

Phloem loading and unloading of sugars and amino acids

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ABSTRACT

In terrestrial higher plants, phloem transport delivers most nutrients required for growth and storage processes. Some 90% of plant biomass, transported as sugars and amino nitrogen (N) compounds in a bulk flow of solution, is propelled through the phloem by osmotically generated hydrostatic pressure differences between source (net nutrient export) and sink (net nutrient import) ends of phloem paths. Source loading and sink unloading of sugars, amino N compounds and potassium largely account for phloem sap osmotic concentrations and hence pressure differences. A symplasmic component is characteristic of most loading and unloading pathways which, in some circumstances, may be interrupted by an apoplasmic step. Raffinose series sugars appear to be loaded symplasmically. However, sucrose, and probably certain amino acids, are loaded into minor veins from source leaf apoplasts by proton symporters localized to plasma membranes of their sieve element/companion cell (se/cc) complexes. Sucrose transporters, with complementary kinetic properties, are conceived to function as membrane transporter complexes that respond to alterations in source/sink balance. In contrast, symplasmic unloading is common for many sink types. Intervention of an apoplasmic step, distal from importing phloem, is reserved for special situations. Effluxers that release sucrose and amino acids to the surrounding apoplast in phloem loading and unloading are yet to be cloned. The physiological behaviour of effluxers is consistent with facilitated membrane transport that can be energy coupled. Roles of sucrose and amino acid transporters in phloem unloading remain to be discovered along with mechanisms regulating symplasmic transport. The latter is hypothesized to exert significant control over phloem unloading and, in some circumstances, phloem loading.

Key-words: amino nitrogen; apoplasmic; loading; phloem; sink; source; sugar; symplasmic; unloading.

Abbreviations: *A*, cross-sectional area; AAP, amino acid permease; *C*, assimilate concentration; *D*, diffusion coefficient; GFP, green fluorescent protein; HXT, hexose transporter; *K_m*, Michaelis–Menten constant; *J_v*, volume flux; *L_p*, hydraulic conductivity; π , osmotic pressure; η , viscosity; *P*, hydrostatic pressure; PCMBs, para-chloromercuribenzenesulphonic acid; Pd, plasmodesma (Pds – plural; Pdl, –

adjective); pmf, proton motive force; ψ , water potential; *R_d*, diffusion rate; *R_f*, bulk flow rate; RFO, raffinose family oligosaccharide; se/cc, sieve element-companion cell; SUT (SUC), sucrose transporter.

INTRODUCTION

Successful colonization of land by green plants depended upon co-evolution of organs specialized to extract water and mineral ions from the soil linked with organs hoisted aloft into the non-viscous aerial environment to capture light for photosynthetic reduction of carbon dioxide. The resulting nutritional interdependence of soil and aerial organs was solved by exchange of acquired nutrients between these regions. For terrestrial macrophytes, distances separating these assimilatory organs exceeded the capacity of simple diffusion to deliver nutrients at rates sufficient to meet demands for their cellular maintenance and growth. In vascular plants, these requirements were met by evolving specialized conduction tissues (phloem and xylem; see van Bel, this volume) that transport nutrients at high rates over long distances by bulk flow.

Xylem elements support an upward bulk flow of mineral-containing sap driven by evaporative loss of water from aerial organs reducing pressure in their cell walls. By contrast, osmotically generated pressure differences move photosynthetic products and inorganic nutrients (assimilates) by bulk flow from leaves (sources – net assimilate exporters) to heterotrophic organs (sinks – net assimilate importers) through the phloem. The sieve element/companion cell (se/cc) complex forms the long-distance transport unit (see van Bel, this volume). Most assimilates are phloem delivered to sinks as these organs characteristically have low rates of transpiration and hence xylem import.

How structural and functional properties of the phloem contribute to bulk flow is formalized in the pressure flow hypothesis (Münch 1930). The product of volume flux (*J_v*), path cross-sectional area (*A*) and concentration (*C*) of a transported assimilate (Eqn 1) determines bulk flow rate (*R_f*). *J_v* is set by the product of hydraulic conductivity (*L_p*) of the axial phloem path and hydrostatic pressure difference between source (e.g. leaf minor veins) and sink (e.g. flowers, meristems, roots) ends of the path ($P_{\text{source}} - P_{\text{sink}}$; Eqn 2):

$$R_f = J_v AC \quad (1)$$

$$R_f = L_p (P_{\text{source}} - P_{\text{sink}}) AC \quad (2)$$

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Hydraulic conductances ($L_p A$) of axial phloem paths do not limit transport rates in vivo (Wardlaw 1990). As a consequence, bulk flow rates through the phloem are modulated by assimilate concentrations and hydrostatic pressure differences (see Eqn 2). Hence, assimilate loading and unloading of se/cc complexes play central roles in phloem transport as loading sets P_{source} and C and unloading P_{sink} . Furthermore, differences in P_{sink} exert a major influence on assimilate partitioning between competing sinks and hence crop yield (Wardlaw 1990).

Photosynthetically reduced carbon contributes to some 90% of plant biomass and is transported from photosynthetic source leaves to heterotrophic sinks principally as sugars and amino nitrogen (N) compounds. Sucrose is an ubiquitous constituent of all phloem saps but, in some plant families, is supplemented by raffinose family oligosaccharides (RFOs) and/or sugar alcohols (Zimmermann & Ziegler 1975). Commonly aspartate and glutamate and their corresponding amides are the principal forms of amino N compounds transported in the phloem (Delrot *et al.* 2001). Together with potassium (reviewed elsewhere, see Patrick *et al.* 2001), sugars and amino N compounds are the principal osmotic components of phloem saps and hence impact on rates of phloem transport (see Eqn 2) and assimilate partitioning patterns. In this context we review current understanding of phloem loading and unloading of sugars and amino N compounds.

Minor vein networks of source leaves support the highest rates of loading and are irreversibly committed to loading se/cc complexes (collection phloem – van Bel 1996a). In contrast, reversible interchange between loading and unloading is characteristic of phloem paths interconnecting major vein networks of leaves to sink organs (for further detail, see van Bel this volume). Unloading predominates in phloem of sink organs (release phloem – van Bel 1996a).

TRANSPORT PHENOMENA COMMON TO PHLOEM LOADING AND UNLOADING

Exchange of sugars and amino acids between phloem and surrounding tissues has been shown to occur through plasmodesmata (Pds) and cell cytosols (symplasmic loading/unloading) and/or across plasma membranes via intervening cell walls (apoplasmic loading/unloading). We choose to use the term ‘plasmic’ to describe transport through these two compartments rather than ‘plastic’ which is more appropriately reserved to describe growth deformation (see Erickson 1986).

Symplasmic transport is likely to be rate limited by movement through Pdl pores. Pdl transport of small molecular weight compounds has been found to conform to diffusion kinetics in staminal hairs (Tucker & Tucker 1993). The rate of diffusion (R_d) through Pds can be derived from Fick’s first law of diffusion as:

$$R_d = n[DA(C_1 - C_2)/l] \quad (3)$$

where n is the number of Pds occupying a cross-sectional area available for transport (A); D , the diffusion coefficient of the diffusing solute within Pdl pores; C , cytosolic solute concentrations of adjacent cells (C_1 and C_2); l , length of Pd substructure that most limits transport.

Pds at some locations within assimilate transport paths from source to sink allow transport of molecules with Stokes radii that are double those transported in staminal hairs (e.g. Fisher & Cash-Clark 2000a) and their conductances are likely to allow bulk flow (Eqn 5). Poiseuille’s law predicts hydraulic conductivities (L_p) of capillaries as:

$$L_p = \pi r^4 / 8\eta l \quad (4)$$

where r is the Pdl radius; η , viscosity of flowing solution; l length of Pds substructure rate-limiting transport.

For bulk flow to proceed, Pds must be insensitive to changes in hydrostatic pressure differences between contiguous cells or if pressure sensitive, the pressure difference must be less than that causing Pdl closure (Oparka & Prior 1992). If these conditions are satisfied, bulk flow rate (R_f) is given by:

$$R_f = nL_p[(\pi_1 - \psi_a) - (\pi_2 - \psi_a)]AC \quad (5)$$

In this context, volume flux (J_v , Eqn 1) is governed by hydraulic conductivity of Pds (Eqn 4) for a Pdl pore cross-sectional area summed for all Pds (nA) and the turgor difference. At water equilibrium, cell turgor is determined by the difference between cell osmotic potential (π) and water potential of the surrounding apoplasmic fluid (ψ_a).

For apoplasmic transport, rates (R_v) of facilitated membrane transport of sugars and amino acids can be modelled in terms of a solute saturable component described by Michaelis–Menten kinetics combined with a solute nonsaturable component obeying by first order kinetics as:

$$R_v = [V_{\text{max}}C/(K_m + C)] + kC \quad (6)$$

where V_{max} is maximal velocity, K_m , Michaelis–Menten constant, C , solute concentration and k the first-order rate constant. For proton-coupled transport, maximal velocity will be influenced by the electrochemical gradient of protons (proton motive force – pmf) across the membrane.

