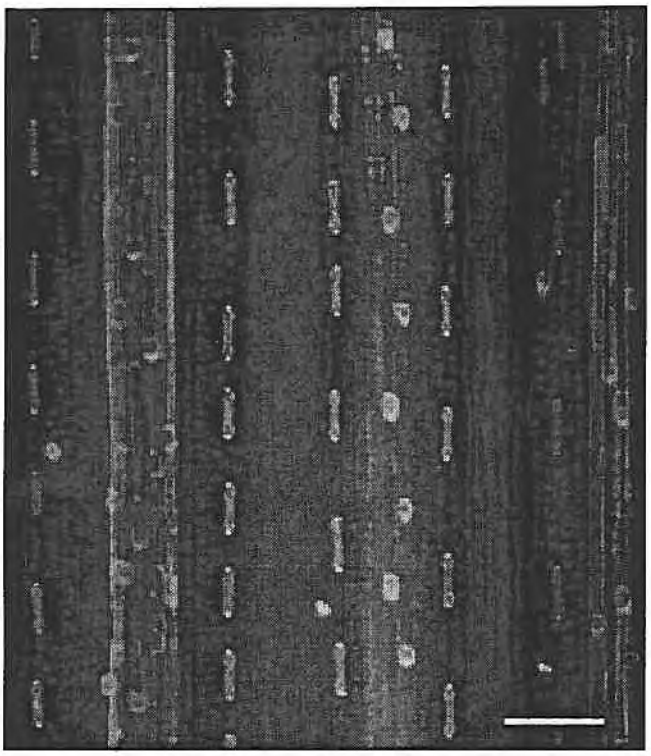
5 *Vascular integration and resource storage*



Surface view of cleared whole mount of a wheat leaf showing large and small parallel veins (mauve). Lines of stomata (orange guard cells) lie along the flanks of these veins. Water evaporates from the wet walls of mesophyll cells below the stomata, drawing water from the veins through sheath cells. Bar represents 100 μm (see Colour Plate xx)
(Photograph courtesy Margaret McCully)

> ... what quantities of moisture trees do daily imbibe and perspire: now the celerity of the sap must be very great, if that quantity of moisture must, most of it, ascend to the top of the tree, then descend, and ascend again, before it is carried off by perspiration.
>
> *(S. Hales, Vegetable Staticks, 1727)*

Chapter outline

Introduction

Evolutionary changes were necessary for plants to inhabit land. All resources reach aquatic plants from the surrounding water whereas terrestrial plants are nourished both from soil and directly from the atmosphere. Roots growing into soil absorb water and nutrients, while leaves, supported by a stem superstructure in the aerial environment, intercept sunlight and CO_2 for photosynthesis. This division of labour results in assimilatory organs of land plants being nutritionally interdependent; roots depend on a supply of photoassimilates from leaves, while shoots (leaves, stems, flowers and fruits) depend on roots to supply water and mineral nutrients. Long-distance transport is therefore a special property of land plants. In extreme cases like the Australian mountain ash (*Eucalyptus regnans*), sap must move up to 100 m and overcome gravity to rise to tree tops.

Specialised vascular tissues have evolved to support the rates at which resources assimilated from the environment need to be transported within land plants. Mature xylem vessels conduct water and nutrients upwards by mass flow through a complex of non-living cells, energised during the day by evaporative water loss from leaves (Section 5.1). Fine vascular networks in leaves control distribution of this sap to recipient cells (Section 5.2). Phloem is responsible for redistributing nutrients, water and photoassimilates through plants along a living pathway (Section 5.3). The balance between supply and demand is integrated in phloem transport through sensitivity of conducting cells (Section 5.4), assimilate loading processes (Section 5.5) and unloading processes (Section 5.6) to developmental events and environmental constraints. Fine tuning of resource delivery to various organs is achieved through interchange of xylem and phloem contents, a process that has been quantified using lupin as a model plant.

5.1 Long-distance transport of water and nutrients

5.1.1 Introduction

Water loss by plants accompanies photosynthesis and nutrient acquisition. In most plants, this water loss reaches a maximum during daylight hours, as gas exchange peaks. Leaves are the primary evaporating surfaces of shoots but stems, fruits and flowers can be important sites of water loss too. Roots must therefore extract large amounts of soil water and deliver it to heights of up to 100 m in a *transpiration stream*. This transpiration stream carries with it nutrients into the shoot canopy (Section 5.1.5; see Case study 5.1).

Water is difficult to extract from plants in any quantity, but as Australian Aborigines found, long tree roots can be detected as raised soil contours and can be dug up fairly easily to yield water (White 1994). If such a root is cut or broken into short lengths and held vertically, watery sap runs from the lower end where it can be collected to drink. The tenacious hold of plant tissues on entrained water has been broken by wounding, allowing air to enter and displace the captive sap.

Such release implies water-filled conduits, but does not reveal the mechanism for long-distance movement of sap in plants. Xylem flow has been the subject of endless debate for experimentalists trying to identify forces involved. A summary below of the early history of experiments dealing with vertical lift of sap into shoots shows that some accord has been reached but details of the mechanism of sap transport are still being debated.

5.1.2 Experimental history: how the Cohesion Theory came to be accepted

Around 1905 great plans were made to resolve the mystery of the ascent of sap in trees by Professor E.J. Ewart in Melbourne, using eucalypts as a model plant. At that time, Australian mountain ashes (*Eucalyptus regans*) vied with American dawn redwoods (*Sequoia gigantea*) as the tallest trees in the world, being well over 100 m tall. Ewart climbed eucalypt trees using special scaffolding, removed lengths of branch and measured the pressures required to push water through these stems. These investigations led him to conclude 'The ascent of water

is, therefore, a vital problem in so far as it depends upon conditions which hitherto can only be maintained in living wood'. If water transport required living cells, it could not be supported by discovery of a pump akin to that in animals. Even roots, which sometimes could pump water by root pressure (Section 3.6), lacked the necessary positive pressures to push water so far aloft, especially around midday when water was most needed.

