tr>
<td>Sunflower</td>
<td>S</td>
<td>Sugar uptake &lt;5% of import rate</td>
<td>McNeil (1976)</td>
</tr>
<tr>
<td>Pea</td>
<td>S</td>
<td>Sugar uptake &lt;5% of import rate; non-specific unloading</td>
<td>Schmalsstig and Cosgrove (1990)</td>
</tr>
<tr>
<td>Castor bean</td>
<td>A?</td>
<td>Apoplastic solutes in cortex (turgor changes much larger than changes)</td>
<td>Meshcheryakov et al. (1992)</td>
</tr>
<tr>
<td>Non-elongating, with secondary growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>A</td>
<td>Free space washout; microautoradiography</td>
<td>Patrick and Turvey (1981)</td>
</tr>
<tr>
<td>Bean</td>
<td>A</td>
<td>Free space washout; PCMBs; plasmolysis</td>
<td>Minchin and Thorpe (1984), Minchin et al. (1984)</td>
</tr>
<tr>
<td>Bean</td>
<td>A or S</td>
<td>EM/morphometric/flux analysis</td>
<td>Hayes et al. (1985)</td>
</tr>
<tr>
<td>Bean (decapitated)</td>
<td>A or S</td>
<td>Comptl analyses; PCMBs; plasmolysis; path depended on growing season</td>
<td>Hayes et al. (1987)</td>
</tr>
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<td>Ray cells</td>
<td></td>
<td></td>
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<tr>
<td>Poplar</td>
<td>S</td>
<td>EM/morphometric/flux analysis</td>
<td>Sauter and Kloth (1986)</td>
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<tr>
<td>Storage parenchyma</td>
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<tr>
<td>Sugar cane</td>
<td>S</td>
<td>High apoplastic sugar conc; apoplastic barrier between phloem and storage parenchyma</td>
<td>Moore and Cosgrove (1991)</td>
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<tr>
<td>Storage root</td>
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<tr>
<td>Sugar beet</td>
<td>A</td>
<td>Apoplastic sugar uptake</td>
<td>Wyse (1979)</td>
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<tr>
<td>Tuber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>S?</td>
<td>EM</td>
<td>Oparka (1986)</td>
</tr>
<tr>
<td>Potato</td>
<td>S</td>
<td>Dye coupling (injection into parenchyma); plasmolysis</td>
<td>Oparka and Prior (1987, 1988)</td>
</tr>
<tr>
<td>Potato</td>
<td>S</td>
<td>Dye coupling (injection into phloem)</td>
<td>Oparka et al. (1992)</td>
</tr>
<tr>
<td>Potato</td>
<td>A?</td>
<td>Apoplastic yeast invertase</td>
<td>Heineke et al. (1992)</td>
</tr>
</tbody>
</table>
lists the sinks examined and an abbreviated summary of the evidence provided for the pathway of transport. Most experimental systems were actively expanding organs or were engaged in active storage. Non-elongating stems are the exception, although some secondary growth was occurring. In a majority of cases, post-phloem movement was judged to follow a symplastic pathway. Only in a few instances have observations implicated an apoplastic pathway later in development (Ruan and Patrick, 1995). The path during pre-ripening appears to be less well characterized. Apoplastic transport may occur in at least the latter portion of the post-phloem transport pathway in citrus fruits, since movement continued in the vesicle stalks even after the stalks were frozen and thawed (Koch et al., 1986). However, the path taken during earlier stages of this unusually long pathway (up to 3 cm) appears less certain.

**The structural approach: do plasmodesmata provide an adequate pathway for transport?**

Plasmodesmatal frequency has often been used to evaluate the capacity of the symplast to accommodate transport. This approach has been applied with various degrees of thoroughness, ranging from a subjective impression of frequency to quantitative determination of frequency, calculation of the solute fluxes involved, and the probable concentration gradient available to drive transport. This range of approaches is noted in Table 1. In general, the degree of confidence attached to the results increases with the amount of information included in the analysis.

