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DARWIN REVIEW

Phloem transport: a review of mechanisms and controls

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Abstract

It is generally believed that an osmotically generated pressure gradient drives the phloem mass flow. So far, this widely accepted Münch theory has required remarkably few adaptations, but the debate on alternative and additional hypotheses is still ongoing. Recently, a possible shortcoming of the Münch theory has been pointed out, suggesting that the Münch pressure flow is more suitable for herbs than for trees. Estimation of the phloem resistance indicates that a point might be reached in long sieve tubes where the pressure required to drive the Münch flow cannot be generated. Therefore, the relay hypothesis regained belief as it implies that the sieve tubes are shorter then the plant's axial axis. In the source phloem, three different loading strategies exist which probably result from evolutionary advantages. Passive diffusion seems to be the most primitive one, whereas active loading strategies substantially increase the growth potential. Along the transport phloem, a leakage-retrieval mechanism is observed. Appreciable amounts of carbohydrates are lost from the sieve tubes to feed the lateral sinks, while a part of these lost carbohydrates is subsequently reloaded into the sieve tubes. This mechanism is probably involved to buffer short-term irregularities in phloem turgor and gradient. In the long term, the mechanism controls the replenishment and remobilization of lateral stem storage tissues. As phloem of higher plants has multiple functions in plant development, reproduction, signalling, and growth, the fundamental understanding of the mechanisms behind phloem transport should be elucidated to increase our ability to influence plant growth and development.

Key words: Carbon transport, leakage-retrieval mechanism, loading, Münch theory, phloem, plant defence, relay hypothesis, signalling, sink, sources, sugar transport, unloading.

Introduction

The evolutionary journey of plants onto land involved the differentiation of the plant body into decentralized organs, such as leaves, roots, stem, and branches. These organs are interconnected at the whole-plant level by long-distance transport. Besides water, sugars are one of the most important components involved in this transport. The phloem tissue is the principal sugar conductive tissue in plants. Over 80 years ago, Ernest Münch (1930) proposed the now widely accepted mechanism for phloem transport. According to his theory, the mass flow in the phloem is driven by an osmotically generated pressure gradient. As the sieve pores interconnect the protoplasts of the sieve tubes, the transport in the sieve tube itself is a mass flow driven by a pressure (or turgor) gradient. Because the sieve tubes are separated by a plasma membrane from the surrounding plant cells, a higher solute concentration indirectly implies a higher turgor pressure as water will enter the sieve tubes by osmosis (Gould *et al.*, 2005). The pressure gradient in the sieve tubes is generated by the accumulation (loading) of sugars and other osmotic substances at the sources and by their release (unloading) at the sinks (Fig. 1). The sources are mainly leaves, whereas all energy-demanding or storage tissues are sinks (e.g. roots, fruits, and meristematic tissues).

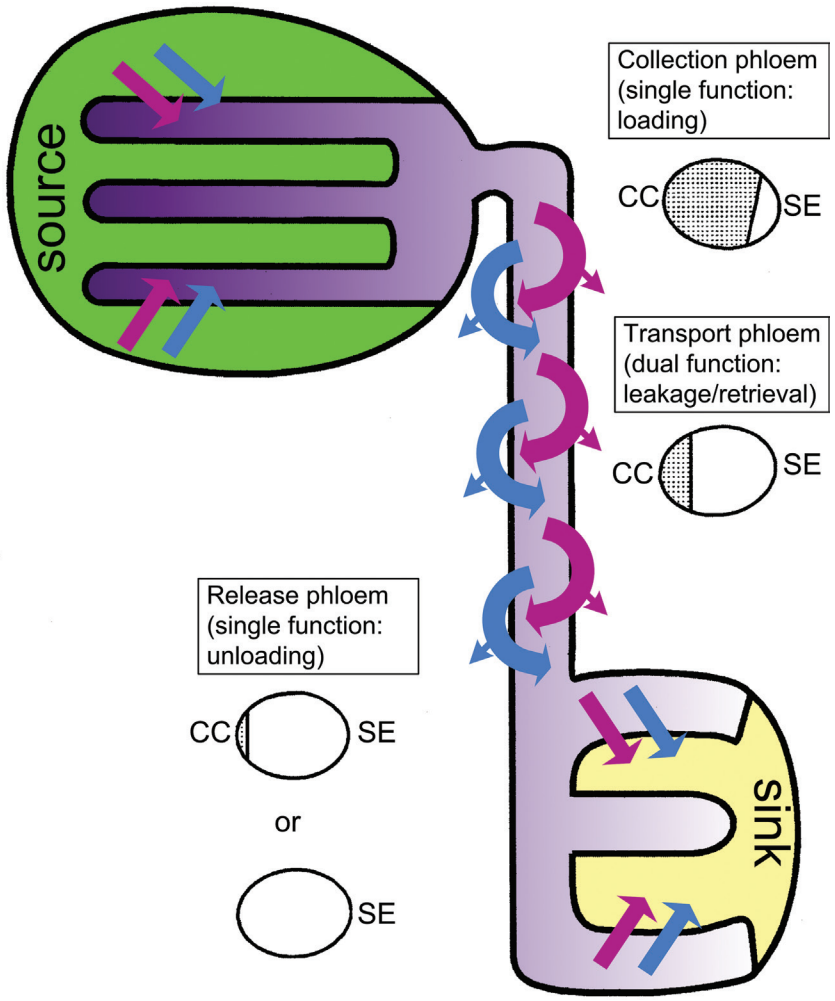


Fig. 1. A dynamic version of Münch's pressure flow model, the local photoassimilates (violet arrows) and water (blue arrows), and the relative proportion of sieve elements (SEs) and companion cells (CCs) in the respective phloem zones. Photoassimilates are translocated via the phloem through essentially leaky instead of hermetically sealed pipes. The solute concentration, and implicitly the turgor, are controlled by release/retrieval mechanisms in the sieve element–companion cell complexes (SECCCs). Differential release/retrieval balances control the influx/efflux of sugars (violet arrows) and water (blue arrows) in the various phloem zones. In the collection phloem (phloem loading), the uptake or retrieval dominates; in the release phloem (phloem unloading), the release dominates. In the transport phloem, having a dual task (nourishment of lateral and terminal sinks), the balance between release and retrieval varies with the requirements of the plant. The gradual loss of solutes and commensurate amounts of water towards the sink, where massive delivery of water and solutes takes place, has been ascribed to the relative size reduction of CCs along the source to sink path, which may explain a decreasing retrieval capacity of the SECCCs in the direction of the sink (van Bel, 2003*b*. The phloem, a miracle of ingenuity. *Plant, Cell and Environment* 26, 125–149 with permission © John Wiley & Sons Ltd; Reprinted from *Vascular transport in plants*. van Bel and Hafke, 2005. Physiological determinants of phloem transport. In: Holbrook NM, Zwieniecki MA, eds. Burlington: Elsevier, 19–44, with permission from Elsevier).