Suction from the shoots was an alternative explanation but manmade suction pumps could not fulfil the task without inducing formation of air bubbles (embolisms) in the xylem and blocking flow. One clue to the solution came from Dixon and Joly (1894) who claimed that very pure water molecules would be held together by powerful cohesional forces provided the water was especially clean (much cleaner than in manmade pumps).

Ewart did not agree with the unorthodox proposal that the suction of pure water through xylem vessels underpinned transpiration. However, Dixon (1914) ultimately postulated the Cohesion Theory, based on those properties of water which distinguish it as an ideal biological solvent — cohesion, adhesion to walls of the vessels and surface tension being central features. In short, in the absence of microscopic gas bubbles water could withstand quite enormous tensions.

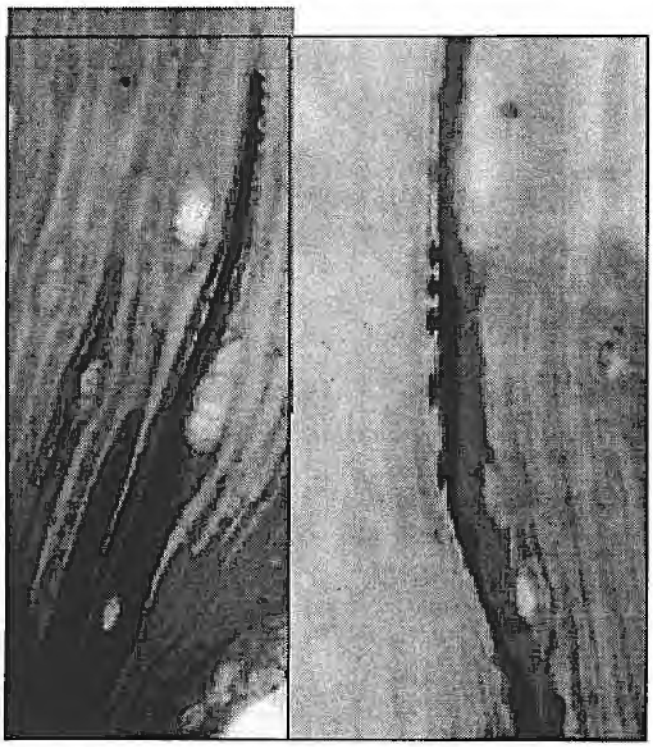


Figure 5.1 (a) Mercury sucked into tracheids of pine (*Pinus radiata*) by transpirational pull generated in the shoots. The water–mercury interface is powerful enough to hold this vertical column of mercury in stems. The height to which the dark column of mercury rises is used to calculate suctions created in xylem vessels. Note the generally small heights, reflecting the high specific gravity of mercury. About 2 MPa suction is produced in these xylem vessels. (b) Mercury enters bordered pits but remains connected to the vertical column of mercury in xylem vessels. While mercury can pass through the pit apertures, it cannot pass the finely porous 'pit membranes' because it is much more cohesive than water. Seen laterally, the half-aspirated bordered pits appear as disks (? see Colour Plates xx and xx)