Nonetheless, several cases of apoplastic unloading have been inferred for active sinks. Very low plasmodesmatal frequencies have been reported for growing corn leaves (Evert and Russin, 1993) and for sugar beet roots (Mierzwa and Evert, 1984). Meshcheryakov et al. (1992) provide water relations evidence, based on pressure probe measurements, of high solute concentrations in the apoplast of growing castor bean stems. An interesting situation occurs in tomatoes, where movement appears to be symplastic in young fruit, but switches to an apoplastic pathway later in development (Ruan and Patrick, 1995). A similar change in pathway may also occur in grapes, in which the ripening phase ('veraison') is accompanied by a general breakdown of compartmentation (Lang and During, 1991). The path during pre-ripening appears to be less well characterized. Apoplastic transport may occur in at least the latter portion of the post-phloem transport pathway in citrus fruits, since movement continued in the vesicle stalks even after the stalks were frozen and thawed (Koch et al., 1986). However, the path taken during earlier stages of this unusually long pathway (up to 3 cm) appears less certain.

**Table 1. (continued)**

<table>
<thead>
<tr>
<th>Organ, species</th>
<th>Path&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Evidence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>A</td>
<td>Comptbl analysis; asymm suc</td>
<td>Damon et al. (1988)</td>
</tr>
<tr>
<td>Tomato</td>
<td>A (old)</td>
<td>EM/morphometric/flux analysis; dye coupling (CFDA loading); [14C]glu uptake, movement, PCMBS</td>
<td>Offler and Horder (1992)</td>
</tr>
<tr>
<td>Grape</td>
<td>A</td>
<td>Breakdown of compartmentation (fruit xylem exudate fruit sap)</td>
<td>Ruan and Patrick (1995)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>A?</td>
<td>Sugar presence in, uptake from, apoplast</td>
<td>Forney and Breen (1986), Pomper and Breen (1995)</td>
</tr>
</tbody>
</table>

<sup>a</sup>A = apoplast; S = symplast.

<sup>b</sup>EM: presence or absence of plasmodesmata; EM/morphometric: quantified plasmodesmatal frequencies; EM/morphometric/flux: calculated flux through plasmodesmata using transport rates for that system; EM/morphometric/flux/gradient: compared calculated flux values to those expected from sugar gradients, plasmodesmatal conductance.

In general, the degree of confidence attached to the results increases with the amount of information included in the analysis.
even more, it seems feasible that combined diffusive and convective solute transport could account for the observed rate of solute movement in corn root tips.

The physiological approach

The principal methods used to trace symplastic transport along the post-phloem pathway are fluorescent tracers, microautoradiography and asymmetrically-labelled sucrose. CF has been particularly valuable for this purpose because it can be introduced into the phloem without specialized equipment (Grignon et al., 1989). Either a slightly acid solution of CF can be used, or it can be ‘ester-loaded’ using the diacetate. In favourable material the arrival, unloading and post-phloem movement of CF can be followed in living tissue, in real time (Plates 1–3). In most cases, however, fluorescence must be examined periodically in fresh sections.

Only a few attempts have been made to introduce fluorescent tracers into the post-phloem pathway by cell injection. Oparka and coworkers used this approach with potato tubers to follow movement out of the SE/CC complex (Oparka and Prior, 1987, 1988) and, after injection into parenchyma, to demonstrate symplastic mobility in the post-phloem pathway (Oparka et al., 1992). Considerably more use of this approach is needed, not only to establish the pathway available for post-phloem movement, but to determine their SEL.

It should be noted that fluorescent compounds have definite shortcomings as tracers for photoassimilates. The fluorescence of CF (and fluorescein), often used in these studies, is strongly pH-dependent (Grignon et al., 1989). Commonly-used tracers for symplastic movement are negatively charged and are distinctly larger molecules than sugars and amino acids (e.g. the hydrodynamic radii of LycC, CF, and sucrose are 0.68, 0.61 and 0.47 nm, respectively (Wang and Fisher, 1994b)). Most importantly, because of their chemical dissimilarity, they will not follow the same distribution pattern when membrane transport occurs. Thus, fluorescent tracers will not be useful for detecting unloading into the apoplast and, after symplastic unloading, they may not assume the same compartmental distribution as assimilates. In some sink tissues, for example, sequestration by vacuoles may hinder the movement of dye along the post-phloem pathway (Wright and Oparka, unpublished observations).

The movement of normally-translocated compounds along the post-phloem pathway can be followed by autoradiographic studies of freeze-dried or freeze-substituted material. The cell-level resolution provided by microautoradiography is usually necessary, but tissue-level autoradiography has been useful when supplemented by other information.