COLLECTION PHLOEM – A HIGH FIDELITY LOADER

Phloem loading is considered to include transport of assimilates from their cellular sites of acquisition/storage to the lumens of se/cc complexes (van Bel & Oparka 1992). This definition, which we adopt here, has a number of important caveats. It ignores the dependence of cytosolic sucrose levels on leaf metabolism/compartimentation (Komor 2000) and excludes the more rigorous requirement for selective accumulation of an assimilate species in the se/cc complexes (Geiger 1975). Thus, as defined ‘loading’ also describes circumstances where sugars (e.g. Turgeon & Medville 1998) and some amino acids (e.g. Lohaus *et al.* 1995)

may be transported from mesophyll cells into the phloem down, rather than up, their concentration gradients.

Contribution of vein classes to phloem loading

Minor veins are considered to be the major site of phloem loading of sugars in source leaves but not exclusively so (van Bel 1993). Surprisingly, this important conclusion rests largely on correlative evidence as outlined below.

For developing dicot leaves, differentiating minor veins do not engage in phloem unloading (Roberts *et al.* 1997; Imlau, Truernit & Sauer 1999). Moreover, their basipetal wave of maturation corresponds with the onset of phloem loading and export of assimilates (Turgeon 1989). A number of structural features of minor veins are considered to facilitate phloem loading. These include: (i) their proximity to all mesophyll cells (2–3 cell diameters – Wylie 1939); (ii) collection lengths per unit leaf area that are an order of magnitude greater than those of higher order veins (e.g. Geiger & Cataldo 1969); (iii) bigger cc diameters compared to transport and release phloem (see van Bel, this volume). The most compelling evidence that minor veins are the major sites of phloem loading has been obtained at a molecular level. Thus, the low affinity/high capacity sucrose transporter (SUT4) is expressed strongly in minor veins (Weise *et al.* 2000) of the apoplasmic loading species, *Arabidopsis* (Haritatos, Medville & Turgeon 2000b; Gottwald *et al.* 2000). Here it is anticipated to support high sucrose fluxes into the se/cc complexes (Weise *et al.* 2000). Another molecular example, supporting minor veins as the principal site of phloem loading, is the localized expression of galactinol synthase (*CmGAS*) cloned from melon. This enzyme is specifically expressed in the intermediary cells (cc equivalents) of minor veins (Haritatos, Ayre & Turgeon 2000a) where it is considered to form part of a symplasmic phloem loading mechanism (polymer-trap hypothesis; Turgeon 1996). Consistent with minor veins being the principal sites of phloem loading, heterologous *CmGAS* expression in putative apoplasmic phloem loading species is confined to these veins (Haritatos *et al.* 2000a).

Similar, but less-well substantiated conclusions, are drawn for leaves of C₃ and C₄ grass species. Here small and intermediate bundles comprise some 80–90% of the leaf vasculature (Kuo, O'Brien & Canny 1974; Dannenhoffer, Ebert & Evert 1990 and references cited therein) and are interlinked by small transverse veins to the larger veins (Kuo *et al.* 1974). Monitoring progression of ¹⁴C photoassimilates by autoradiographic imaging, following fixation of a ¹⁴CO₂ pulse, shows that small and intermediate veins are responsible for loading photoassimilates. Loaded ¹⁴C photoassimilates then move, via transverse veins, to lateral veins for export from the leaf (Lush 1976; Altus & Canny 1982; Fritz, Evert & Nasse 1989).

Major leaf veins and the remainder of the phloem path are considered to principally function as a long-distance transport conduit (transport phloem – see van Bel, this volume). In fulfilling this role, the phloem path retains high solute concentrations by retrieval of solutes leaked to the

surrounding apoplasm. Retrieval is carried out by a complex of low (Barker *et al.* 2000; Schulze *et al.* 2000; Weise *et al.* 2000) and high affinity (Stadler *et al.* 1995; Kühn *et al.* 1997; Noiraud, Delrot & Lemoine 2000; Noiraud, Marrouset & Lemoine 2001) sugar transporters localized to the se/cc complexes (Fig. 1B).

Movement from mesophyll to minor vein boundary – an undisputed but poorly chartered route

The cellular pathway of loading collection phloem depends upon whether assimilates originate from mesophyll cells or the xylem transpiration stream. Direct xylem-phloem transfer clearly involves membrane transport into phloem cells. This transport phenomenon has not received a great deal of attention but is relevant for amino N compounds delivered from roots (Fig. 1A). For example, xylem-delivered amides are transferred directly from xylem to phloem in leaves of castor bean (Jeschke & Pate 1991) and lupin (Atkins 2000).

Triose phosphates are released from chloroplasts into the cytosol of mesophyll cells during the day (Fliege *et al.* 1978) whereas glucose is released during the night from amylolytic turnover of starch (Weber *et al.* 2000). The released substrates are rapidly interconverted into sucrose (Quick & Schaffer 1996) and additionally, in some species, into sugar alcohols such as sorbitol and mannitol (Loescher & Everard 1996). Movement of sugars from mesophyll to minor veins is rapid whereas exchange with vacuolar pools is much slower (Outlaw *et al.* 1975). In contrast, amino acids and nitrate imported into mesophyll cells from the xylem transpiration stream have longer residence times in leaves of lupin prior to being exported as amino acids (Atkins 2000; Fig. 1A). How general this phenomenon is awaits study of other plant species. Xylem-imported ureides are de-aminated and the released ammonia is incorporated into amino acids some of which are exported (Winkler *et al.* 1988). As leaves enter senescence, export rates of remobilized amino N compounds characteristically increase (Delrot *et al.* 2001).

There is a general, but poorly justified, acceptance that sugars, and presumably amino acids, move symplasmically from mesophyll to bundle sheath/phloem parenchyma cells of minor veins (see reviews by van Bel 1993; Turgeon 2000; Fig. 1A). High densities of Pds interconnecting cells along this route provide notional support for this claim (Beebe & Russin 1999). Pd capability for solute transport has been investigated in several monocots (Erwee, Goodwin & van Bel 1985; van Kesteren, van der Schoot & van Bel 1988; Farrar, Minchin & Thorpe 1992; Botha & Cross 1997) and dicots (Madore, Oross & Lucas 1986; Fisher 1988; Turgeon & Hepler 1989) by micro-injection of small molecular weight membrane-impermeant fluorescent probes. In all cases (see References above), dye coupling was demonstrated to exist between mesophyll and minor vein cells. However vascular cell types importing the dye could not be resolved with confidence. Whether conductive capacity of interconnecting Pds is sufficient to support observed rates