Along the phloem pathway, three successive functional sections can be defined, each with a specific task (Van Bel and Hafke, 2005): collection, transport, and release (Fig. 1). In the collection phloem, assimilates are loaded into the sieve element–companion cell complex (SECCC) of the minor leaf veins after being produced in the leaf mesophyll. Next, assimilates are transported towards the sinks via the transport phloem. The transport phloem is located in the major veins, petioles, branches, stem, and roots. The transport phloem has a dual function: it transports assimilates not only to terminal sinks (e.g. the roots and shoots) but also to lateral sinks along the path (e.g. the cambium) which are responsible for growth and tissue maintenance. These lateral sinks support the continuous leakage and retrieval of solutes along the pathway. The main task of the release phloem in the sinks is to unload assimilates from the SECCCs into growing or storage cells. The decreasing volume ratios between the companion cells and the sieve elements along the phloem stretch (Fig. 1) may be related to a decreasing energy requirement for assimilate retention in collection, transport, and release phloem (van Bel, 1996, 2003*b*).

Over time, the original Münch theory has required remarkably few adaptations (Thorpe *et al.*, 2005). It is generally believed that loading and unloading sites are both essential to

maintain the osmotic gradient and control the rate of phloem translocations (Gamalei, 2002). The loading and unloading mechanisms can be active or passive, and apoplastic (the sugars enter the apoplast at least once by crossing the cell membrane) or symplastic (entirely through the plasmodesmata-connected cytosol of cells) (Turgeon, 2010*b*). The apoplast refers to the continuous system of cell walls and xylem vessels. In Münch's original theory, the loading and unloading processes only occur in the sources (collection phloem) and sinks (release phloem), respectively. However, loading and unloading also take place along the phloem pathway (transport phloem) (van Bel, 2003*a*; Thorpe *et al.*, 2005). This is called the leakage-retrieval mechanism. As such, the sieve tubes are considered as permeable instead of impermeable pipes. Recently, Thompson (2006) hypothesized that the pressure gradients along the phloem pathway should be low or negligible in order to regulate easily the solute exchange between the SECCCs and the surrounding tissues. His hypothesis, based on a detailed model study (Thompson and Holbrook, 2004), seems physiologically acceptable because in the decentralized plant body, without a nervous system, individual cells can only sense and modify their own turgor and not the pressure gradients between them. Hence, according to this hypothesis, the transport phloem regulates the phloem turgor rather than the turgor gradient (Thompson, 2006). In addition to this hypothesis, several 'alternative' hypotheses are still under debate. The most recent findings and questioned hypotheses are reviewed herein.

Münch theory and tall trees

Recently, a possible shortcoming of Münch's theory, especially in large trees, has been pointed out. According to Münch's theory, the phloem flow (F^{Pc}) is defined by the pressure (P) difference between sources and sinks and influenced by the resistance of the phloem pathway (R^{Pc}):

$$F^{Pc} = \frac{P_{source} - P_{sink}}{R^{Pc}} \quad (1)$$

Several experimental studies (Fisher, 1978; Köckenberger *et al.*, 1997; Jahnke *et al.*, 1998; Windt *et al.*, 2006; De Schepper *et al.*, 2013) showed that phloem speed (proportional to F^{Pc}) across a wide range of angiosperm plants is roughly within the same range, namely around 1 cm min^{-1}. Windt *et al.* (2006) hypothesized that the phloem is scaled and regulated to maintain a constant and relatively slow flow. In angiosperm trees the phloem speed seems to increase with tree height (Dannoura *et al.*, 2011). Via a theoretical study, Thompson (2006) added that the pressure gradient along the phloem pathway (P_{source}–P_{sink}) should be low or negligible. Furthermore, Thompson and Holbrook (2003*a*) stated that the transit time of sucrose through a sieve tube is inversely proportional to the square of the sieve tube's length. The phloem resistance (R^{Pc}) in their model was mainly determined by the considered sieve plates whose number increased with the number of sieve elements and, thus, with the length of the sieve tube. Taking these findings into account, a contradiction arises for large plants, including trees. Because sources and sinks are symplastically connected according to Münch's theory (1930), it is generally assumed that the sieve tube's length equals the plant's axial length. In large trees, this implies that the phloem resistance becomes very large. According to Equation 1, a high phloem resistance (R^{Pc}) results either in a large pressure difference (P_{source}–P_{sink}) or in a low phloem velocity (F^{Pc}), which contradicts the above-mentioned findings. According to Münch's theory, the phloem pressure in trees should be higher than those of herbaceous species since the required pressure difference increases with the resistance of the sieve tubes and, hence, the plant's length. However, literature data suggest the opposite as the lowest measured values of phloem pressure are found in trees (Hammel, 1968; Wright and Fisher, 1980; Sovonick-Dunford *et al.*, 1981; Pritchard, 2007). As such, phloem pressure does not scale to plant size (Turgeon, 2010*a*). Moreover, estimations of phloem resistance based on the detailed architecture of sieve plates suggest that in long sieve tubes (as in large trees), a point might be reached where the pressure gradient required to drive the Münch flow exceeds the turgor pressure that can be generated (Mullendore *et al.*, 2010). As such, the Münch pressure flow seems more suitable for herbs than for trees (Turgeon, 2010*a*).