Some sink tissues have a strong acid invertase activity in their cell walls. In those cases, phloem-translocated, asymmetrically-labelled sucrose has been used to determine whether an apoplastic step might be involved in post-phloem movement. If so, hydrolysis, reabsorption of the hexoses and resynthesis of sucrose from a common hexose phosphate pool should lead to the loss of asymmetric labelling in the sink sucrose. Retention of asymmetry is taken to indicate a completely symplastic pathway of movement into the sink tissues. However, judging from the measurements of axial sugar gradients in corn root tips (Sharp et al., 1990) and similar, less extensive, data from other meristems, this rationale needs closer examination. They found that hexoses, representing virtually all of the sugar content of the terminal 10 mm, declined toward the tip. Sucrose actually showed a slight reverse gradient (i.e. highest concentration at the tip). Because vacuoles are virtually absent from the tip, these must be cytosolic gradients, indicating that hexoses, not sucrose (as assumed by Bret-Harte and Silk, 1994), are the transported form of sugars along the post-phloem pathway. Evidently, almost all of the imported sucrose is converted to free hexoses, perhaps by inversion, as it enters the post-phloem pathway. Retention of asymmetric labelling in the small proportion of remaining sucrose would shed no light on the path taken by hexoses. This also raises questions about the ‘protected derivative’ hypothesis concerning the transport form of translocated sugars (Arnold, 1968).

Somewhat ironically, perhaps, the methods available for evaluating apoplastic assimilate transport are more limited than for symplastic transport. As noted above, phloem-translocated fluorescent compounds can not be expected to trace the transmembrane movement that would initiate transport along an apoplastic post-phloem pathway. Thus, it is difficult to be certain, in those cases where post-phloem movement follows an apoplastic pathway, that movement was initiated by unloading from the SE/CC complex itself. Unloading could initially be symplastic, with movement into the apoplast occurring a short distance away from the SE/CC complex. This would have the advantage of utilizing a larger membrane area for unloading, and of depositing the assimilates into a larger, and presumably more conductive, apoplast volume. In the case of mature bean stems, however, sound evidence has been obtained for apoplastic unloading from the SE/CC complex itself (Hayes et al., 1987). Here, translocated 14C-assimilates continued to appear in the apoplast even after the symplast had been disrupted by plasmolysis.

Because of its small volume and microcapillary structure, and because of the potential for symplast/apoplast exchange, methods for obtaining solutes from the cell wall space are often indirect and sometimes subject to conflicting interpretation. For post-phloem transport, compartmental analysis (used here to include a single brief wash period) has been the most frequently-used approach to the estimation of apoplastic solute content.
In most instances, compartmental analysis was in fact supplemented by other methods (microautoradiography, plasmolysis, PCMBS treatments).

**Gradients**

Transport along the post-phloem pathway will be driven by gradients in concentration and/or pressure. However, the number of measurements made of gradients is limited. In some cases, plasmolysis has been used to measure osmotic gradients. While this approach may be suitable for inferring large concentration differences, it can not be used with confidence for quantitative work on a system where there is rapid solute turnover. To do so, one must implicitly accept the proposition that, during osmotic treatment, solute movement into and out of cells will be undisturbed, or at least equally affected. This is highly unlikely, especially in excised tissues, and may be reasonably expected to lead to substantial error for cells whose entire solute content turns over each second (sieve elements) to a few minutes (the post-phloem pathway). For this reason, plasmolysis results will not be included.

Fairly extensive measurements of gradients in a sink have been made with growing roots, primarily in the context of cell expansion. In viewing these measurements, it must be noted that two distinct regions of post-phloem transport are involved, one radially away from the protophloem in the zone of elongation, and the other longitudinally away from the protophloem terminus in the terminal 1–5 mm of the root tip. Most measurements have been made along the root axis. In general, there is little evidence for an axial turgor gradient (Pritchard, 1994; Spollen and Sharp, 1991; Tomos and Pritchard, 1994). If anything, turgor was slightly higher in the tip region under some conditions. In root tips of water-stressed corn seedlings, total osmotic concentration was lower toward the root tip (Sharp et al., 1990), despite the absence of an axial turgor gradient (Spollen and Sharp, 1991), strongly suggesting the presence of apoplastic solutes in the meristem, declining in concentration toward the tip. Pritchard (1994) cites several additional lines of evidence indicating the presence of apoplastic solutes in root tips. As noted above, Sharp et al. (1990) also found that free hexoses, representing most of the sugar content in the terminal 10 mm, declined toward the tip. The gradient was steeper in water-stressed seedlings, and was correlated with a higher rate of hexose deposition in the terminal 4 mm.