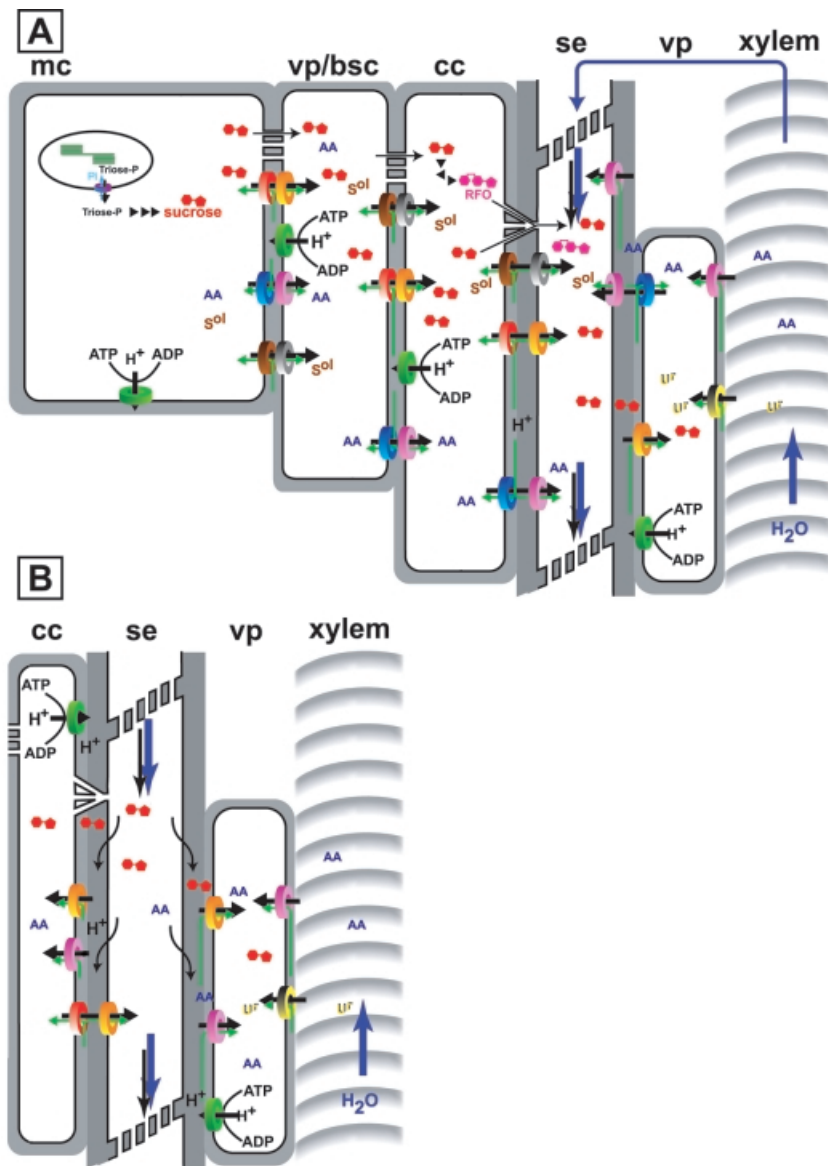


Figure 1. Phloem loading. (A) Sucrose (red), synthesized in the leaves, moves from mesophyll cells to sieve elements either by a symplasmic path through plasmodesmata or by an apoplastic path through specific transporters. Influx of sucrose (orange transporter) involves cotransport of sucrose with protons; whereas efflux (red transporter) is predicted to involve antiport or facilitated diffusion. Sugar alcohols (S^{ol} ; brown) are actively loaded into sieve elements from their site of synthesis in mesophyll cells. However, the exact locations of influx (grey) and efflux (brown) carriers are not known. In species with numerous plasmodesmata (Type 1), raffinose (RFO; pink molecule) are synthesized in the intermediary cell (companion cell). According to the 'polymer trap' hypothesis, these sugars cannot diffuse back to the mesophyll because of their molecular size. However, they can be loaded symplasmically into sieve elements. Amino acids and amides (AA; blue) are synthesized in mesophyll cells or imported through the xylem. Similar to sucrose, amino acids/amides can be loaded symplasmically or apoplastically. Ureides, synthesized in roots, are delivered in the xylem to leaves where they are retrieved via a cotransport system (yellow transporter). The apoplastic step of AA/amides loading requires influx (pink; symporter) and efflux (blue; antiporter) transporters. All of these transporters are driven by the proton motive force generated by a H^+ -ATPase (green) located at the plasma membrane. (B) Long-distance transport of nutrients in the transport phloem. Along the transport phloem, nutrients leaked from the sieve elements are retrieved from the phloem apoplasm. Here, the companion cell is normally smaller than the sieve element. mc, mesophyll cell; vp/bsc, vascular parenchyma/bundle sheath cell; cc, companion cell; se, sieve element.

of photoassimilate transport to minor vein networks remains to be verified. The most compelling evidence for symplasmic transport of assimilates to minor veins has been demonstrated by a maize mutant (*sucrose export defective1 - sxd1*; Russin *et al.* 1996). The lesion results indirectly in structurally deformed Pds located between bundle sheath and vascular parenchyma cells of minor veins. This deformation is linked specifically with a significant reduction in photoassimilate export (Russin *et al.* 1996; Botha *et al.* 2000; Mezitt-Provencher *et al.* 2001).

The ultimate step – transport from bundle sheath/vascular parenchyma cells to se/cc complexes

The cellular pathway followed by photoassimilates in entering se/cc complexes determines candidate mechanisms that contribute to their loading. This issue has attracted consid-

erable interest and debate (Geiger 1975; Giaquinta 1983; van Bel 1993; Turgeon 1996). Observations of Pdl frequencies interconnecting se/cc complexes to adjacent phloem parenchyma/bundle sheath cells and cc ultrastructure have been used to erect hypotheses for putative pathways of phloem loading (Gamalei 1989). However, ultimate proof rests with functional tests, the design of which demands care to avoid ambiguities that have plagued many past studies (Lucas 1985). Direct tests for symplasmic loading of minor vein se/cc complexes have not been performed. To date testing has focused on manipulating membrane transport capacity of vascular cells putatively contributing to apoplastic loading.

One of the least ambiguous and routine tests for apoplastic phloem loading is to determine sensitivity of photoassimilate export to inhibiting plasma membrane transport (in general) with the membrane impermeant sulphhydryl reagent, para-chloromercuribenzenesulphonic acid

(PCMBs), and concurrently testing for any confounding effect on photosynthesis (van Bel, Ammerlaan & van Dijk 1994). Using this approach, responses consistent with apoplasmic phloem loading have been found for a wide range of plant species. These include monocots (e.g. Thompson & Dale 1981; van Bel *et al.* 1992, 1994; Ng & Hew 1996) and dicots (e.g. Giaquinta 1976; Turgeon & Wimmers 1988; Bourquin, Bonnemain & Delrot 1990; van Bel *et al.* 1992, 1994; Flora & Madore 1996; Moing, Escobar-Gutierrez & Gaudillere 1997; Goggin, Medville & Turgeon 2001). Similar findings also apply to phloem loading of amino acids in soybean (Servaites, Schrader & Jung 1979) and broad bean (Despeghel & Delrot 1983). Putative transporters responsible for phloem loading are localized to plasma membranes of *se/cc* complexes (Fig. 1A). This has been clearly established for sucrose symporters (SUTs, also named SUCs) by their observed immunolocalization patterns in leaves of *Arabidopsis* (Truernit & Sauer 1995; Stadler & Sauer 1996; Weise *et al.* 2000), *Plantago major* (Stadler *et al.* 1995) and the *Solanaceous* species, potato (Riesmeier, Hirner & Frommer 1993; Kühn *et al.* 1997; Weise *et al.* 2000) and tomato (Barker *et al.* 2000). Definitive proof that sucrose loading takes place at the *se/cc* complex boundary is provided by genetic evidence. Here, photoassimilate export is inhibited by antisense repression of SUT expression in transgenic potato (Riesmeier, Willmitzer & Frommer 1994; Kühn *et al.* 1996; Lemoine *et al.* 1996) and tobacco (Bürkle *et al.* 1998) or by knock-out mutation of *SUC2* in *Arabidopsis* (Gottwald *et al.* 2000). The above observations show that apoplasmic loading is independent of high Pds frequencies (e.g. Haritatos *et al.* 2000b; Goggin *et al.* 2001). Apoplasmic loading occurs in species with (Turgeon & Wimmers 1988; Bourquin *et al.* 1990; Flora & Madore 1996; Gottwald *et al.* 2000) or without (Giaquinta 1976; van Bel *et al.* 1992, 1994; Reismeier *et al.* 1994; Flora & Madore 1996; Moing *et al.* 1997; Goggin *et al.* 2001) their *ccs* modified as transfer cells. Moreover, both sucrose and sugar alcohols (Moing *et al.* 1997; Noiraud *et al.* 2000, 2001) can be loaded into *se/cc* complexes through an apoplasmic pathway (Fig. 1A).

Less certainty applies to the cellular site of photoassimilate release to the leaf apoplasm. Vascular parenchyma cells are considered to be the most probable site but, in their absence, bundle sheath cells could perform this role (see Giaquinta 1983; van Bel 1996b). Evidence for this claim is based on structural observations including Pdl connectivity (Beebe & Russin 1999) and modification of vascular parenchyma cells as transfer cells (B type) with secondary wall ingrowths polarized towards *se/cc* complexes (Pate & Gunning 1969). Blockage of export by structural disruption of Pds linking bundle sheath and vascular parenchyma cells in the maize sucrose export defective (*sxd1*) mutant (Russin *et al.* 1996) provides the most convincing evidence for this conclusion.

A PCMBs-insensitive export of photoassimilate from source leaves has been interpreted to be indicative of *se/cc* complex loading through an entire symplasmic route (Madore & Lucas 1987; Turgeon & Gowan 1990; van Bel *et al.* 1992, 1994; Flora & Madore 1996; Hoffmann-Thoma, van Bel & Ehlers 2001). In these cases, a weak PCMBs inhibition of photoassimilate export has been attributed to a slowing of sugar retrieval from leaf and phloem path apoplasts (van Bel *et al.* 1994). *Se/cc* complexes of all PCMBs-export insensitive species exhibit Pdl frequencies greater than $1 \mu\text{m}^{-2}$ (i.e. Type 1 and Type 1–2a, Gamalei 1989). The structural corollary of this conclusion has been shown not to hold as several species with Type 1 and Type 1–2a configuration have been found to function as apoplasmic loaders (e.g. Haritatos *et al.* 2000b; Gottwald *et al.* 2000; Goggin *et al.* 2001). In contrast, no exceptions have been reported for Type 1 species that transport high concentrations of RFOs. However, these species also transport sucrose and a sucrose transporter has been isolated from melon phloem sap (Ruiz-Medrano, Xoconostle-Cazares & Lucas 1999). Intermediary cells of these species are characterized by a dense cytoplasm containing large numbers of small vacuoles and high densities of tubular endoplasmic reticulum; high frequencies of distinctive Pds ($> 10 \mu\text{m}^{-2}$) interconnect these cells with adjacent bundle sheath cells (Turgeon 1996).

Overall strong evidence indicates that apoplasmic and symplasmic modes of phloem loading occur in minor vein networks. Moreover, these loading paths may coexist. For instance, *se/cc* complexes in the same minor vein have been found to exhibit Pdl connectivities consistent with apo- and symplasmic loading routes (van Bel *et al.* 1992). Physiological evidence for such coexistence has been detected in *Ricinus communis* that normally functions as an apoplasmic loader (Orlich *et al.* 1998). A parallel behaviour is found in stems where unloading appears to switch between apo- and symplasmic routes depending upon the source/sink ratio (Patrick & Offler 1996).

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Transport mechanisms driving phloem loading

High osmotic concentrations of solutes in *se/cc* complexes and sugar selectivity are common (van Bel 1993), but not exclusive (Altus & Canny 1985; Turgeon & Medville 1998), hallmarks of phloem loading. These features are satisfied by proton symporters that load *se/cc* complexes from the leaf apoplasm with sucrose (Riesmeier *et al.* 1993; Stadler *et al.* 1995; Truernit & Sauer 1995; Stadler & Sauer 1996; Kühn *et al.* 1997; Barker *et al.* 2000; Weise *et al.* 2000) or with mannitol (Noiraud *et al.* 2001). Sucrose symporters are colocalized to plasma membranes of *se/cc* complexes with H^+ -ATPases (Bentwood & Cronshaw 1978; Bouché-Pillon *et al.* 1994; DeWitt & Sussman 1995) that generate the required *pmf* to drive symport (Fig. 1). Based on chemical profiles of leaf and phloem sap amino acid composition (Lohaus *et al.* 1995), broad specificity amino acid/amide symporters (Fischer *et al.* 1998) could function to load these compounds into *se/cc* complexes from the apoplasm (Fig. 1). Amino acid loading into the phloem is also carried out by H^+ -coupled transport (Fischer *et al.* 1998; Ortiz-Lopez, Chang & Bush 2000; Delrot *et al.* 2001; Williams & Miller 2001).