Based on these contradictions, several studies (Thompson, 2006; Hölttä *et al.*, 2009; Jensen *et al.*, 2009; Knoblauch and Peters, 2010; Mullendore *et al.*, 2010; Turgeon, 2010*a*) suggested that, at least in some cases (e.g. large trees), the sieve tubes are shorter than the plant's axial length. The proposed translocation pathway would be composed of series of shorter, overlapping sieve tubes with apoplastic loading steps between them. Lang (1979) called these intervening loading steps 'relays'. In this relay system, solutes are energetically transported from one unit to the next, providing a boost in pressure at the relays along the transport pathway. The relay hypothesis does not conflict with Münch's vision of phloem transport: the osmotically generated pressure flow is still the main mechanism of moving solutes between sites of active transport, but the speed and direction are additionally controlled near the relays (Thompson and Holbrook, 2003*a*). However, the difficulty with the relay hypothesis is that phloem sap is rich in organic molecules and ions (Turgeon, 2010*a*). Therefore, transfer across the plasma membrane requires an elaborated set of transporters unless the composition of the sap changes at each step (Knoblauch and Peters, 2010; Turgeon, 2010*a*). Nevertheless, the leakage-retrieval process in the transport phloem is in favour of the relay theory (Willenbrink, 2002; Thompson, 2006; Knoblauch and Peters, 2010). This leakage-retrieval mechanism, which is further discussed in a separate section, is interpreted by van Bel (2003*b*, 2005) as a dynamic relay mechanism. However, the only experimental study that tested the relay hypothesis in castor bean (*Phaseolus vulgaris*) favoured hydrostatic continuity (Murphy and Aikman, 1989), and the anatomical data currently available do not support the relay hypothesis.

A recent study (Jensen *et al.*, 2011, 2012) pointed out that, in addition to sieve tube conductivity, phloem speed

is strongly influenced by the osmotic resistances present in the collection and release phloem. Most phloem studies (e.g. Thompson and Holbrook, 2003*a*) neglect these osmotic resistances through surface area. In contrast to the resistance in the transport phloem which is proportional to the plant length, the resistances in the collection and release phloem are *inversely* proportional to the source (e.g. leaf length) and sink (e.g. root length) length. By taking into account these resistances, a universal scaling law for phloem dimensions was discovered, namely the third power of the sieve tube radius linearly relates to leaf length multiplied by stem length. Because this relationship applies for all studied vascular plants (herbs and trees), it seems that all plants employ the same basic mechanism for an optimal phloem transport. As such, active transport facilitation, such as the relay mechanism, seems unlikely, because this would have altered the observed relationship in this study (Jensen *et al.*, 2011, 2012).

Table 1 summarizes the major differences regarding phloem transport between herbaceous plants and trees based on hypotheses and data found in the available literature. In addition, differences exist between angiosperm and gymnosperm trees due to their different phloem anatomy.

Three loading strategies and their ecophysiological function

Phloem loading is the starting point for the long-distance sugar transport: the accumulation of sugars osmotically increases the hydrostatic pressure in the sieve tubes. The route of sugar movement from mesophyll cells to sieve elements can be apoplastic or symplastic. The symplastic mode does not involve crossing the plasma membrane, while the apoplastic mode does. Besides the symplastic–apoplastic discrepancy, a distinction can be made between active and passive loading. In active loading, metabolic energy is used to pump assimilates into the phloem against a concentration gradient. In this view, three types or strategies of phloem loading can be defined in the collection phloem of the minor veins: active apoplastic loading, active symplastic polymer trapping, and passive symplastic diffusion (Table 2). Each loading type corresponds to a specific type of companion cell in the SECCCs (van Bel, 2003*b*; Van Bel and Hafke, 2005) (Table 2).

During the process of active apoplastic loading, the sucrose destined for export enters the apoplast at some point and can only be pumped into the phloem from the apoplast by a carrier that is specific for sucrose (Lalonde *et al.*, 2003; Van Bel and Hafke, 2005; Pittermann, 2010). The apoplastic loading is driven by a proton-motive force, maintained by proton pumps, and sucrose carriers (e.g. sucrose-symporter SUT4) in the plasma membrane of the SECCCs. Because of this active mechanism, a steep osmotic gradient can be created, which requires a commensurate influx of water, generating a high local turgor in the sieve tubes (Van Bel and Hafke, 2005). The companion cell, specialized in apoplastic loading, is called a transfer cell and is characterized by (i) many cell wall invaginations to increase the plasma membrane surface and (ii) the presence of few plasmodesmata which connect with the mesophyll cell. Hence, the mesophyll and the SECCC operate virtually uncoupled as the symplastic connectivity between both is largely reduced (van Bel, 2003*b*).

Polymer trapping occurs in species that translocate the raffinose family of the oligosaccharides. In a first step, assimilates synthesized in the mesophyll cells passively diffuse from the bundle sheath cells through abundant plasmodesmata into special companion cells, which are called intermediary cells. In these intermediary cells, the diffused assimilates are converted into the larger oligosaccharides, raffinose and stachyose (polymers made of three or four hexose sugars) (Lalonde *et al.*, 2003; Turgeon and Ayre, 2005). A fundamental feature of this mechanism is that the branched plasmodesmata between the intermediary cells and the mesophyll cells are slightly narrower than those at many other interfaces. The size exclusion limits of these plasmodesmata allow diffusion of sucrose into the intermediary cell, but do not permit diffusion of the larger sugars, raffinose and stachyose, in the opposite direction. Hence, raffinose and stachyose accumulate in high concentrations in the SECCCs and sucrose continues to diffuse passively from the mesophyll cells, where it is synthesized, into the intermediary cells, where it is utilized (Lalonde *et al.*, 2003; Turgeon and Ayre, 2005). Polymer trapping is

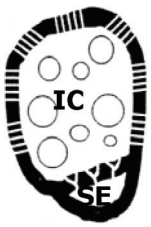
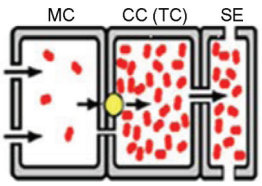
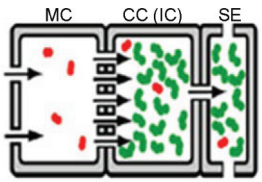
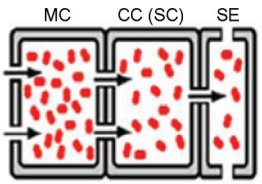
Table 1. *Phloem-related characteristics which differ between herbs and trees*

Phloem characteristics	Herbaceous angiosperms	Angiosperm trees	Gymnosperm trees	References
Phloem pressure	Relatively high	Relatively low	Relatively low*	e.g. Turgeon (2010*a*)
Pressure difference between sources and sinks	Low*	Low*	Low*	Thompson and Holbrook (2003*b*); Turgeon (2010*a, b*)
Phloem loading	Active	Passive	Passive	Van Bel and Hafke (2005); Turgeon (2010*b*); Liesche *et al.* (2011)
Phloem speed	Relatively high (± 1 cm min^{-1})	Relatively high (± 1 cm min^{-1})	Relatively low* (<1 cm min^{-1})	Windt *et al.* (2006); Dannoura *et al.* (2011); Jensen *et al.* (2012); Liesche *et al.* (2013)
Stem sieve plate radius	An increase with plant length	No increase with plant length	No increase with plant length	Jensen *et al.* (2012)
Stem sieve plate shape	Circular	Circular	Rectangular	Rosner *et al.* (2001); Mullendore *et al.* (2010); Jensen *et al.* (2012)

* Hypotheses which are not yet experimentally proven.