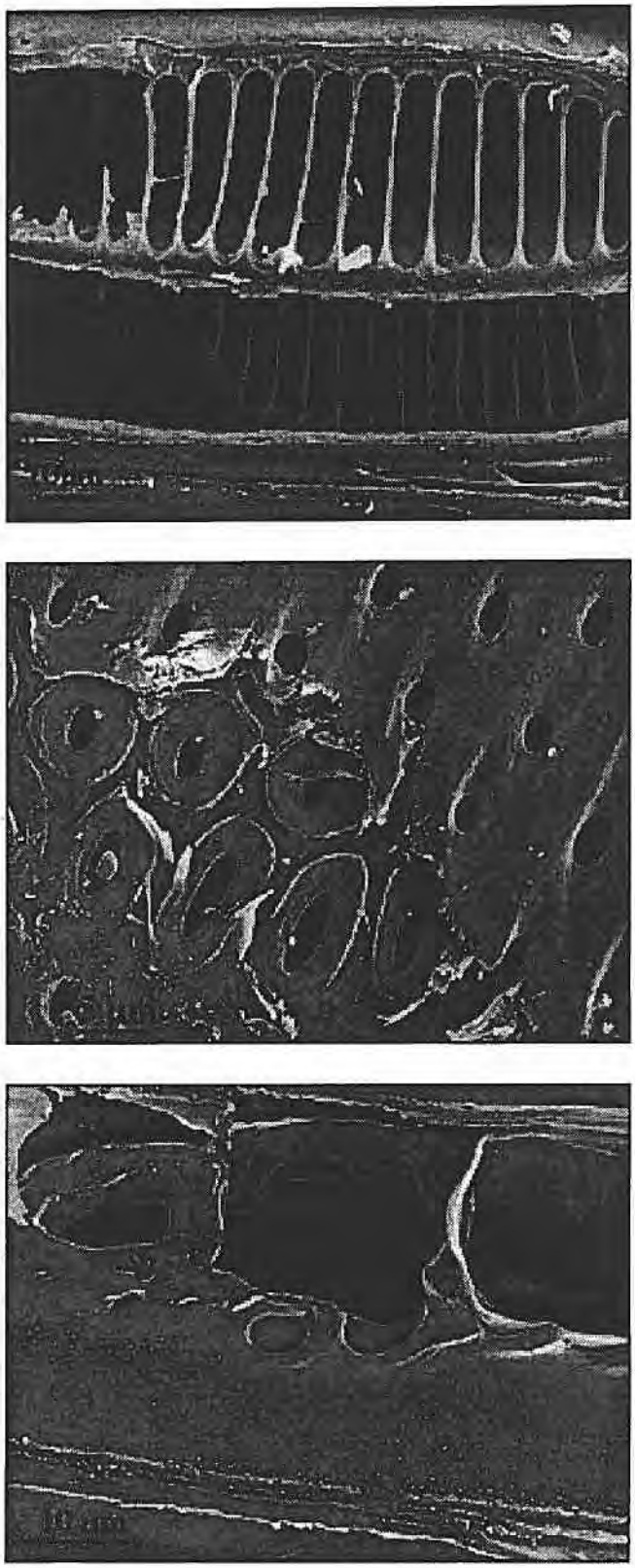


Figure 5.2 (a) Scanning electron micrograph (SEM) of the finely sculptored scalariform *perforation plates* in poplar (*Populus italica*) xylem. Water passes easily from one xylem vessel to another by this route. (b) SEM illustrating bordered pits connecting two vessels in *Eucalyptus*. The pits can be seen in surface view (top right) and the borders of pits can be seen when this surface is broken away (bottom left). The borders appear as cup-shaped depressions. (c) SEM of poplar wood illustrating the close connections between ray cells (note three adjacent cells) and xylem conduits (xylem wall with pits lie beside these ray cells). Simple pits can occur in fields allowing transfer of ions from xylem sap into ray cells or release of photosynthates into the xylem stream. Magnifications appear on the micrographs (see Colour Plate xx)

Through the evaporative power of the atmosphere, a continuous 'chain' or 'catena' of water, well below atmospheric pressure, could be drawn up to a leaf canopy. The tensions created in this way could even suck water from the surrounding soil. We now recognise that the evaporative energy is supplied as the latent heat of vaporisation ultimately derived from solar power. This cohesive property of water gave rise to the 'Cohesion Theory for the Ascent of Sap'.

Two other properties of water are also essential for long-distance water transport: surface tension, and the adhesion of water to solid surfaces such as the xylem vessels within trees. Dixon claimed that if water could 'hang together', the enormous evaporative energy of the air (the same power which dries the washing hanging on a line) could be harnessed to lift sap, which is mainly water, vertically. This would entail no metabolic energy on the part of the plant. This theory of sap flow accorded with earlier experiments by Strasburger (1893) showing that a tall oak tree trunk, severed at the base, could draw poisons and dyes up to the leaves by some wick-like action. If metabolism energised sap flow, poison should have inhibited it. This was well illustrated in later experiments (Figure 5.1a) in which mercury was drawn through fine tracheids of pine stems purely through the suction created by transpirational water loss from the shoot above. The tension required to achieve this is about 2 MPa.

However, the physical properties of plants had to be more complex than those of simple pipes conducting water. As mentioned, manmade pumps failed through embolisms if used to suck water higher than 10 m, whereas hundreds of litres of water reaches the canopies of tall trees daily. Even overlapping sawcuts in tree trunks, which should allow a massive quantity of air to flow into xylem vessels when under suction and cause trees to die from embolisms, did not stop all water flow to leaves. If water was under such suction, how could trees keep air bubbles out of the sap when the trunk was cut? This additional problem was not resolved until the very complex anatomical structures of trunks were much better understood. Xylem (Figure 5.2) is not composed merely of pipes: it is made up of *partially sealed units* (technically vessels, tracheids, and fibres, called collectively *conduits*) which most effectively limit the spread of introduced gases and thus maintain water flow in some conduits despite very severe disruption from embolisms in others.

5.1.3 Xylem as an effective conduit for sap

For xylem sap to sustain tensions required in tall trees, there must be *no gas bubbles* in the system. Cohesion breaks down if there is a single 'nucleation site' on which bubbles can form and enlarge. On the other hand, sap normally contains dissolved gases which, surprisingly, do *not* disrupt the system provided there are no nucleation sites available. Even the rigid walls of xylem vessels are compatible with high xylem tensions, attracting water by adhesion, which is essential for transport.

Surface tension acts as an interfacial water–air stopper, preventing air from being sucked into the many millions of tiny pores present in all plant cell walls. For example, water delivered to leaf cells by xylem vessels passes through these tiny menisci, which act effectively as non-return valves, so preventing air from being sucked into the xylem (Section 5.2). Surface tension also explains how water in leaves remains under strain within an essentially porous system through which water flows.

Xylem anatomy seems to be highly significant. Vascular transport systems have evolved to become amazingly reliable despite the metastable condition of the sap. From primitive, thickened, hollow cells, increasing specialisation has produced greater elongation and thickening of the tubes. Xylem walls contain *pits*, in which zones of the primary wall known as 'pit membranes' allow water to be transmitted between vessels efficiently, while preventing a gas phase spreading through the interconnected system of vessels and blocking transport through *embolisation* (the blockage of a fluid channel with a bubble of gas). No living membrane is present in these wall structures. However, the efficiency with which 'pit membranes' isolate adjacent vessels is shown in Figure 5.1(b) where mercury, a highly cohesive liquid, is drawn into specialised bordered pits of pine tracheids without being able to exit into neighbouring tracheids.