Estimates of sucrose fluxes across plasma membranes of *se/cc* complexes in minor vein configurations committed to apoplasmic loading (Giaquinta 1983; Wimmers & Turgeon 1991) are maximal for facilitated membrane transport (approximately 10^{-7} mol m^{-2} s^{-1}). Consistent with this conclusion, transport studies using isolated mesophyll cells and leaf veins indicate that vein loading is mediated by high capacity/low affinity sucrose and amino acid transporters (van Bel, Mol & Ammerlaan 1986). Michaelis–Menten constant (K_m) values of 5–20 mM, derived for sucrose uptake by leaf discs (Giaquinta 1983) or from modelling sucrose export from intact leaves (Moing *et al.* 1994), are markedly different to those of 1 mM for plasma membrane vesicles (Bush 1993). This could reflect symporters of different affinities dominating in each of these experimental systems. For example, antisense down-regulation of *StSUT1* only was detected in plasma membrane vesicles prepared from transgenic potato leaves when antisense *StSUT1* cDNA was under control of the constitutive *CaMV 35S* promoter (Lemoine *et al.* 1996). To date most functionally characterized sucrose symporters have K_m values consistent with those found for plasma membrane vesicles (Lemoine 2000). However, two recently cloned sucrose symporters display much higher K_m values when functionally expressed in yeast (AtSUT4, K_m of 5.9 mM at pH 4 – Weise *et al.* 2000; AtSUT2, K_m of 11.7 at pH 4 – Schulze *et al.* 2000 but cf. Meyer *et al.* 2000). Assuming K_m values of 1 and 10 mM for the symporters, Michaelis–Menten kinetics predict that high- and low-affinity symporters would approach maximal transport velocities at apoplasmic sucrose concentrations of 10 and 100 mM, respectively. Estimates of whole-leaf apoplasmic concentrations of sucrose are 1–5 mM (Delrot *et al.* 1983; Tetlow & Farrar 1993; Lohaus *et al.* 1995; Voitsekhovskaja *et al.* 2000). We predict apoplasmic sucrose concentrations of 27–133 mM in the vicinity of minor veins. Our estimates are derived from whole-leaf apoplasmic concentrations (see above) on the grounds that minor veins contain 80% of leaf sucrose (Geiger 1975) and occupy 3% of total leaf volume (Geiger 1975; Ding *et al.* 1988; Winter, Robinson & Heldt 1993). At these concentrations, the low affinity/high capacity transporter would be working at 73–100% of its maximal velocity of 10^{-7} mol m^{-2} s^{-1} whereas the high affinity/low capacity transporter would be at maximal velocity. A dependence on energized membrane transport of amino acids into *se/cc* complexes of minor veins is possible. This claim is suggested by phloem sap to mesophyll amino acid ratios of 2.2 for spinach (Lohaus *et al.* 1995), 1.5 for maize (Lohaus *et al.* 1998), and 1.6–2.9 for cultivars of *Brassica napus* (Lohaus & Moellers 2000). This transport step could be mediated by amino acid/ H^+ symporters detected in plasma membrane vesicles prepared from leaves (Bush 1993; Weston, Hall & Williams 1995). Magnitudes of *pmfs* established across *se/cc* complex plasma membranes are estimated to account for observed accumulation ratios of amino acids and sucrose (Lohaus *et al.* 1995) given a stoichiometry of 1 : 1 between solute and proton transported (Bush 1993).

Little is known about release of sucrose and amino acids to the leaf apoplasm from bundle sheath/vascular parenchyma cells to supply substrate for loading into minor vein *se/cc* complexes (van Bel 1996b). Facilitated membrane transport is suggested by estimates of sucrose efflux derived from measures of cell surface areas and known rates of sucrose export (Giaquinta 1983). This conclusion is supported by observations of sucrose and amino acid efflux from leaf discs, protoplasts and plasma membrane vesicles (see Laloi, Delrot & M'Batchi 1993 and references therein). In this context, AtSUC3 protein (equivalent to AtSUT2 – Barker *et al.* 2000) was immunolocalized to phloem parenchyma cells of minor veins where it may function as a facilitator for sucrose release (Meyer *et al.* 2000). Whether transport is energy coupled is equivocal (Laloi *et al.* 1993) but precedents for sucrose/proton antiport located on plasma membranes have been detected in coats of developing seeds (see pp 48).

The mechanism of symplasmic transport is unknown but could involve intracellular cytoplasmic streaming (e.g. see Lansing & Franceschi 2000) arranged in series with passive diffusion through interconnecting Pds (see Eqn 3). For the shared symplasmic path for both apo- and symplasmic loaders, a favourable osmotic difference, and hence a presumptive concentration difference, to drive sugar and amino acid diffusion, has been detected between mesophyll to bundle sheath/vascular parenchyma cells in all species examined. Elegant single cell sampling of barley leaves confirms a favourable sucrose/photoassimilate concentration difference irrespective of variations in leaf metabolism (Koroleva *et al.* 1998). However, bulk flow is an equally plausible mechanism. For instance, as pointed out by van Bel (1996b), photoassimilate concentration gradients directed toward minor veins will generate considerable gradients in turgor pressure to drive bulk flow and these latter gradients may be amplified by osmotic withdrawal of water from proximal cells to load the phloem.

How assimilate accumulation and selectivity in *se/cc* complexes is achieved by symplasmic transport is less certain. A number of models have been put forward. The simplest is that sucrose (and amino acids) is transferred symplasmically from mesophyll to *se/cc* complexes by intercellular diffusion across Pds (Altus & Canny 1985; Turgeon & Medville 1998). Current validation of the model rests upon estimates of cytoplasmic sucrose concentrations which assume a mesophyll compartmentation of sucrose to higher concentrations in the cytosol compared to vacuole (Turgeon & Medville 1998; cf proposed facilitated diffusion across tonoplasts of mesophyll cells – Martinoia, Masson-Neau & Frange 2000). A more straightforward test of the model will be to determine PCMBs-sensitivity of photoassimilate export (see earlier discussion). Interestingly, PCMBs-insensitive export and hence presumptive symplasmic loading of sucrose has been demonstrated in polyol translocating parsley (Flora & Madore 1996). Whether this finding applies to sucrose loading in RFO translocating species awaits resolution (Turgeon 2000). However, bulk estimates of sucrose concentrations in cucumber leaves suggest

that sucrose is loaded against a concentration gradient (Haritatos, Keller & Turgeon 1996) and this is consistent with the presence of sucrose transporter transcript in *se/cc* complexes of pumpkin leaves (Ruiz-Medrano *et al.* 1999). The elaborated endomembrane system, extending from mesophyll cells to *se/cc* complexes of RFO species, is postulated to be a transport domain (Gamalei *et al.* 1994, 2000). This proposal gains some credibility from the demonstrated intercellular transport of micro-injected fluorescent dyes into the endomembrane network of stem epidermal cells (Cantrill, Overall & Goodwin 1999). However, to concentrate sucrose in intermediary cells using such a route would require a sophisticated osmoregulatory mechanism to prevent severe swelling or shrinkage of endomembranes (cf. Gamalei *et al.* 2000). Setting sucrose accumulation aside, the most plausible explanation for concentrating RFOs in intermediary cells (Haritatos *et al.* 1996), and supported by persuasive experimental evidence, is the 'polymer trap' model (Turgeon 1996, 2000). The model envisions sucrose to move symplasmically from mesophyll to intermediary cells where it is converted to raffinose and stachyose (Fig. 1A). Size exclusion limits of Pds allow passage of synthesized RFOs from intermediary cells to sieve elements but prevent leakage back to bundle sheath cells thus accounting for their accumulation in *se/cc* complexes (Turgeon *loc. cit.*)

Control of phloem loading

Control of phloem loading could be exercised on any component of photoassimilate movement from mesophyll cytoplasm to *se/cc* complexes of minor veins (Fig. 1A). Symplasmic loading rates would be expected to be limited by diffusion (Eqn 3) through Pdl pores of such dimensions that viscous drag would prevent bulk flow in the reverse direction (Eqn 5). In these circumstances, Eqn 3 predicts a linear dependence of export rate on mesophyll assimilate concentration with the slope being a function of Pdl conductance. This relationship has been found to apply to a range of C_3 and C_4 species including verified apoplasmic and symplasmic phloem loaders (Grodzinski, Jiao & Leonardos 1998; Leonardos & Grodzinski 2000). This observation infers that apoplasmic phloem loading may be rate limited by symplasmic delivery to bundle sheath/vascular parenchyma cells. A conclusion consistent with results of a flux control analysis suggesting that some 80% of the control of photoassimilate transport in potato plants was upstream of photoassimilate efflux to the leaf apoplasm (Sweetlove & Hill 2000). However, the linear relationship between sucrose synthesis and export breaks down under elevated CO_2 (Grodzinski *et al.* 1998; Leonardos & Grodzinski 2000) and, in some plants, better conforms to facilitated membrane transport (Eqn 6 and see Moing *et al.* 1994; Komor 2000). Together, these observations suggest that control of loading could vary according to the physiological state of the leaf. This proposition is exemplified in *Ricinus* cotyledons where it has been shown that phloem loading can be switched between sym- and apoplasmic routes by

blocking the alternative pathway (Orlich *et al.* 1998; Schobert, Zhong & Komor 1998).