Table 2. *Relationship between the phloem loading strategy at the minor vein and the ultrastructure of the companion cell*

(i) When apoplastic loading occurs, sucrose is pumped from the apoplast into the transfer cells (TCs) of the minor vein phloem by transporters (yellow circle), which enables the leaf to minimize the overall sucrose concentration in the leaf blade. Transfer cells (TCs) possess cell wall invaginations to increase the plasma membrane surface and have virtually no plasmodesmata at the MC–CC interface. (ii) When polymer trapping takes place, sucrose diffuses through plasmodesmata into the minor vein intermediary cells (ICs) and is converted to oligosaccharides, thus actively elevating the transport sugar concentration gradient in the phloem. ICs are characterized by numerous cytoplasmic vesicles and many plasmodesmata at the wall interface between mesophyll (MCs) and companion cells (CCs). (iii) If passive diffusion takes place, assimilates passively diffuse through plasmodesmata into simple companion cells (SCs) of the minor vein. The assimilate concentration in MCs, and therefore in the entire leaf, is high; the assimilate concentration in the veins is slightly lower. SCs, without particular properties, have a moderate plasmodesmal density at the MC–CC interface (adapted from van Bel, 2003b. The phloem, a miracle of ingenuity. Plant, Cell and Environment 26, 125–149 with permission © John Wiley & Sons Ltd; Turgeon 2010b. The role of phloem loading reconsidered. Plant Physiology 152, 1817–1823. www.plantphysiol.org, Copyright American Society of Plant Biologists).

Loading strategy	Apoplastic loading	Polymer trapping	Diffusion
Active or passive	Active	Active	Passive
Symplastic or apoplastic	Apoplastic	Symplastic	Symplastic
Type of companion cell	Transfer cells (TCs)	Intermediary cells (ICs)	Ordinary or simple companion cells (SC)
			
Number of plamodesmata at MC–CC interface	Few	Abundant	Moderate
Schematic diagram of the loading strategy			
Transport sugar	Sucrose ( )	Sucrose ( ) oligosaccharides ( )	Sucrose ( ), often sugar alcohols
Uphill sugar gradient	Yes	Yes	No

an active mechanism, although it does not involve the active transport in the formal sense of moving ions and molecules across a membrane. It is thermodynamically active since energy is used to create a concentration difference between the mesophyll cells and the SECCCs (Turgeon, 2010*b*).

Just recently, passive loading by diffusion has been recognized as a valuable loading strategy (Turgeon, 2010*b*). Recent data (Rennie and Turgeon, 2009) indicate that a large number of woody species, especially trees, load assimilates passively by maintaining high sucrose concentrations, and in some cases sugar alcohols, in the mesophyll cells. By definition, passive loading requires no energy input and is energetically downhill, with the sugar levels being higher in the mesophyll than in the phloem. Ions and molecules diffuse through plasmodesmata at each interface, without a concentrating step. The driving force for transport comes from the creation of a high solute concentration in the photosynthetic cells. These solutes diffuse from ordinary companion cells into the SECCCs through regularly appearing plasmodesmata. The corresponding hydrostatic pressure, engendered by the solutes in the sieve elements of the source phloem, sustains mass flow (not diffusion) towards the sinks (Turgeon, 2010*b*). In this loading strategy, passive transport through plasmodesmata is referred to as diffusion, although a bulk flow may also be possible in plasmodesmata with sufficiently large radii (Fisher and Cash-Clark, 2000). In contrast to active loading, passive loading is not accompanied by an uphill sucrose gradient from mesophyll to phloem. As such, this passive loading strategy can explain why the phloem osmotic potential and turgor pressure is low in trees (Turgeon, 2010*a*). Passive loading has almost exclusively been observed in trees, making it difficult to argue that active loading is essential for efficient phloem transport. This is in agreement with Münch's original theory which did not consider active loading (Turgeon, 2010*b*).

Evaluation of the different loading strategies in an ecophysiological context revealed their evolutionary advantage (Pritchard, 2007; Turgeon, 2010*a*, *b*). Active loading increases

the efficiency to move a large amount of assimilates because the higher level of soluble carbohydrates in the sieve tubes maximizes the amount of carbon and energy delivered per unit of volume (Turgeon, 2010*a*). Furthermore, active loading reduces the amount of non-structural carbohydrates (NSCs) in the leaf mesophyll without compromising export. Several studies (Harper, 1989; Turgeon, 2010*b*) suggested that reducing non-productive carbon in the leaves significantly increases the plant growth potential. Another possible advantage of a low sugar concentration in the leaf mesophyll is the possible reduction of the feedback inhibition of photosynthesis (Turgeon, 2010*b*). Therefore, active loading strategies substantially increase the growth potential of plants as they decrease the unnecessary inventory of NSCs in the leaves (Turgeon, 2010*b*). Active loading is especially desired in herbaceous species, as their growth is rapid and their leaf production continuous (progressive addition of new leaves throughout the growing season). In trees, leaf production mostly occurs in periodic flushes, and much of the carbon used to fuel these flushes comes from storage. Therefore, the advantages of active loading are less important for trees, which often have relative growth rates well below that of herbs. Measured predawn NSC levels in leaves confirm this hypothesis: the NSC levels of herbaceous crop plants are in general lower than those of woody plants (Rennie and Turgeon, 2009). If this is true, storage of extra foliar NSC reserves in leaves of herbs can be considered as a safety factor, which becomes redundant in cultivated conditions. Hence, manipulation of storage in leaves could result in significant increases in growth potential of crop plants (Turgeon, 2010*b*). It seems that the passive symplastic loading mode is the most primitive loading system, which evolved to active symplastic and apoplastic loading depending on the species requirements and growth conditions (Lambers *et al.*, 1998).