Vascular systems have evolved from plant species possessing only fibres and tracheids, for example the more primitive *Tasmannia*, to more advanced plants possessing *vessels* which resemble the unicellular tracheids in structure but are much wider and longer and originate from a number of cell initials fused together. Lignin thickening patterns have also evolved. Some thickening designs, such as *annular* and *spiral*, allow the tubes to extend longitudinally while supplying growing organs. When growth has ceased, an organ can be provided with more efficient pipes of larger bore and with stronger thickenings, in *reticulate* and *scalariform* patterns (Figure 5.2a). Pit fields which allow water transport across vessel walls can also be simple, unreinforced structures (simple pits) or more elaborate bordered pits in which secondary cell walls mechanically support the pit membrane. Pits prevent air in an air-filled conduit from spreading to adjacent conduits which are conducting water under strong suction. Reinforcement of the walls around pits (Figure 5.2b) allows 'pit membranes' to be as large as possible and thereby maximise water exchange between vessels.

5.1.4 Speed of sap flow

A number of methods have been used to determine the speed of sap flow. This task is not easy because xylem is not a

uniform conduit supporting a single flow rate. Some of the main approaches are outlined below.

(a) Dyes

Dyes have been used to detect water flow for centuries. Such methods developed a poor reputation until dyes were selected for their ability to move freely in the xylem: dyes which associate with the negatively charged vessel walls move much more slowly than the sap in which they were dissolved. Modern dyes, generally negatively charged ions, have been especially useful to illustrate pathways of water flow (Section 5.2). However, if dyes are to be used to interpret flow rates, caution must be exercised. Applying a dye solution, at atmospheric pressure, to plant tissue with vessels under tension can lead to the dye being sucked into vessels artefactually. This will be most acute in water-deficient tissues. Interpreting the direction and rate of sap flow in this case will tell little about the steady-state condition. Also, when dyes are used to assess mass transfer, the capacity of a vessel (related to its width) will influence the amount of dye passing through that vessel. So, fine capillaries might transport only a small amount of dye but at a rapid rate.

(b) Radioisotopes

Radioisotopes are useful tracers of water flow, but they suffer from the same problems as dyes. Cationic isotopes, especially divalent cations, tend to bind to the xylem walls. Radioactive phosphorus makes a good tracer because it is anionic and a strong emitter.

(c) Heat flow methods

Heat-flow and heat-pulse methods are widely used for the non-invasive measurement of water flow in stems and tree trunks. One ideal approach introduces a steady source of heating to the stem or trunk. This is surrounded by temperature detectors (thermistors or thermocouples). If there is virtually no sap flow, say, late at night, temperatures around the heater adopt a symmetrical 'zero-flow' profile. When water flow occurs, the thermal profile is dragged upwards, providing a temperature shift which can be converted by appropriate computer circuitry into a continuous measurement of flow. Automatic devices of this kind are now being used widely for irrigation scheduling.

5.1.5 Solute transport via transpiration

Xylem sap is very dilute when compared to both phloem sap and the contents of cells lining the transport pathway and for this reason it is often regarded as virtually pure water. Indeed, the osmotic pressure of xylem sap rarely exceeds 0.1 MPa (about 40 mOsmol l^{-1}). This dilute sap effectively transports large amounts of inorganic nutrients by mass flow (see Case study 5.1), providing the supply of water and nutrients to roots can be maintained. In a rapidly growing plant such as an annual herb, powerful mechanisms for concentrating essential ions in the roots (see Case study 4.1) combine with transpiration of water from leaves to sustain nutrient supply to shoots.

(a) Composition of xylem sap

While most solutes in xylem sap are inorganic ions (e.g. nitrate, potassium, magnesium and calcium), other important solutes are also present (Table 5.1). Organic molecules in xylem sap can be present in substantial concentrations, sugars reaching 5 mM in maize xylem sap (Canny and McCully 1989). Many trees including eucalypts are host to boring insects at particular times of the year when sugar and nitrogen content of the sap is nutritionally valuable. Even though sugar concentrations in xylem sap are much lower than in phloem sap, the high nitrogen to sugar ratios and low osmotic pressures make it a good substrate for many predators. More extreme examples of the carbohydrate content of xylem sap are temperate deciduous trees such as maple which have traditionally been tapped to yield a sugary solution in the period prior to budburst. This indicates that xylem can be a conduit for carbon remobilisation in addition to its central role as a pathway for water and nutrient transport. In secondary tissues, rapid transfer of solutes into and out of the xylem is partly achieved through close association of living ray cells and xylem vessels (Figure 5.2c).

Table 5.1 Concentrations of major solutes (mM) in mid-stem phloem and stem base xylem (tracheal) sap of Banksia prionotes *sampled in natural habitat in Yanchep, Western Australia*

Solute mM	Xylem sap	Phloem sap
Sucrose	absent	493
Total amino acids	0.53	2.35
Malate	0.42	4.28
Potassium	2.39	15.2
Sodium	1.84	24.1
Magnesium	0.55	6.36
Calcium	0.48	5.96
Phosphate	0.111	0.60
Nitrate	0.01	0.38
Chloride	2.92	26.5
Sulphate	0.25	1.06

(After Pate and Jeschke 1993)

Other organic molecules act to transport inorganic nutrients to the shoots. Nitrate and ammonium are assimilated into organic forms, such as amino acids, in the roots of many plants. In legumes, nodules deliver an even wider selection of nitrogenous compounds to the xylem, including ureides and amides (Figure 3.22). These often constitute the dominant form of nitrogen reaching shoots and are therefore a major component of the sap. Other examples of complexed forms of inorganic nutrients in xylem sap are metal ions such as zinc, copper and iron which are almost exclusively chelated to organic acids.