Coarse symplasmic control could be imposed by developmental events determining Pdl frequencies and their differentiation from primary to secondary states (e.g. Ding *et al.* 1993). Fine control is likely to be exercised by changes in Pdl conductance as illustrated by effects of apoplasmic pH (Madore & Lucas 1987) and viral movement protein (Almon *et al.* 1997) on phloem loading and export. As our understanding of Pdl function increases (Schulz 1999; Roberts *et al.* this volume), greater opportunities will be provided to discover the nature of controls regulating Pdl gating along the symplasmic loading pathway. Currently, cloning of sucrose and amino acid symporters offers more scope to examine the control of apoplasmic loading.

Since sucrose concentrations in the minor vein apoplasm are likely to saturate the *se/cc* complex sucrose symporters (see flux estimates above), then regulation of apoplasmic loading must be mediated through alterations in membrane protein levels of transport-capable symporter (Eqn 6). This prediction is borne out by the finding that enhanced export rates from peach leaves, induced by increases in sink : source ratio, were accounted for by changes in maximum velocity of export (Moing *et al.* 1994). In this context, symporter activity could be regulated by sucrose pool sizes responding to changes in source : sink ratio. Here sucrose in the minor vein apoplasm provides a measure of source supply and sink demand. In this context, expression of *ZmSUT1* (Aoki *et al.* 1999) and the closely related *LeSUT2* (Barker *et al.* 2000) are up-regulated in excised leaves loaded with sucrose through the transpiration stream. However, SUTs differ in their response to sucrose signals as shown by *SUT1* expression being unaltered (e.g. *StSUT1* – Barker *et al.* 2000) or repressed (*BvSUT1* – Chiou & Bush 1998) in response to transpirationally delivered sucrose. Phytohormones also function as source : sink signals and undoubtedly interact with sugar-signalling pathways (Paul & Foyer 2001). These molecules up- or down-regulate phloem loading of sucrose (Patrick 1987) possibly through alterations in *SUT* expression (Harms *et al.* 1994). Cytokinins, imported through the transpiration stream, are the strongest candidates for sink signals transmitted to source leaves to integrate sink demand (Roitsch & Ehneß 2000) with phloem loading (Patrick 1987). The simplest model is regulation of root : shoot ratios by root-produced cytokinins integrating photoassimilate supply with root demand. This model also extends to shoot sinks, such as developing seeds, where seed-produced cytokinins could be delivered to source leaves in phloem-imported water recycled through the xylem (Emery, Ma & Atkins 2000). Other phytohormones, synthesized locally in mature leaves (abscisic acid, auxins and gibberellins), are likely to regulate phloem loading (Patrick 1987) through slowing or accelerating processes of leaf senescence (Paul & Foyer 2001).

Instantaneous transmission of alterations in sink demand could be transmitted to source leaves as changes in *P* (Eqn 2) of the *se/cc* complexes. For example, under sink-limited conditions, export rates respond rapidly (in min-

utes) to changes in sink demand (e.g. Moorby, Troughton & Currie 1974) and this is believed to be consistent with local turgor regulating phloem loading (Smith & Milburn 1980a, b, c). Turgor regulation of H⁺-ATPase activity in the phloem (Daie 1987) provides a common mechanism to control amino acid and sucrose loading by changing the *pmf*. Turgor shifts could be sensed through a signal cascade (Yuasa *et al.* 1997) resulting in phosphorylation/dephosphorylation regulating activity of the H⁺-ATPase (Sze *et al.* 1999) or transporters (Roblin *et al.* 1998).

RELEASE PHLOEM – DO SINKS SUCK OR ARE THEY FREE LOADERS?

At sinks, photoassimilates move from *se/cc* complexes to sites of utilization/storage (phloem unloading – van Bel & Oparka 1992; Fig. 2). Thus, phloem unloading includes transfer across the *se/cc* complex boundary (sieve element unloading) arranged in series with cell-to-cell transport to sites of photoassimilate utilization (post-sieve element transport). For most sinks, phloem unloading follows sym-

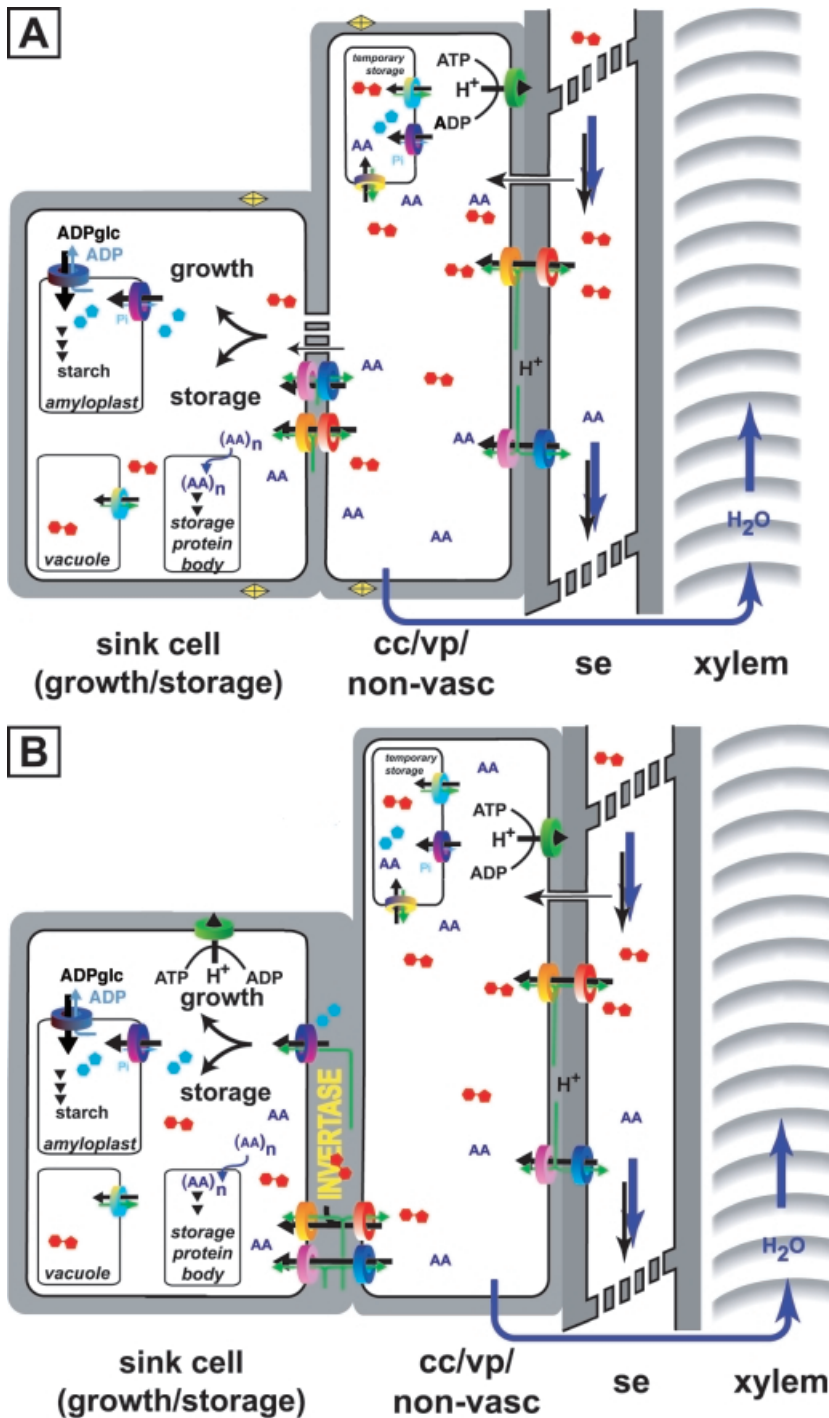


Figure 2. Phloem unloading and postphloem unloading. During certain stages of sink development, phloem unloading has been shown to follow a symplasmic route (A) or this path is interrupted by an apoplasmic step (B). For some sinks, sucrose influx has been described to occur by facilitated diffusion (not shown) or by proton symport. For other sinks, an extracellular invertase hydrolyses sucrose into hexoses (light blue molecules) that are then subsequently taken up by sink cells via hexose transporters (blue/purple). A strategically-positioned apoplasmic barrier of cell wall encrustations (yellow diamond) impairs the back flow of nutrients. In both cases (symplasmic and apoplasmic), sucrose, amino acids/amides, and hexoses may be accumulated into vacuoles for temporary or more long-term storage by specific transporters. Alternatively storage may occur as polymerized products in amyloplasts (starch) or storage protein bodies (AA). *cc*, companion cell; *mc*, mesophyll cell; *vp*, vascular parenchyma; *se*, sieve element; colour coded as in (Fig. 1).

plasmic routes (Patrick 1997; Fig. 2A). This is illustrated by movement of green fluorescent protein (GFP), synthesized in companion cells of source leaves, the 27 kDa molecule is transported through the phloem to enter a range of sink organs (Imlau *et al.* 1999). However, in some cases, symplasmic transport is interrupted by an apoplasmic step in the post-sieve element pathway (Fig. 2B). This occurs where photoassimilate transport takes place across genome interfaces (biotrophic relationships; host/parasite; maternal/filial generations) and in sinks accumulating osmotic to high concentrations (Patrick 1997). Irrespective, a component of unloading is to the phloem apoplasm from *se/cc* complexes driven by large transmembrane sucrose concentration differences (Patrick 1990). In most sinks, this flux is predicted to be relatively small constrained by the limited plasma membrane surface areas of protophloem sieve elements and metaphloem *se/cc* complexes (Patrick 1997; Schulz 1998).