Loading plays a crucial role in controlling the phloem hydrostatic pressure. In the collection phloem, loading can be regulated at two levels: at the level of leaf mesophyll cells or at the SECCC level. First, in the mesophyll, photosynthesis is the main process which indirectly controls loading as it determines the carbohydrate availability for export. The sugar concentration in the mesophyll cells directly influences the diffusion that is essential for symplastic loading. Regarding apoplastic loading, the mesophyll (apoplastic) solute concentration seems to control the sucrose carrier activity (Barker *et al.*, 2000; Komor, 2000; Patrick *et al.*, 2001). Plants can control this carbohydrate availability for export as a sugar excess down-regulates photosynthesis by feedback mechanisms (Foyer, 1988; Lewis *et al.*, 2000; Cheng *et al.*, 2008). These feedback mechanisms mostly prevent overproduction of photoassimilates, thus keeping supply to the SECCCs within appropriate limits (Van Bel and Hafke, 2005). Secondly, phloem loading seems to be controlled by three state variables in the SECCCs: (i) the turgor pressure; (ii) the sucrose level; and (iii) the presence of phytohormones. Orlich *et al.* (1998) hypothesized that the SECCC turgor regulates the conductance of the plasmodesmata leading into the companion cells (Turgeon and Ayre, 2005), as such influencing the symplastic loading. In addition, the SECCC turgor regulates the H^+-ATPase activity which influences the proton-motive force that drives the sucrose carriers needed for apoplastic loading (Daie, 1989; Patrick *et al.*, 2001; Lalonde *et al.*, 2003). The sucrose level in the SECCCs directly controls the diffusion of symplastic loading, while the apoplastic sucrose level regulates the activity of the apoplastic sucrose carriers (Patrick *et al.*, 2001; Vaughn *et al.*, 2002; Lalonde *et al.*, 2003). High turgor and/or sucrose levels in the SECCC will decrease phloem loading. In addition, phytohormones can up- or down-regulate phloem loading (Lalonde *et al.*, 1999, 2003). Possible loading-regulating phytohormones are cytokinins, abscisic acid, auxins, and gibberellins (Lalonde *et al.*, 2003).

Leakage-retrieval mechanism

The major function of the transport phloem is the translocation of carbohydrates from sources to sinks. While sources are specific tissues in which photosynthesis or remobilization takes place, sinks are present everywhere since maintenance respiration takes place in all living cells. Also more demanding sinks exist, needing a larger carbon influx to sustain growth (e.g. cambium) or storage (e.g. roots) (Thorpe *et al.*, 2005). Hence, the transport phloem nourishes not only the terminal sinks but also many lateral sinks along the plant axis (van Bel, 2003c; Thorpe *et al.*, 2005; Van Bel and Hafke, 2005). To feed the lateral sinks, appreciable amounts of carbohydrates are lost from the SECCCs along the phloem pathway. However, a part of these lost carbohydrates is subsequently reloaded, or retrieved, into the SECCCs. This behaviour is called the leakage-retrieval mechanism and has been demonstrated in several studies using radioactively labelled assimilates (Thorpe and Lang, 1983; Minchin and Thorpe, 1987; Minchin *et al.*, 2002; Gould *et al.*, 2004; Thorpe *et al.*, 2005; De Schepper *et al.*, 2013). This flexible mechanism allows rapid restoration of any osmotic disturbance along the pathway and, hence, locally buffers short-term irregularities in the turgor potential and gradient in the SECCCs (Gould *et al.*, 2004; Van Bel and Hafke, 2005). These irregularities can be induced by temporal changes in source and/or sink activities. The leakage-retrieval mechanism controls the pressure gradient between the sources and the sinks to allow a constant flow between them and, as such, it decouples the activity of the sources and sinks in the short term (Thorpe *et al.*, 2005).

The leakage-retrieval mechanism is based on a balance between leakage (or unloading) of carbohydrates out of the SECCCs into the surrounding apoplast and their subsequent retrieval (or reloading) (Thorpe *et al.*, 2005). Today, most of the evidence indicates that the leakage mechanism follows an apoplastic route and is a passive diffusion process in which carbohydrates diffuse out of the SECCC symplast into the phloem apoplast (Patrick *et al.*, 2001; Thorpe *et al.*, 2005). Under some specific conditions, such as high source–sink ratios, the leakage mechanism can change to a symplastic route (Patrick and Offler, 1996; Patrick *et al.*, 2001). The SECCCs in the transport phloem are, under normal conditions, symplastically isolated from the surrounding tissues due to few, virtually closed plasmodesmata between the SECCCs and the phloem parenchyma cells (van Bel, 2003*b*, *c*). These

plasmodesmata can open under sink-limiting conditions, allowing symplastic leakage and, hence, the storage of the excess assimilates in the stem parenchyma (Patrick and Offler, 1996; Patrick *et al.*, 2001; van Bel, 2003*c*). Unlike the leakage process, the mechanism of the retrieval process is active and mediated by sucrose symporters (e.g. SUT1 and SUC2) (Patrick *et al.*, 2001). These symporters can be located at the plasma membrane of both the companion cell and the sieve element (Kuhn *et al.*, 1997; Thorpe *et al.*, 2005). Due to their different mechanisms, the leakage and retrieval processes are controlled in a different way. The leakage diffusion process is mainly controlled by the symplastic SECCC assimilate concentration, as the apoplastic concentration is very low and changes in it will have little effect on the leakage diffusion (Patrick, 1990; Thorpe *et al.*, 2005). The retrieval process, depending on sucrose carriers, will, in contrast, be sensitive to the apoplastic sucrose concentration and to the SECCC turgor (Patrick *et al.*, 2001; Thorpe *et al.*, 2005). Furthermore, the apoplastic retrieval can be influenced by pH, potassium (K^+), and calcium (Ca^{2+}) concentrations (Thorpe *et al.*, 2005; Van Bel and Hafke, 2005). These variables may manipulate the proton-motive force, which drives the sucrose carriers. K^+ and Ca^{2+} channels have been found on the plasma membrane of the SECCCs in the transport phloem (Deeken *et al.*, 2000, 2002; Van Bel and Hafke, 2005).