Phytohormones are also found in xylem sap but often in concentrations several orders of magnitude lower than those considered necessary to elicit a physiological response. This

does not preclude xylem sap as a critical source of these substances because delivery of solutes in the xylem must also take into account rates of sap flow; mass transfer of phytohormones such as abscisic acid (Jokhan *et al.* 1996) and cytokinins (Nooden *et al.* 1990) might be significant even if it is delivered in a dilute solution.

(b) Modification of xylem contents

Xylem sap composition is highly variable, and modified according to requirements of shoot tissues. Toxic ions can be removed from sap and essential nutrients recycled intensively as described in Case study 5.1. Unidirectional flow of sap in the xylem stream (although not invariably in the same direction) contrasts with bidirectional flow in the phloem and the two consort to deliver resources to where they are most needed. In particular, transfer from xylem to phloem provides a means of diverting essential elements from the main transpiring surfaces, the older leaves, to growing tissues where they are required (see Case study 5.1).

Discovery of transfer cells (Pate and Gunning 1972) showed how these specialised cells differentiate in order to effect a rapid transfer of solutes, in this case into and out of the xylem stream (see Case study 4.2). Transfer cells lie adjacent to xylem vessels and are highly modified to carry out rapid ion exchange of xylem and phloem elements. While exchange can occur in both directions, concentrations of most nutrients and organic solutes are much lower in the dilute xylem sap than in adjacent phloem elements, requiring active transport mechanisms for xylem to phloem transfer (Table 5.1). Such energetically 'uphill' transport is thought to be energised by proton pumps in the membranes of transfer cells. Solutes such as potassium in cereals and amino acids in soybeans are known to be transferred to the phloem by this route, particularly through nodes in the stem.

Xylem parenchyma cells, which are believed to participate in loading of ions into xylem vessels in roots (Figure 3.31), also contribute to modification of ion levels along the xylem pathway. Amino acids, potassium, calcium, magnesium and even micronutrients such as molybdenum are exchanged between xylem vessels and the accompanying parenchyma cells to achieve a xylem sap composition appropriate to shoots. This process is species dependent. For example, molybdenum is sequestered in the roots of beans and participates in nodule function, whereas tomato has less capacity to withdraw molybdenum from xylem sap (Hecht-Buchholz 1973). In secondary tissues, rapid transfer of solutes into and out of the xylem is partly achieved through close association of living ray cells and xylem vessels (Figure 5.2c).

Even weaker associations between ions and cell walls of both xylem vessels and surrounding cells can retard the upward movement of inorganic ions like cadmium, copper, zinc and calcium. For ions such as copper, this is not desirable unless toxic quantities are likely to reach the shoot during brief periods of oversupply. Retarded translocation of cadmium and other heavy metals, on the other hand, might be advantageous to a plant by preventing a rapid supply that cannot be diluted by growth.

5.2 Vein endings and export pathways

5.2.1 Introduction

Evaporation from wet cell walls of substomatal cavities in leaves creates a large tension (negative pressure; suction) which is transmitted via xylem conduits, pulling more sap from roots to leaves. Fine pores in cell walls provide sufficient suction to draw water to the crown of even a lofty tree: a curved interface in a 10 nm pore can store a pressure of -30 MPa.

A rapidly transpiring leaf can evaporate its own fresh weight of water in 10 to 20 min, though many plants such as cacti, mangroves and plants in deep shade have much smaller rates of water turnover. Leaf veins must carry this water to all parts of a leaf to replace evaporated water, and maintain cell hydration and turgor. When water supply fails to meet this demand, shoots wilt.

5.2.2 The pipeline: leaf vein architecture

The simplest vein architecture is found in conifer needles where a single unbranched strand of xylem and phloem is surrounded by mesophyll (Figure 5.3). Vascular strands are enclosed by an endodermis that separates them from the mesophyll, and are embedded in a mixture of parenchyma cells and tracheids called transfusion tissue. Water from the xylem permeates radially outward through transfusion tissue, endodermis and mesophyll to evaporate below lines of stomata in the epidermis.

Leaves of angiosperms have much more complicated venation than conifer needles. Look at a grass leaf with your hand lens. Parallel veins run the length of the leaf, but they are not all the same size. A few large veins have several small veins lying between them (see Chapter 5 frontispiece). On closer inspection with a light microscope, all these parallel veins are connected at intervals by very small transverse veins (Figure 5.4). There are in fact two vein systems with different functions: large veins supply water rapidly to the whole length of a leaf blade while small veins and their transverse connections distribute water locally, drawing it from the large veins. Water in large veins flows only towards the tip, but in small veins it can flow either forwards or backwards along the leaf blade or transversely between adjacent parallel veins. The distinction of flow patterns in large and small veins arises as a result of different vessel sizes. Large veins have wide vessels (*c.* 30 μm