The situation with respect to radial gradients in roots is less satisfactory. Conflicting experimental reports were reconciled by the observation that both turgor and osmotic gradients were absent in the roots of non-transpiring plants, but appeared in the meristematic and mature regions with the onset of transpiration (Pritchard et al., 1989; Rygol et al., 1993). Both turgor and osmotic concentration declined, by 0.3–0.5 MPa, from the inner cortex toward the epidermis. Because the gradients are opposite to that expected for entering water and solutes, these observations have considerably complicated interpretations of water and solute uptake by roots. Among other matters, they suggest that symplastic transport might be blocked under these conditions (Oparka and Prior, 1992), raising questions about the mechanism of radial post-phloem transport even though the turgor and osmotic gradients are in the right direction for such movement. The issues raised are complex and can only be touched on here, but they are thoughtfully reviewed by Clarkson (1993).

Water relations measurements have also been made on elongating stems of castor bean (Meshcheryakov et al., 1990), pea (Malone and Tomos, 1992) and soybean (Nonami and Boyer, 1993). The results differ on several important points. Meshcheryakov et al. (1992) found steep radial gradients in turgor and osmotic pressure, declining, as in the case of roots, outwardly toward the epidermis. The gradient amounted to about 0.7 MPa over a distance of only 470 μm; there was no gradient in water potential (0 under their conditions), either radial or axial. Restricting the water supply by removing the roots (remained 0) caused the turgor to drop by 0.5 MPa or more, and the turgor and osmotic gradients disappeared. On resupplying water, turgor was rapidly restored (c. 2 min) and the gradient returned. The turgor increase exceeded the increase in water potential by as much as 0.7 MPa, indicating that apoplastic solutes had probably accumulated to almost 0.3 M while water movement was restricted, and were swept away upon resumption of water flow.

Malone and Tomos (1992), working with pea, also found no axial gradient in water potential or in osmotic

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Plate 1. Part of the root system of *Arabidopsis thaliana* translocating the fluorescent probe carboxyfluorescein (CF). The growing, intact root was imaged using confocal laser scanning microscopy. In places, the two phloem traces are clearly visible in both the primary root and in first-order lateral roots. Note that the conducting phloem behaves as an isolated domain. The arrow indicates a developing lateral root primordium where dye unloading has commenced (from Oparka et al., 1995).

Plate 2. Phloem unloading in the primary root tip of *Arabidopsis*. Following its arrival in the protophloem, CF was unloaded radially into the region of cell elongation and axially towards the root tip, resulting in a ‘tear-drop’ shaped unloading zone. The consecutive images were taken at 3 min intervals (for details, see Oparka et al., 1994).

Plate 3. Fluorescence recovery after photobleaching (FRAP). The unloading zone was subjected to a brief (5 s) exposure to a 100 mW argon laser, resulting in the bleaching of dye within the unloading zone. Immediately, there was a rapid resumption of longitudinal transport in the protophloem sieve elements (arrow), followed by a slower radial unloading to surrounding cells.
concentration in elongating stems. In contrast to Meshcheryakov et al. (1992), however, no radial gradients were present. Their data suggest the possible presence of apoplastic solutes, since cell water potentials were lower in slower-growing stems (i.e. with less water movement).

Nonami and Boyer's (1993) results with soybean hypocotyls contrast with those from both castor bean and pea. Water and osmotic potentials were lower in elongating tissues than in mature tissues. Radial gradients were absent in non-elongating regions. Turgor was uniform in elongating tissues, but osmotic potential and, as a result, water potential, decreased radially toward the epidermis. This gradient, evidently caused by growth-induced water movement into expanding cells, was relatively small (about 0.15 MPa) and, as they point out, such a gradient may be detectable only when transpiration is entirely absent. They achieved this by working in near darkness and by coating the seedlings with vaseline, speculating that a growth-induced gradient might be undetectable if even low rates of transpiration occurred.

Pressure probe experiments with wheat leaf meristems indicates that apoplastic solutes contribute as much as 0.6 MPa to the meristematic water potential and, further, that they are important to the regulation of turgor pressure in the meristem (Tomos and Pritchard, 1994).