It has been speculated that, in addition to short-term buffering, the leakage-retrieval mechanism also plays a role in maintaining the pressure gradient along the phloem pathway (van Bel, 2003*b*, 2005). It is believed that the transport corridors in the sieve plates are so narrow that appreciable losses in turgor pressure arise at every sieve plate. Therefore, van Bel (2003*b*; van Bel and Hafke, 2005) hypothesizes that solutes are released in the sieve tube apoplast at the proximal side of the sieve plate and that they are retrieved at the distal side (Fig. 2). Membrane channels should facilitate these leakage and retrieval processes around the sieve plates. In this view, the leakage-retrieval system would lead to a re-establishment of the turgor pressure behind every sieve plate. This proposed mechanism is a variation on the original relay hypothesis, as van Bel (2003*b*; van Bel and Hafke, 2005) does not state that sieve tubes are shorter than the plant's axis. In their concept, relays are present at the sieve plates of the sieve tube, while in the original relay hypothesis relays are only present where individual 'shorter' sieve tubes are in vertical contact.

Furthermore, it is suggested that the flow between the long-term lateral sinks and the SECCCs is controlled by their apoplastic carbohydrate concentration (McQueen *et al.*, 2005; Thorpe *et al.*, 2005). These long-term sinks relate to long-term buffering of stored carbohydrate reserves in the

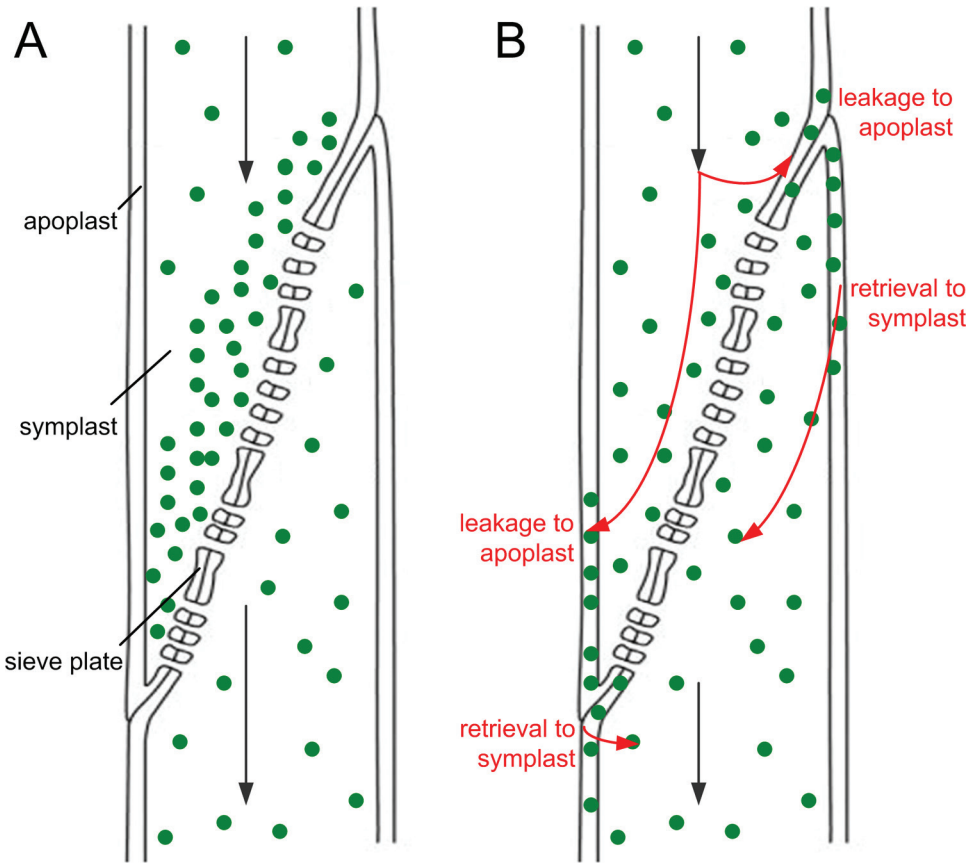


Fig. 2. The pressure drop around the sieve tubes is reduced if the leakage-retrieval mechanism acts as a dynamic relay system. (A) Pressure drop over the sieve plates due to the plates' higher resistance towards sucrose transport. (B) A reduced pressure drop due to sucrose leakage proximal of sieve plates and retrieval at the distal site.

symplast of parenchyma tissues and stem ray cells. The proposed hypothesis for long-term buffering assumes that the short-term apoplastic phloem concentration is rather constant (van Bel, 1990). Whenever the carbohydrate concentration in the apoplast is either substantially increased or decreased by short-term buffering, the long-term symplastic pool will be replenished or depleted, respectively (Fig. 3). According to this hypothesis, the apoplastic sugar concentration controls the replenishment and remobilization of the long-term lateral stem storage tissues. The short-term buffering capacity depends on the volume of the apoplastic pool and its apoplastic sugar concentration, while the long-term buffering depends on the amount of available storage tissues and their carbon concentration. When the limits of long-term lateral buffering are reached, the activity of the sources and sinks needs to be regulated. The storage capability is probably adapted to predictable imbalances of supply and demand, such as in diurnal and seasonal trends, or it could develop as the need arises (Thorpe *et al.*, 2005).