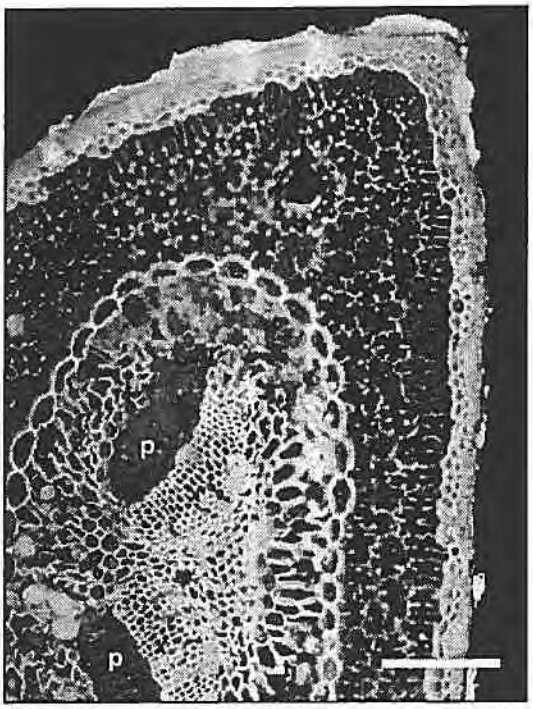


Figure 5.3 Transverse section of a pine needle. Note the simplicity of its venation in comparison to that of a grass (Figures 5.4 and 5.5) and a dicotyledon (Figure 5.7). A single central vein has two strands of phloem (p) and xylem (x), embedded in transfusion tissue (t). These vascular tissues are separated from chlorophyllous mesophyll (red) by an endodermis (e) with Casparian strips and suberised lamellae, through which water must pass outwards from the xylem and sugar must pass inwards to the phloem. Stomata in the epidermis appear bluish. Rhodamine B stain, fluorescence optics. Bar represents 100 μm
(Courtesy M. McCully)

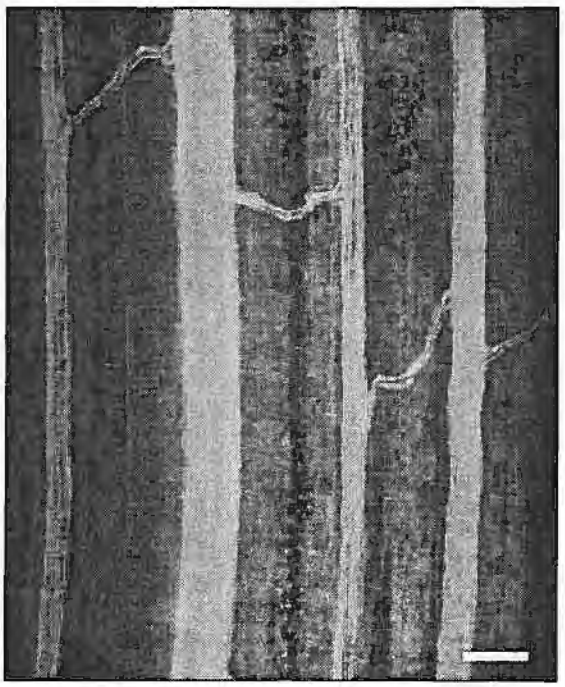


Figure 5.4 The same leaf as in the frontispiece for Chapter 5, showing a large and three small longitudinal veins and transverse veins connecting them. Water moves along the leaf only in large (supply) veins, and enters small (distribution) veins through transverse veins. In small veins it can move in either direction in response to local pressure gradients. Small veins could carry up to two-thirds of the evaporated water. Semi-dark-field optics, blue filter. Bar represents 100 μm (see Colour Plate xx)
(Courtesy of M. McCully)

diameter), while small veins have narrow vessels (*c.* 10 μm diameter). Applying Poiseuille's Law (Section 5.4.5(a)) for a fixed pressure gradient, volume flow in a large vessel will be $30^4/10^4 = 810$ times the flow in a small vessel. Put another way, pressure gradients along the leaf in large vessels will be very slight, but steep pressure gradients can develop locally in the mesophyll that will direct local flows in narrow vessels. Large veins supply water rapidly over the whole lamina while small veins distribute it locally and slowly.

You do not see the vessels with your hand lens, you see the sheaths that surround xylem and phloem, much as an endodermis surrounds vascular strands of a conifer needle. Grass leaf veins have one (maize) or two (wheat) sheaths of parenchyma cells enclosing the parallel veins and containing the xylem and phloem tissues, and all materials passing out of or into the transport tissues must go through these parenchymatous sheaths (Figure 5.5).

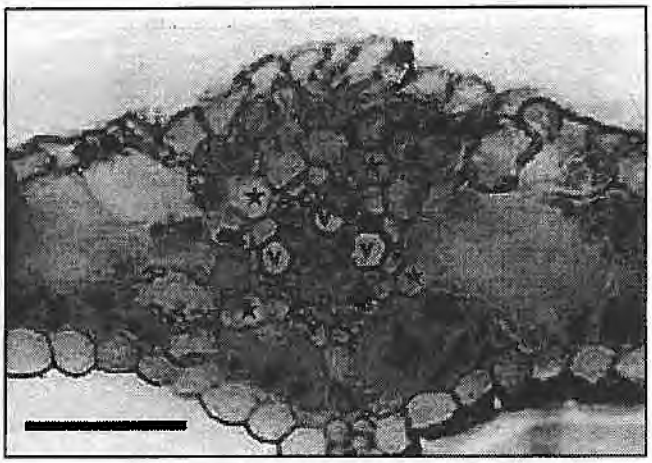


Figure 5.5 Transverse hand-section of a fresh wheat leaf showing a single large (supply) vein comprising three large vessels (v). The vein is surrounded by two sheaths of living cells, the inner mestome sheath (arrowheads), and outer parenchyma sheath (*). The mestome sheath of these veins is impermeable to water. There is no apoplasmic path for water through the mestome sheath of large veins, except through a connecting transverse vein. In small veins, by contrast, two or three mestome sheath cells next to the xylem permit flow of water and solutes through the cell wall apoplasm. Water enters the symplasm at the inner boundary of the parenchyma sheath (Canny 1990). Phloem (p). Toluidine blue stain, bright field optics. Bar represents 100 μm (see Colour Plate xx)

A dicotyledonous leaf contains the same two vein systems as a grass leaf, but these are differently arranged. Large supply veins are prominent, comprising a midrib and *two orders* of branches off it, often standing out from the surface of the lamina. These contain wide vessels and carry water rapidly to the leaf margins. Between them lie distribution veins, another *two branch orders* of small veins dividing the mesophyll up into islets about 1–2 mm across, and within these islets a *fifth and final order* of branches of the finest veins (Figure 5.6). The fourth- and fifth-order branches have only narrow vessels. As in grass leaves, these vascular tissues are enclosed by bundle sheath cells through which materials must pass when leaving the xylem or entering the phloem (Figures 5.7 and 5.22).