In general, observations to date suggest that apoplastic solutes may contribute significantly to water relations and the regulation of turgor pressure in meristematic tissues. Clearly, they may, in this role, have a considerable influence on the control of post-phloem transport via the symplast. However, the apoplastic solutes themselves may not be transported. Unfortunately, the solutes involved have not been identified. This would be useful in determining whether they might be transported, and is necessary for understanding their possible role in apoplast/symplast exchange.

The unusually long post-phloem pathway (up to 3 cm) in citrus fruit has been used to advantage by Koch and coworkers (Koch et al., 1986; Koch and Avigne, 1990) to measure sugar and osmotic gradients along the pathway. Movement is slow, and occurs against a sugar concentration gradient during most of fruit growth. Movement apparently occurs as bulk flow of a 0.2–0.4 M sucrose solution which is accommodated by the expansion of the juice vesicles, although apolastic solutes may also lower vesicle turgor. Flow through at least part of the pathway may be apoplastic, since movement through vesicle stalks continued after the stalks were frozen and thawed.

Gradients in sucrose concentration and water potential have also been measured for the post-phloem pathway (vascular parenchyma to the endosperm cavity; a distance of about 400 μm) in developing wheat grains. In normally-importing grains, there was a concentration difference between the ends of the pathway of about 150–200 mM (Fisher and Wang, 1995). Presumably, the total osmotic gradient is larger, because other solutes are moving as well as sucrose. The sucrose gradient disappeared under conditions where transport ceased (grain excision, PCMBS treatment or plasmolysis). Despite the evident osmotic gradient, estimated to be 0.5–0.7 MPa, there was probably little or no turgor gradient. Partly because of an apoplastic barrier between the vascular tissues and the endosperm cavity (Wang and Fisher, 1994b), the water potential in the endosperm cavity was more positive, by about 0.4–0.6 MPa (Fisher and Gifford, 1986; Fisher, 1985, and unpublished measurements). Thus movement along this pathway appears to be mostly by diffusion.

**Domains**

**Apoplastic**

Sinks often exhibit a considerable degree of hydraulic isolation from the rest of the plant. This isolation can arise simply from the absence of fully-differentiated xylem vessels, as in the case of terminal meristems like roots, or it may be a consequence of structural features which restrict the movement of water into and out of the sink. Because the concentration of apoplastic solutes in seeds and fruits is often substantial, hydraulic isolation may be important in preventing the loss of these solutes via the xylem (Bradford, 1994). The xylem discontinuity in the pedicel of wheat and barley grains, characterized by Zee and O'Brien (1970), may be a general feature of Festucoid grasses (Pizzolato, 1990, and references therein). A xylem discontinuity is absent in rice (Zee, 1972) and perhaps in other Oryzoid grasses (Pizzolato, 1990). In tomato and grape, a similarly high resistance to water movement occurs in the xylem connection to the fruit, although the structural basis differs. In tomato, xylem connections across the abscission layer in the ‘knuckle’ region of the fruit pedicel are poorly developed (Lee, 1989). In grapes, xylem resistance is low in unripe berries, but increases rapidly soon after the onset of ripening (‘veraison’). The structural cause of increased resistance is not fully known, but the stretching and rupturing of xylem vessels that accompanies this stage of rapid fruit expansion may cause blockage by allowing embolisms to occur (Findlay et al., 1987).

Apoplastic barriers may also restrict the movement of water and solutes within the sink itself. In small grains (wheat, barley and rice), cell wall deposits in the chalaza (the ‘pigment strand’) strongly hinder apoplastic water and solute movement between the vascular tissues and the embryonic tissues. This leaves the symplast as the only route for assimilate movement (Oparka and Gates, 1981; Wang and Fisher, 1994b; Wang et al., 1994) and may explain the relative insensitivity of phloem unloading in wheat grains to high osmotic concentrations in the
endosperm cavity (Wang and Fisher, 1994a). There, the vascular tissue apoplast appears to be a domain by itself, separate from the rest of the plant and from the embryo and endosperm. In sugar cane stems, the sclerenchyma sheath around vascular bundles prevents the movement of apoplastic solutes from the storage parenchyma into the xylem (Jacobsen et al., 1992; Welbaum et al., 1992). As a result, turgor pressure in the parenchyma of excised tissue is fairly constant at about 0.4 MPa, due to apoplastic solutes, even as the stored sugar concentration increases from 1 MPa to 2.5 MPa (Welbaum and Meinzer, 1990). If this is the situation in situ, maintenance of low turgor in the storage parenchyma might be important to the accumulation of sugars into a sink with such a high solute concentration. However, this supposed effect would be attenuated, perhaps completely if, as Welbaum and Meinzer argue, positive pressure developed in the apoplast, keeping the apoplast water potential close to that of the xylem. Recently, Dong et al. (1994) provided support for that interpretation by demonstrating that the intercellular spaces of sugar cane storage tissues are solution-filled, rather than gas-filled, suggesting that they may be pressurized.