The easy way out – symplasmic unloading

Structural precedents exist for symplasmic unloading in all sink types. Thus, Pds interconnect non-phloem tissues with protophloem sieve elements in root apices (Bret-Harte & Silk 1994; Schulz 1995) and sink leaves (Ding *et al.* 1988; Haupt *et al.* 2001 but cf. Evert & Russin 1993) and metaphloem *se/cc* complexes in storage sinks (Offler & Patrick 1984, 1993; Oparka 1986; Offler & Horder 1992; Wang, Offler & Patrick 1995a; Fig. 2A). Characteristically, a Pdl constriction is located at the protophloem/vascular parenchyma boundary of root tips (Schulz 1998) and *se/cc* complex/vascular parenchyma boundary of storage sinks (Offler & Patrick 1984, 1993; Offler & Horder 1992; Wang *et al.* 1995a). At specific stages of organ development, Pds interconnecting sink cells with *se/cc* complexes are transport capable as shown by passage of phloem-imported membrane-impermeant fluorochromes and macromolecules, including foreign ones such as GFP and dextrans. In this context, a potential for symplasmic unloading has been demonstrated in root apices (Oparka *et al.* 1994; Wright & Oparka 1996; Imlau *et al.* 1999) and developing leaves of dicots (Roberts *et al.* 1997; Imlau *et al.* 1999) and monocots (Haupt *et al.* 2001; Fig. 2A). Similar observations have been made for storage sinks including developing fruits (Patrick & Offler 1996), tubers (Viola *et al.* 2001) and maternal tissues of seeds (Patrick, Offler & Wang 1995; Wang *et al.* 1994; Wright & Oparka 1996; Imlau *et al.* 1999; Fisher & Cash-Clark 2000a; Ruan, Llewellyn & Furbank 2001; Fig. 2A). Photoassimilates appear to follow these symplasmic routes of phloem unloading in roots apices and expanding leaves as photoassimilate unloading is found to be insensitive to PCMBs (Giaquinta *et al.* 1983; Schmalstig & Geiger 1985; Turgeon 1987; Schmalstig & Cosgrove 1990; Farrar *et al.* 1995). Similar conclusions, drawn on less convincing evidence, have been drawn for developing fruit (Ruan & Patrick 1995 and references therein), maternal tissues of seeds (Patrick & Offler 2001) and tubers (Oparka & Prior 1987; Viola *et al.* 2001). Dye coupling studies have identi-

fied symplasmic domains that canalize photoassimilate flows to elongation zones in root tips (Oparka *et al.* 1994) and to sites of photoassimilate release in coats of developing seeds (Patrick *et al.* 1995). These patterns undoubtedly reflect selective Pdl gating.

In a number of sink types, symplasmic transport is turned up or down at certain stages of development. For sink leaves, dye coupling of *se/cc* complexes with adjacent phloem parenchyma ceases abruptly coincident with transition to net export of photoassimilates; an event closely correlated with a developmental change from simple to branched Pds (Oparka *et al.* 1999). This phenomenon may also account for the onset of symplasmic isolation of metaphloem *se/cc* complexes (Oparka *et al.* 1994) in developing root tips (Zhu, Lucas & Rost 1998). Significantly, simple Pds interconnect cells along post-sieve element unloading pathways in developing seeds including maternal (Offler & Patrick 1984, 1993; Wang *et al.* 1995a) and filial (Wang *et al.* 1995b) components. However, this generalization is not universal as demonstrated by carboxyfluorescein transport through branched Pd of cotton fibres (Ruan *et al.* 2001).

Shifts between apo- and symplasmic routes have been detected during sink development of tubers (Viola *et al.* 2001) and fruits (Ruan & Patrick 1995; Patrick & Offler 1996). In both cases, the switch coincides with commencement of a grand phase of photoassimilate import. Prior to initiation of tuber development, Pds are closed and unloading from *se/cc* complexes follows an apoplasmic route (Viola *et al.* 2001). Coincident with tuber swelling, developing internal and external phloem strands of tubers commence to unload symplasmically (Viola *et al.* 2001). In contrast, developing tomato fruit exhibit a reverse behaviour switching from symplasmic to an apoplasmic path of unloading (Ruan & Patrick 1995; Patrick & Offler 1996). A major difference between potato tuber and tomato fruit sinks is that the former accumulates starch and the latter osmotically active hexoses. Furthermore, the recent finding that cotton fibre Pd are reversibly gated across their development (Ruan *et al.* 2001) could equally apply to other unloading pathways allowing for greater flexibility in regulating photoassimilate fluxes from the phloem.

The observations described above provide persuasive evidence that a route of symplasmic unloading exists in a wide range of sinks. However, whether these symplasmic paths have the capacity to support observed rates of photoassimilate transport is more open for debate. Modelling symplasmic transport depends upon making assumptions about Pdl conductances. A model based on estimated Pdl pore diameters of 4 nm suggested that Pdl conductances could not account for observed transport rates in root apices (Bret-Hart & Silk 1994). However, more recent studies have found much larger size exclusion limits for Pds in developing leaves (Imlau *et al.* 1999; Oparka *et al.* 1999), root apices (Imlau *et al.* 1999) and maternal seed tissues (Imlau *et al.* 1999; Fisher & Cash-Clark 2000a). A careful appraisal suggests pore diameters could be as high as 8 nm (Fisher & Cash-Clark 2000a). This translates into a 16-fold

higher hydraulic conductance (see Eqn 4) and an approximate similar outcome for diffusive conductance (see Fisher & Cash-Clark 2000a and references therein) compared to earlier estimates (Bret-Hart & Silk 1994) and these can account for observed photoassimilate fluxes. Some experimental observations support the modelling conclusions. For instance, symplasmic water entry from the phloem accounts for 50% of the cell volume increase in elongating hypocotyls (Schmalstig & Cosgrove 1990) and 80% in elongating roots (Bret-Hart & Silk 1994; Pritchard, Winch & Gould 2000).

Mechanisms and controls of symplasmic unloading

In the absence of evidence to the contrary, it is assumed that ions and small molecular weight compounds (approximately 800 Da) move passively through Pdl canals by diffusion (Eqn 3; cf Tucker & Tucker 1993) or possibly by bulk flow (Eqns 4 and 5). A concentration gradient of a major osmotic solute will translate into an osmotic gradient. Thus, symplasmic unloading of photoassimilates includes diffusion and bulk flow components and their relative contributions will depend upon path conductances and driving forces for each transport mechanism.

To our knowledge, cytosolic concentration gradients required to drive diffusion of photoassimilates through sink symplasm have not been measured. However, this concern does not apply to root tip cells that are highly cytoplasmic with minimal vacuolation and to maternal seed tissues where rapid exchange between vacuole and cytosol results in these two compartments kinetically behaving as one (Fisher & Wang 1993). In this context, favourable concentration gradients for symplasmic diffusion of sugars have been detected in root tips (Winch & Pritchard 1999) and maternal seed tissues (Fisher & Wang 1995). Consistent with diffusion contributing to unloading, photoassimilate import rates into root tips (Schulz 1994) and stems (Patrick & Offler 1996) immersed in sucrose solutions were inversely related to bath sucrose concentrations. In developing wheat seeds, post-sieve element transport of photoassimilates is considered to occur by diffusion. This conclusion is based upon a number of diagnostic observations. These include: (i) a sucrose concentration gradient sufficient to drive diffusion at normal rates of import (Fisher & Wang 1995); (ii) membrane-impermeant fluorochromes can move against the incoming photoassimilate flow (Wang & Fisher 1994b; Fisher & Cash-Clark 2000a); (iii) the absence of a pressure gradient along the post-sieve element pathway (Fisher & Cash-Clark 2000b). In contrast, a component of sieve element unloading is likely to occur by bulk flow (Eqn 5) driven by large drops in osmotic concentration of 0.7–1.0 MPa across this boundary in root tips (Warmbrodt 1987; Pritchard 1996) and developing seeds (Fisher & Wang 1995; Fisher & Cash-Clark 2000b). In some sinks, movement by bulk flow could extend to post-sieve element transport.