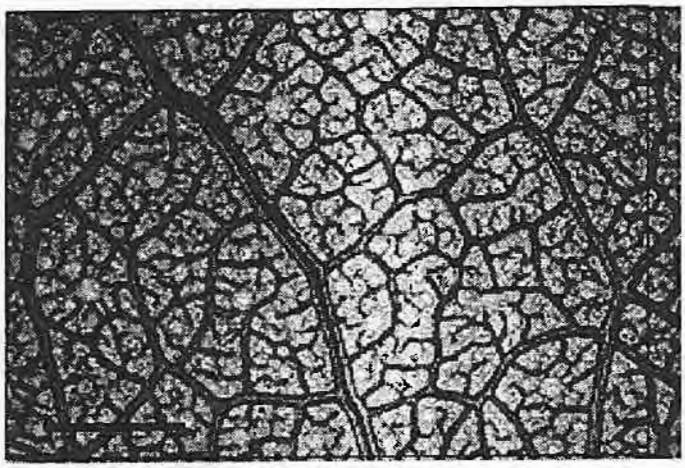


Figure 5.6 Whole mount of a cleared leaf of *Eucalyptus crenulata* showing the more complicated arrangement of supply and distribution veins characteristic of a dicotyledonous leaf. Islands marked out by large veins with large vessels, in which water is moved rapidly all over the lamina, surround islets of small veins with small vessels in which water is slowly distributed locally to the mesophyll. The ratio of small to large veins in a dicotyledonous leaf is much larger than in a grass leaf (see Figure 5.4). A factor of 10 is not uncommon, suggesting that *c.* 90% of evaporated water comes from the small veins. Partial phase-contrast optics. Bar represents 1 mm (see Colour Plate xx)

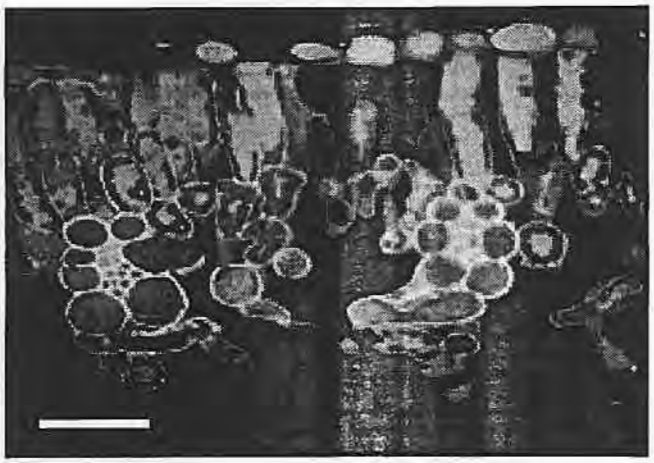


Figure 5.7 Transverse section of a leaf of soybean showing two of the smallest veins surrounded by bundle sheath cells. Veins of this size are at the end of the branching network shown in Figure 5.6, and supply most water that is evaporated. Before processing, this leaf had been transpiring in a solution of fluorescent dye for 40 minutes. The small vessels in each vein contain dye solution which has become concentrated by water loss to the symplasm and out through the bundle sheath. Dye has started to diffuse away from small vessels in the cell wall apoplasm of bundle sheath cells. Anhydrous freeze-substitution and sectioning, fluorescence optics. Bar represents 50 μm (see Colour Plate xx)

Any distribution network such as the branching vessels of decreasing size in leaves is found to obey Murray's Law. This states that the cube of the radius of a parent vessel is equal to the sum of cubes of the radii of the daughter vessels (e.g. a 50 μm vessel would branch into five 30 μm vessels). Such a pattern of branching produces optimal flow in several senses: minimum energy cost of driving that flow, minimum energy cost of maintaining the pipeline, constant shear stress at the walls of pipes, and rapid flow in supply pipes with slow flow in distributing pipes to permit exchange through the pipe walls (LaBarbera 1990).

5.2.3 Damage control

Transpiration operates by suction, but leaves are especially liable to damage by grazing and mechanical forces. Leaf vessels therefore need special protection against air embolisms spreading in the vessel network that would block liquid flow (Section 5.1). This is achieved at all points in the leaf distal to the node (i.e. petiole, large veins, small veins) by the vessels being very short. That is, files of vessel elements joined to make a single pipe with a terminal end-wall are much shorter in the leaf than in the rest of the plant. Water flows through vessel end-walls with little extra resistance, but an air–water interface cannot be pulled through an end-wall or pit membrane (Section 5.1). The force needed to curve the interface into a meniscus small enough to pass through the end-wall is the same force as is generated by evaporation from wet cell walls (Section 5.2.1). To pull the interface through a hole of diameter D μm requires a pressure of $0.3/D$ MPa. While only 0.1 MPa can pull an interface through a 3 μm hole, 6 MPa is required to pull air through a 0.05 μm hole: cell walls have pores much smaller than 0.05 mm. So an embolism formed from cavitating water fills one vessel but does not spread beyond it.

Short vessels are easily demonstrated by allowing a leaf to transpire in a fine colloidal suspension that cannot pass end-walls. Latex paint, diluted 100 times with water and allowed to settle for a week or two, provides such a suspension. Leaves that have drawn up this suspension for an hour or so during transpiration can be cleared by dissolving out the chlorophyll and soaking in lactic acid. Progress of the paint is then readily seen (Figure 5.8). Very few vessels exceed 1 cm in length.