**Symplastic**

Aside from restricted movement resulting simply from the absence of plasmodesmata, instances have been noted where transport in the post-phloem pathway was restricted even though plasmodesmata were nevertheless present. In contrast to apoplastic domains, which most probably affect the rate of movement by influencing tissue water relations, symplastic domains appear to have a ‘channeling’ influence on solute movement. Thus, in the wheat grain, there was minimal solute movement, either of labelled assimilates (Fisher and Wang, 1993) or of fluorescent tracers (Wang and Fisher, 1994b) from the vascular parenchyma into adjoining pericarp chlorenchyma or into the nucellus. The latter point contrasts with rice, where the nucellus is an important pathway for circumferential movement around the grain (Oparka and Gates, 1981). Seed coats of *Vicia* and *Phaseolus* appear to be divided into three concentric symplastic domains (Patrick et al., 1995). However, their role in post-phloem transport differs markedly for the two species, with the lateral transport occurring via the central domain of ground parenchyma in *Phaseolus*, whereas in *Vicia* the ground parenchyma was symplastically isolated from the phloem. Instead, lateral transport occurs in the inner and outer tissue layers (thin-walled transfer cells and chlorenchyma, respectively). In all cases, plasmodesmata have been observed between adjacent cells of the apparent domain boundaries (Morrison, 1976; Offler and Patrick, 1984; Offler et al., 1989; Wang et al., 1995).

**Future directions**

Most investigations to date of post-phloem transport have been concerned primarily with distinguishing between apoplastic versus symplastic pathways of movement. More remains to be done in this area, particularly in cases where the apoplast is involved. Fortunately, fluorescent tracers, particularly carboxyfluorescein diacetate, have considerably simplified the detection of symplastic unloading and post-phloem movement, although their limitations for tracing assimilates must be kept in mind. Another qualitative question, the identity of sugars moving along the post-phloem pathway in meristems, requires some re-evaluation.

Quantitative aspects of post-phloem transport, required to evaluate the role of sinks in assimilate partitioning, are in a far less satisfactory state. Values for such fundamental pathway characteristics as hydraulic and diffusive conductances are almost entirely lacking for both the apoplast and the symplast. Clearly, these are challenging technical problems, especially given the diversity in sink anatomies. Nonetheless, it should be possible to obtain fairly readily more extensive information on some of the component factors of conductances, such as the SEL for symplastic movement and diffusion coefficients in cell walls. One of the more difficult path parameters to obtain may be the hydraulic conductance of the symplast, since it appears to be sensitive to pressure gradients. In this regard, it should be noted that available values for \( L_p \), nominally designated as ‘membrane hydraulic conductivity’, are in fact composite values for symplastic and transmembrane water movement. Perhaps some way can be found of estimating their relative contributions to \( L_p \).

Measurement of the driving forces for transport likewise pose difficult technical problems, compounded by the necessity of making measurements on pathways which are sufficiently intact to be still functioning. Micro-sampling methods of various kinds, pressure probe measurements, and microanalytical measurements hold the key to much of this work.

The role of apoplastic solutes and of apoplast–symplast solute exchange merits particular attention. Even if they are not moving themselves, apoplastic solutes are a potentially important contributor to driving forces in the symplast. Conceivably, localized apoplastic–symplastic solute exchange could play a significant role in controlling the relative proportion of diffusive to convective transport in the symplast by, for example, maintaining constant turgor. This does not require that solutes be restricted to a discrete apoplastic domain, only that they be much less mobile than in the symplast, a condition that probably occurs in most meristematic and storage tissues. The possibility that some apoplastic domains may be pressurized should not be overlooked. Here, too, the pressure probe appears to offer a suitable approach for addressing
some of these questions, particularly for real-time measurements. Clearly, however, additional methods are urgently needed to identify and measure the solutes involved.

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