For instance, photoassimilate import has been found to respond positively to down-regulation of sink cell turgor pressure in developing seeds (Wolswinkel 1992 but cf. Wang & Fisher 1994a), root tips (Williams, Minchin & Farrar 1991; Schulz 1994) and stems (Patrick & Offler 1996). In addition to delivering nutrients, bulk flow of sap from the phloem provides a mechanism to meet water requirements for volume growth in expansion sinks (e.g. Bret-Hart & Silk 1994; Farrar *et al.* 1995; Pritchard *et al.* 2000). However, in storage sinks this raises problems for separating incoming flows of photoassimilates from exiting phloem water (see Patrick & Offler 2001). Another impediment for bulk flow is where osmotically active assimilates are accumulated to high concentrations (e.g. developing fruit) such that differences in P between sieve elements and sink cells could be attenuated (see Eqn 5). A solution to this latter impasse is anatomical separation of vascular and sink cell apoplasts by wall deposits of solute impermeable encrustations in cells surrounding the vascular bundles (Fig. 2A). As a consequence, vascular and sink cell turgor are independently regulated by adjustments in their apoplasmic sap osmotic pressures as osmotic concentrations rise in sink cell protoplasts (for example, sugarcane stems and see Moore 1995).

The diffusion equation (see Eqn 3) predicts that unloading is linked to metabolism and intracellular compartmentation through the activities of these processes determining cytosolic concentrations of amino N compounds and sucrose in sink cells. A similar linkage is predicted for bulk flow in growth and polymer storage sinks where metabolic interconversion to non-osmotic species maintains a low cell Π , hence P (see Eqn 5). However, a number of observations indicate that other phenomena share control of unloading in addition to lowered photoassimilate pool sizes in sink cells. For instance, antisense transgenic potato tubers in which sucrose synthase activity was down-regulated by 95% resulted in only a 50% decrease in tuber dry matter (Zrenner *et al.* 1995). Similarly, turgor differences between sieve elements and sink cells only partially account for differences in rates of photoassimilate import into elongating root tips (Pritchard *et al.* 2000). These outcomes, at least in part, are conjectured to result from the relatively high osmotic concentrations of sucrose and amino N compounds in phloem sap compared with those in recipient sink cells. As a consequence, changes in sink cell osmotic concentrations cause a less than proportionate change in concentration and osmotic pressure differences against the respective values in sieve elements. Experimental support for this proposal is found in the observation that import of radio-labelled sucrose is depressed by 90% into excised tomato fruit in which sucrose synthase activity is down-regulated by a corresponding amount by transformation with antisense sucrose synthase (D'Aoust, Yelle & Nguyen-Quoc 1999). For excised fruit, phloem sap sucrose concentrations are likely to be greatly reduced and, as a result, differences with sink values will be relatively small. In these circumstances, minor shifts in sink cell concentrations will have

large effects on differences in sucrose concentrations between sieve elements and sink cells (see Eqn 3). However, probably for the reasons given above, this behaviour is not reproduced for fruit volume growth on intact plants (D'Aoust *et al.* 1999). Overall these observations point to Pdl conductance exercising a major control over unloading flux through symplasmic pathways. This control is likely to be regulated at points of lowest conductance located at pro-phloem sieve element/vascular parenchyma boundaries in root tips (Warmbrodt 1987) and metaphloem se/cc complex/vascular parenchyma boundaries in developing seeds (Offler & Patrick 1984, 1993; Wang *et al.* 1995a; Fisher & Cash-Clark 2000b).

Direct evidence for Pdl conductance controlling symplasmic unloading of photoassimilate is scant. One suggestive example is that osmotically elevated rates of photoassimilate unloading in root tips were found to correlate closely with increases in Pdl sleeve area (Schulz 1995) and hence conductances (see Eqn 4). However, this response does not indicate how cell growth/metabolism and Pdl conductance are co-ordinated. Phytohormones have been implicated in regulating Pdl conductances in stems to account for their effects on photoassimilate accumulation when radial gradients of sucrose concentration and water potential are clamped (Patrick & Offler 1996). For growth sinks, phytohormones could co-ordinately regulate expansion growth (Cosgrove 1999) and Pdl conductance through calcium-related signalling pathways (Holdaway-Clarke *et al.* 2000). The latter by altering callose deposition/hydrolysis (Botha & Cross 2000; Baluska *et al.* 2001 but cf. Ruan *et al.* 2001) and physiochemical states of key cytoskeleton components (Baluska *et al.* 2001) located around and in the neck regions of Pds. It is envisioned that callose deposition sets upper limits for Pdl pore diameter whereas fine control is exercised by the cytoskeletal motor system. Overall, these observations suggest that sinks do not suck but free load. They do so by modulating conductances of strategically located Pds to regulate photoassimilate unloading rates to levels commensurate with their metabolic and growth demands using the driving forces of high photoassimilate concentrations and hydrostatic pressures (Eqns 3 and 5) resulting from phloem loading activities in source leaves.

High concentrations of assimilates may be transported over considerable distances through non-vascular symplasmic routes (Patrick 1997), a process that depends upon assimilate retention in the symplasm by retrieval from the sink apoplasm. For instance, high intracellular hexose concentrations in root tips (Pritchard *et al.* 2000) are associated with strong expression (Sauer & Stadler 1993) of functionally active (Xia & Saglio 1988) hexose transporters (HXTs). One or more of amino acid permeases (AAPs) expressed in roots (Fischer *et al.* 1998) may function in retrieval. Similarly, the strong expression of HXTs (e.g. NtSTP1 – Sauer & Stadler 1993) and AAPs (e.g. AtAAP6 – Fischer *et al.* 1998) in leaf sinks are likely to serve a retrieval function (Fig. 2).

Border crossing checkpoint: an apoplasmic step in phloem unloading pathways

Photoassimilates traversing phloem unloading pathways encounter symplasmic discontinuities sited at interfaces between generations of developing seeds (Patrick & Offler 2001) and mutualistic biotrophic relationships (Hall & Williams 2000; Smith, Dickson & Smith 2001; Day *et al.* 2001). In addition, an apoplasmic step has been shown to occur in sinks that accumulate osmotic solutes to high concentrations and lack apoplasmic separation into vascular and sink domains (e.g. developing tomato fruit – Patrick 1997; Fig. 2B). Although these systems share much in common, a major difference is that in mutualistic biotrophic relationships, the plant host derives amino N compounds from the microbial partner in exchange for sugars (Smith *et al.* 2001; Day *et al.* 2001).

Dye-coupling experiments show that symplasmic continuity exists between importing sieve elements and non-phloem tissues in maternal tissues of developing seeds (Patrick & Offler 2001) and in rhizobial root nodules (Brown *et al.* 1995). Except for *Rhizobia* nodules (Day *et al.* 2001), identification of cellular sites of photoassimilate release to the sink apoplasm is circumstantial as membrane proteins responsible for efflux are yet to be isolated and characterized (see below). For developing seeds, structural and physiological findings indicate that maternal cells, most likely to function in photoassimilate release, are located at or proximal to maternal/filial interfaces (Patrick & Offler 2001). Post-sieve element transport through a symplasmic compartment allows for imported nutrients to be sequestered into short-term storage pools and to undergo metabolic interconversion. The former functions to buffer variation in phloem import rates and the latter to modify imported nutrients to meet the nutritional requirements of filial tissues. For instance, extensive post-sieve element pathways in grain legume seeds provide scope for temporary nutrient storage and metabolism (Fig. 2). Some 70–90% of amino acids (Lanfermeijer, van Oene & Borstlap 1992) and sucrose (Patrick 1993) are located in compartments with half-times for exchange consistent with vacuolar storage. Vacuolar amino acid transporters have been characterized in yeast for which there are homologues in plants and could serve for this purpose (Wipf *et al.* 2002). Vacuolar nutrients are readily exchanged with cytoplasmic compartments for release to the seed apoplasm and their pool sizes are estimated to meet embryo demand for 4–12 h (Lanfermeijer *et al.* 1992; Patrick 1994). Amino N compounds, imported mainly as amides, undergo considerable metabolism prior to release to the seed apoplasm (Murray 1987; Rochat & Boutin 1991). In pea seed coats metabolism of asparagine occurs at a high rate resulting in formation of glutamine, alanine and valine which are then unloaded into the apoplasmic space and taken up by the developing cotyledons (Rochat & Boutin 1991).

Pds interconnect sieve elements with non-phloem pericarp tissues in developing tomato fruit and grape berries.

However, once sugar accumulation commences, a symplasmic discontinuity develops at the vascular bundle/storage parenchyma interface in tomato (Patrick 1997). Consistent with an apoplasmic route of unloading, tomato fruit develop a capacity for membrane transport of hexoses commensurate with observed rates of hexose accumulation (Brown, Hall & Ho 1997; Ruan, Patrick & Brady 1997). Similarly, an apoplasmic step in grape berries is suggested by coincident expression of hexose (Filion *et al.* 1999) and sucrose (Davies, Wolf & Robinson 1999; Ageorges *et al.* 2000) transporters with the onset of sugar accumulation. In both cases, symplasmic isolation of vascular and sink tissues ensures that elevated turgor, resulting from rising osmotic concentrations in sink cells (e.g. 1 M hexoses in grape berries), does not compromise phloem turgor driving bulk photoassimilate import into these fruit sinks (Patrick 1997). In grape, possible breakdown in cellular compartmentation (Lang & Düring 1991; Dreier, Hunter & Ruftner 1998) represents an alternative solution to maintaining phloem import against a rising osmotic concentration in the sink tissues. Apoplasmic domains for nutrient exchange between host and fungus have been identified for ecto- and endomycorrhizas in root cortices, but precise cellular locations of solute exchange are not known (Smith *et al.* 2001).