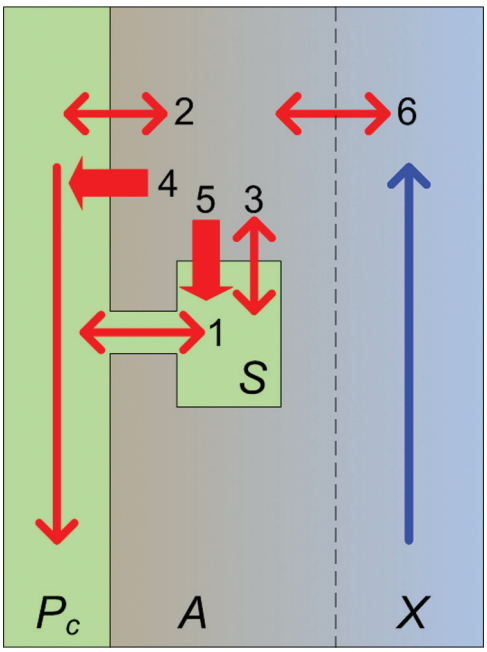


Fig. 3. Schematic presentation of the pathways for lateral transport of carbohydrates in the plant stem. (1) From the phloem sieve tubes (Pc), carbohydrates can move by diffusion via plasmodesmata into the symplastic storage compartment (S) (e.g. ray cells or phloem parenchyma). Carbohydrates can diffuse through the cell membrane (2) into the apoplast (A) or (3) into the storage compartment. From the apoplast adjacent to the phloem, carbohydrates can be (4) actively retrieved (reloaded) into phloem, (5) be loaded into the storage compartment, or (6) diffuse further through the apoplast into the xylem stream (X), where flow is usually in the opposite direction of that in the phloem (Reprinted from *Vascular transport in plants*. Thorpe MR, Minchin PEH, Gould N, McQueen JC. 2005. The stem apoplast: a potential communication channel in plant growth regulation. In: Holbrook NM, Zwieniecki MA, eds. Burlington: Elsevier, 355–371, with permission from Elsevier).

Other phloem functions

Phloem functioning is not limited to translocation of sugars between sources and sinks, but is also involved in long-distance signalling and plant defence.

Long-distance signalling relates to the distribution of a local effect, mostly induced by changes in source and sink activity, over long distances. The first way to conduct this physicochemical information transfer between sources and sinks is in the form of pressure–concentration waves (Thompson and Holbrook, 2003*b*, 2004; Thompson, 2006). A theoretical study (Thompson and Holbrook, 2003*b*) has shown that these pressure–concentration waves will travel faster than the phloem sap itself, but only if the osmotic pressure is high relative to the turgor difference between sources and sinks (Thompson, 2006). This first assumption is one of the reasons why Thompson and Holbrook (2003*b*, 2004; Thompson, 2006) are convinced that the source–sink pressure gradient is small and the osmotic potential of the phloem tubes is high. Furthermore, Thompson and Holbrook (2003*b*, 2004; Thompson, 2006) assume that the total water potential of the apoplast and the symplast of the SECCC are in equilibrium along the phloem pathway. This second assumption implicates that every change in turgor is accompanied by a proportional change in solute concentration. For example, when a turgor drop is induced, the total water potential of the phloem sap will become lower (more negative) compared with that of the surrounding apoplast. As such, the sieve tube is forced to absorb more water from the apoplast and consequentially the phloem sap is diluted (Thompson, 2006). If these two assumptions as previously mentioned are fulfilled, pressure–concentration waves can explain long-distance signalling as depicted in Fig. 4. A local increase in solute concentration leads to an influx of water and, hence, an increase in local turgor (Fig. 4A, B). This turgor disturbance can be propagated as a pressure–concentration wave along the length of the sieve tube. The water potential of the sap transiently increases (becomes less negative) due to the increased turgor (Fig. 4C), resulting in an efflux of water that concentrates the already present solutes until equilibrium in water potential is regained (Fig. 4D) (Thompson and Holbrook, 2004). This example illustrates how the pressure–concentration waves can alter the turgor pressure in the entire sieve tube in response to a local induced disturbance. Therefore, unloading and loading rates along the entire phloem pathway (i.e. source, transport, and sink phloem) will alter correspondingly to the changed turgor pressure, because the (un)loading processes are controlled by the SECCC turgor pressure as previously discussed. This concept is physiologically very interesting because individual cells in the decentralized plant body can only sense and modify their own turgor (Thompson, 2006). If relays are present along the transport phloem, it seems logical that the wave propagation will be stopped at each relay as this physically interrupts the phloem symplast. In the case of the dynamic relay hypothesis based on the leakage-retrieval mechanism, the pressure wave will probably not be halted at the sieve plates as the phloem symplast is not interrupted.