Cellular sites of sucrose and amino acid uptake by filial tissues of developing seeds have been unequivocally identified by immunolocalization of transporters responsible for photoassimilate uptake into these organs. Thus, SUTs and H⁺-ATPases are colocalized to plasma membranes of filial cells abutting maternal/filial interfaces in developing seeds of dicots and temperate cereals during storage product accumulation (Patrick & Offler 2001). A transcript of a wide specificity amino acid permease, PsAAP1, exhibits a similar cellular localization pattern (Tegeder *et al.* 2000b). Thereafter, transport to underlying storage cells is symplasmic (Patrick & Offler 2001). An apoplasmic route is arranged in parallel (Patrick & Offler 2001) but presents a high resistance to solute movement in developing seeds of both dicots (Gifford & Thorne 1985) and temperate cereals (Wang *et al.* 1995b).

Mechanisms and controls of photoassimilate exit to, and uptake from, the sink apoplasm

Proposed mechanisms of photoassimilate efflux to sink apoplasm along post-sieve element pathways are conjectural and range from passive leakage to energy-coupled membrane transport. To date, no putative photoassimilate effluxer has been isolated and functionally characterized.

Depending upon membrane surface areas available for release, passive leakage may account for glucose release from root cortical cells at intercellular interfaces with mycorrhizal partners (Smith *et al.* 2001). Expression of hexose transporters in colonized regions of the plant host (Harrison 1999) could function to regulate net exchange of carbon to fungal hyphae in a pump/leak configuration. If larger

photoassimilate fluxes pertain, then a selective release mechanism is required so that two-way exchange of solutes between host and fungal partners is not compromised. Studies of photoassimilate release from other organs indicate plant genomes may encode proteins that could serve such a function.

The simplest of these is for passive sucrose release regulated by an extracellular invertase-controlling transmembrane differences in solute concentrations. Extracellular invertases are commonly linked with cell expansion sinks (Roitsch *et al.* 2000). These enzymes are also present in storage sinks that take up hexoses from their apoplasmic saps, such as tomato fruit and seeds of tropical cereals (Patrick 1997; Fig. 2B). In this context, a broadly held view is that extracellular invertases function to maintain large transmembrane concentration differences to drive passive leakage of sucrose and prevent its reloading by SUTs located on plasma membranes of se/cc complexes (Eschrich 1980). However, it is sobering to consider that reducing the highest recorded apoplasmic sucrose concentration of approximately 100 mM (Patrick 1997) to zero would only increase a transmembrane concentration difference across the se/cc complexes by 30% against a lower-than average phloem sap sucrose concentration of 300 mM. A more primary role of extracellular invertases may be to double the osmotic contribution of unloaded sucrose to apoplasmic osmotic pressures that affect sieve element turgor and hence rates of photoassimilate import (Eqn 2; Roitsch *et al.* 2000). This function would include all phloem-transported solutes and would apply equally to all sinks, irrespective of their primary pathway of unloading. Elucidating roles of extracellular invertases in photoassimilate release using a molecular approach is not trivial as altered hexose fluxes affect sugar-sensitive components of plant development (e.g. see Tang, Luscher & Sturm 1999). However, developing tomato fruit gives some hints. Here an extracellular invertase functions co-operatively with passive leakage of sucrose from the phloem to the fruit apoplasm (Ruan & Patrick 1995). Hexoses are retrieved by HXTs localized to plasma membranes of storage parenchyma cells (Ruan *et al.* 1997; Gear *et al.* 2000) and symporter activities account for cultivar differences in fruit hexose levels (Ruan *et al.* 1997). HXTs also perform a similar function in filial tissues of tropical cereals (Thomas, Felker & Crawford 1992) and fungal partners of mycorrhizas (Smith *et al.* 2001). In many sinks, entry into a storage phase is accompanied by a developmental down-regulation of extracellular invertases and sucrose is taken up intact (Patrick 1997).

Efflux mechanisms conferring solute selectivity have been detected in developing seeds. These include carriers supporting facilitated diffusion of sucrose in cereals (Porter, Kniewel & Shannon 1987; Wang & Fisher 1995) and sucrose/proton antiporters in French and broad bean (Fieuw & Patrick 1993; Walker *et al.* 1995, 2000). Facilitated diffusion may be mediated by sucrose/H⁺ symporters in the absence of a proton gradient (Lemoine *et al.* 1996). Consistent with this suggestion, release cells of the nucellus pro-

jection in developing wheat grains strongly express a SUT homologue that lacks functional properties of a symporter (Bagnall *et al.* 2000). Energy-coupled sucrose release in seed coats of French and broad bean exhibits properties consistent with sucrose/H⁺ antiport (Fieuw & Patrick 1993; Walker *et al.* 1995, 2000). Interestingly, SUT homologues colocalize with H⁺-ATPases in seed coat release cells of broad bean (Harrington *et al.* 1997) and pea (Tegeger *et al.* 1999) in which sucrose/H⁺ symporter activity is not detectable (De Jong *et al.* 1996). Thus these putative SUT paralogues could function as sucrose/H⁺ antiporters as only subtle differences exist between facilitators and proton-coupled transporters in the major facilitator superfamily (Pao *et al.* 1998). A requirement for energized transport to drive sucrose efflux is not immediately self-evident for phloem unloading. However, estimates of transmembrane concentration differences indicate these are small in French and broad bean seed coats (8 and 30 mM sucrose, respectively, Patrick 1994) and non-existent in wheat (Fisher & Wang 1995). Under these conditions, transport may need to be energized to contribute to the high osmotic concentrations in the seed apoplast and to meet the rapid osmoregulatory adjustments in apoplastic sap osmotic pressure (Patrick 1994).

Energy-coupled sucrose release from French and broad bean seed coats accounts for 50% of their total sucrose flux (Fieuw & Patrick 1993; Walker *et al.* 1995, 2000). The remaining passive flux might occur through non-selective channels reported to support sucrose and amino acid efflux from seed coats of pea (De Jong *et al.* 1996, 1997). Interestingly, a non-selective channel has been detected in release cells of *Phaseolus* seed coats that is permeable to a wide range of electrolytes including large organic ions such as glutamate (Zhang *et al.* 2002). Non-selective channels would compromise the polarized exchange of sugars, amino N compounds and phosphate between host plant and mycorrhizal fungi (Smith *et al.* 2001) and could only function for sucrose if these channels were located separately from sites of phosphate and amino N exchange.

Proton-coupled symporters in filial tissues of grain legumes and temperate cereals retrieve sucrose (Patrick & Offler 2001) and amino N compounds (Hirner *et al.* 1998; Tegeger *et al.* 2000b) released to the seed apoplast (Fig. 2B). Sucrose symporter activity plays a key role in post-sieve element transport as shown by over-expression of *StSUT1* in pea cotyledons enhancing their growth rates (Rosche *et al.* in press). This raises the question as to how metabolic demand is communicated to post-sieve element transport events. Filial pool sizes of sucrose and amino acids are candidates to function as signals because these change in response to altered rates of solute delivery and storage product formation and are known to regulate symporter gene expression through de-repression (Patrick *et al.* 2001). Resulting changes in solute withdrawal from the seed apoplast are sensed by a turgor-homeostat mechanism located in maternal seed tissues that adjusts effluxer activities accordingly (Patrick & Offler 2001). The outcome

is maintenance of relatively high apoplastic sucrose concentrations (Patrick 1994; Thomas, Hetherington & Patrick 2000). As a consequence, transcriptional regulation of filial symporter activity (Weber *et al.* 1997) is fully realized though changes in its maximal velocity (Tegeger *et al.* 2000a). Long-term changes in filial demand are met by shifts in the set point of the turgor homeostat that integrates phloem import rates with those of post-sieve element transport (Patrick 1994). Regulation of symplasmic delivery to sites of solute efflux from maternal tissues also could be mediated by hormonal control of Pdl conductances (see pp 47). Indeed, pharmacological applications of auxin to wheat plants was found to enhance rates of post-sieve element transport of ¹⁴C photoassimilates in developing grains and this effect was attributed to an integrative auxin action to regulate Pdl and membrane transport (Darussalam, Cole & Patrick 1998).

CONCLUDING REMARKS

The history of deducing pathways and mechanisms of phloem loading and unloading from experimental observations provides a wonderful account of the challenges encountered in studying a highly integrated series of transport events not readily accessible to experimental manipulation. These ingredients have generated their fair share of ambiguities that have strongly influenced thinking at particular times in the development of the field (e.g. see Lucas 1985).

Cloning sugar and amino acid/amide symporters have breached this technological impasse in providing access to key influx events involved in apoplastic phloem loading and unloading. In the case of sucrose transporters, molecular genetic manipulation of their expression has allowed unequivocal resolution of their central roles in phloem loading and transport in *Arabidopsis* and *Solanaceous* plants. How these findings apply to other species, and to the loading of amino N compounds, remains to be determined. The case of sucrose loading in RFO transporting species will be of particular interest. Similar advances in understanding the physiological roles of sink-located symporters are anticipated in the near future. A significant impediment to a complete appreciation of apoplastic loading and unloading is the current scant knowledge of the efflux step. Cloning efflux transporters will allow this gap in knowledge to be filled.

There is a growing body of structural and physiological evidence showing that components of phloem loading and unloading involve transport through symplasmic domains. Circumstantial evidence points to modulation of Pdl conductance functioning to regulate phloem loading and particularly unloading. However, despite the development of elegant technologies to study Pdl transport, their role in phloem loading and unloading will remain unclear until molecular manipulation of Pd substructure becomes possible.

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