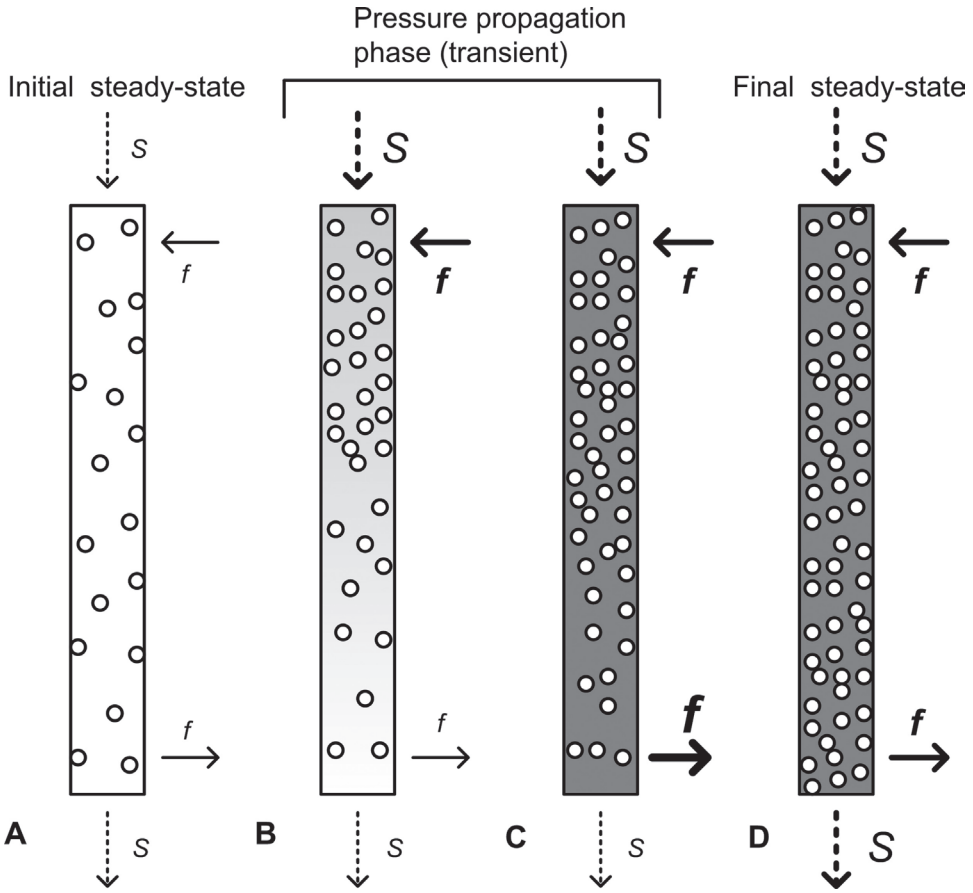


Fig. 4. The propagation of pressure–concentration waves. At steady state, solute (S) and apoplastic water flux (f) into the sieve tube equal the solute and water flux out. The size of these symbols and their associated arrows denote the magnitude of each flux. The concentration of open circles is proportional to the sugar concentration, and the degree of shading in the background is proportional to turgor pressure. It is assumed that in the sieve tube the osmotic potential is high relative to the source–sink pressure gradient and that no external gradient occurs in the apoplastic water potential. (A) The pressure and solute concentration are initially low. (B) Solute loading in the collection phloem increases, which locally increases concentration, membrane water influx, and pressure. (C) This increase in pressure is rapidly propagated along the length of the sieve tube, transiently raising the sap water potential relative to the apoplast and the efflux of water near the release phloem. (D) The terminal efflux of water concentrates the solutes, raising the solute concentration until the sieve sap is in local water potential equilibrium (from Thompson and Holbrook, 2004. Scaling phloem transport: information transmission. *Plant, Cell and Environment* 27, 509–519 with permission © John Wiley & Sons Ltd).

Long-distance signalling by the phloem can also be achieved through transport of hormones (Turgeon and Wolf, 2009) and macromolecules, such as RNA and proteins (Oparka and Cruz, 2000; van Bel, 2003*b*; Thorpe *et al.*, 2005; Pritchard, 2007; Turgeon and Wolf, 2009). It is well accepted that low molecular weight signalling molecules are translocated in the phloem, initiating physiologically amplified responses at a distance from their sites of synthesis (Thompson and Schulz, 1999). Several investigators (Baker, 2000; Friml and Palme, 2002; Turgeon and Wolf, 2009) have detected the presence of the endogenous plant hormone auxin and its native form, indole-3-acetic acid (IAA), in the phloem sap. Peptide hormones, such as systemin, also appear to be transported throughout the plant via the phloem (Narvaez-Vasquez *et al.*, 1995; Thompson and Schulz, 1999). Some mRNA molecules present in the phloem sap are transported with the phloem sap towards the sinks where they seem to induce gene silencing (Oparka and Cruz, 2000; van Bel, 2003*b*). However, the generality of the concept that mRNA molecules act as signals to coordinate developmental processes at the whole-plant level is still under debate (Turgeon and Wolf, 2009). Long-distance trafficking of some protein products in the phloem is associated with the promotion of flowering in several species (Corbesier *et al.*, 2007; Lin *et al.*, 2007; Tamaki *et al.*, 2007; Turgeon and Wolf, 2009). Notwithstanding that some phloem proteins are involved in long-distance signalling, it is believed that many of the phloem proteins have entered the sieve element by non-specific diffusion and not by design (Oparka and Cruz, 2000; Turgeon and Wolf, 2009).

Finally, profound and sudden physiological changes can be communicated over large distances by electropotential waves (Furch *et al.*, 2010). These waves have been recorded in response to stimuli such as wounding, cold, heat, and electrical shocks (Fromm and Spanswick, 1993; Rhodes *et al.*, 1996; Mancuso, 1999; Furch *et al.*, 2010). Along their

path, electropotential waves often cause occlusion of the sieve elements by callose and sealing proteins (Furch *et al.*, 2010).

Besides long-distance signalling, phloem plays a role in plant defence against predators. First, the phloem protects the plant by its high turgor pressure and concomitant high sugar concentration in the SECCCs (Turgeon, 2010*a*). When a sieve tube is wounded, pressure release causes surging and forces cellular debris into the sieve pores, sealing the phloem (e.g. Ehlers *et al.*, 2000). Some insects have adapted and can maintain their feeding due to specific compounds in their saliva (Will and van Bel, 2006), but the majority of them cannot cope with this effective sealing strategy (Turgeon, 2010*a*). In addition, the high osmotic pressure of the phloem discourages the phloem feeders as it desiccates the animal tissues (Turgeon, 2010*a*). Secondly, the plant is protected from predators by phloem proteins and secondary compounds in the phloem. Specific non-dispersive phloem proteins, for example P-proteins or forisomes, are able to seal the sieve elements at the sieve plate after damage so that leaking of the pressurized phloem sap out of the damage sieve tube is prevented (van Bel *et al.*, 2002; Knoblauch and Peters, 2010; Ernst *et al.*, 2012). Several phloem proteins, for example serpin (Petersen *et al.*, 2005) and systemin (Narvaez-Vasquez *et al.*, 1995), inhibit the protein digestion of insects and hence protect against chewing insects (van Bel, 2003*b*; Pritchard, 2007). In addition, the phloem transports several types of secondary compounds, which are toxic and behave as natural pesticides. Examples of such secondary compounds are glucosinolates, iridoid glycosides, pyrrolizidine alkaloids, and cardenolides (Turgeon and Wolf, 2009).

Conclusions

The Münch theory of phloem transport is continuously being refined by including both active and passive phloem (un)loading in the collection and release phloem and by introducing the leakage-retrieval process along the transport phloem. Phloem-related data measured on trees and herbaceous species revealed significant differences between both plant types. Not only do their loading strategies differ, but also the observed phloem pressure is much higher in herbs than in trees, although Münch's theory suggested the opposite. Based on these findings the abandoned hypothesis of a relay system needs to be reassessed, especially for trees. Therefore, we urge that future experiments focus not only on herbs (which is mostly the case in current studies), but also on trees. Most of the phloem hypotheses (e.g. small phloem pressure gradient, leakage-retrieval process, relay system) are based on fragmentary observed data or theoretical experiments. Nevertheless, they are experimentally hard to test due to the technically demanding measurement methods associated with the protective nature of phloem tissue. In particular, information about phloem anatomy (sieve tube length, sieve pore areas) and phloem pressure seems crucial to elucidate the proposed hypotheses. A better fundamental understanding of the phloem system could increase our insight into how information is exchanged between sources and sinks and how growth is finally regulated. Hence, it could be a valuable tool to increase the productivity of and mitigate disastrous environmental impacts on commercial plants and natural forests.

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