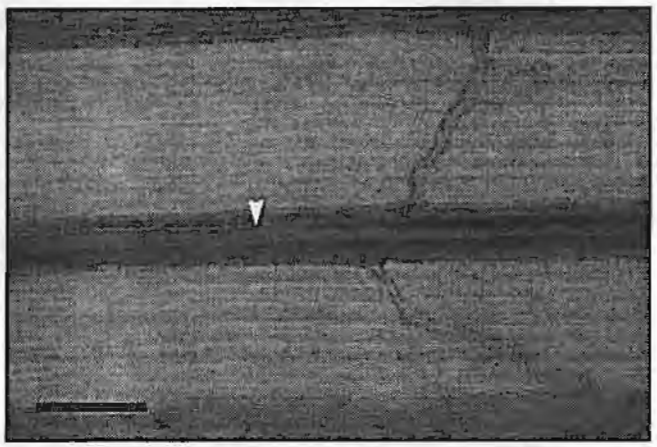


Figure 5.8 Cleared whole mount of a wheat leaf demonstrating the frequent occurrence of end-walls in leaf vessels. The leaf was fed an emulsion of green latex paint in the transpiration fluid from a cut surface 6.5 mm to the right of the picture. At the right side of the picture, two vessels in the central large vein (see Figure 5.5) are carrying paint. Halfway across the picture (at arrowhead) the upper vessel is blocked by an end-wall through which the paint particles could not pass, although water continued to flow. The paint in the lower vessel continued for another 3 mm beyond the left of the picture, where an end-wall in that vessel limited its further progress. Note that paint has not passed out of the large vein into transverse veins where water flowed because pit membranes (Section 5.1) filtered out paint particles. Bright-field optics. Bar represents 100 μm (see Colour Plate xx)

5.2.4 Unit pipe: generalised vein structure

Amidst the great variety of plant leaf structures, pipelines carrying a transpiration stream have certain constant features. They consist typically of tightly packed xylem and phloem tissues surrounded by a parenchymatous or fibrous sheath. Both xylem and the phloem contain living parenchyma cells as well as their characteristic transporting conduits, vessels and/or tracheids, plus sieve tubes. There are no intercellular air spaces, or only very small ones. The sheath acts both as a mechanical barrier that may confine pressure within the vein, and a permeability barrier that can control rates and places of entry and exit of materials. Exceptions are to be found at the ultimate ends of some dicotyledonous fine veins where tracheids or sieve elements may be unaccompanied by other cells, in the transverse veins of grasses which have no sheath, and in some special veins at leaf margins where the sheath is absent on the xylem side (see below).

5.2.5 Water extraction from the pipeline

Vessels are not just pipes to carry water, they are pipes with holes in them (pits) through which water can leak out, fulfilling a principal leaf function of water distribution through the transpiration stream to places where it will evaporate. The branching network of vessels is beautifully adapted to achieve this.

Think about flow in a leaky vessel. The volume of forward flow varies as the fourth power of the radius (Poiseuille's Law). The frequency of leaks through vessel walls varies with surface area of the walls, that is, as the first power of the radius ($2\pi r$). So if the vessel is wide, forward flow is much larger than leakage. The wide vessels in large veins supply water all over the leaf without losing much on the way. As the width of a vessel becomes smaller, the forward flow (a function of r^4) is reduced much more strongly than the leaks (a function of r). The proportion of extracted water increases in relation to forward flow. Indeed, for a fixed pressure gradient there is a critical radius at which all water entering the vessels supplies leaks, and there is no forward flow at all. The finest veins of leaves have vessels of a diameter that is close to this critical value. As sap disperses into the fine ramifications of the network, it moves more and more slowly forward, and leaks increasingly outwards through the sheath to the mesophyll. This is the rationale of the distributing networks of the small branched veins of both dicotyledons and grasses.

Extraction of water from fine veins can be readily demonstrated. Stand a cut leaf in an aqueous solution of dye, such as 0.1% sulphorhodamine G and allow it to transpire for an hour. The solution moves rapidly through large veins all over the leaf in a few minutes. Then it moves increasingly slowly into the network of distributing small veins. By the end of an hour it has reached the ends of the finest veins and, as water is extracted from them, the dye becomes more and more concentrated (Figure 5.8). Sparingly soluble dyes crystallise out as solids in these small vessels. You may see these dye deposits by cutting hand-sections of leaves under paraffin oil and mounting these sections in oil (O'Dowd and Canny 1993). Solid dye is confined to the smallest veins, often in localised deposits (Figure 5.9). Whether dye movements give a reliable picture of the movement of natural solutes will be taken up below.

This experiment reveals something else fundamentally important about water movement. Separation of dye from water is evidence of ultrafiltration. Water passes out of the pits in vessel walls and enters the plasma membrane of bundle sheath cells; dye is excluded from these cells. This experiment shows that water was extracted from vessels into living cells.

5.2.6 Solutes in the transpiration stream

Much information available on the rate of solute transport through cell walls comes from studies of dye movement. Dye molecules are rather large compared to inorganic solutes which make up xylem sap and therefore only give an approximate idea of diffusivity of nutrient ions through a cell wall. Movement of dyes from the finest veins to leaf surfaces 100 mm away takes about 30 min, suggesting that diffusion rather than mass flow is responsible for solute distribution to cells. Water turns over in a whole leaf each 10–20 min, demonstrating that solute movement through the cell wall apoplasm is one or more orders of magnitude slower than water movement.

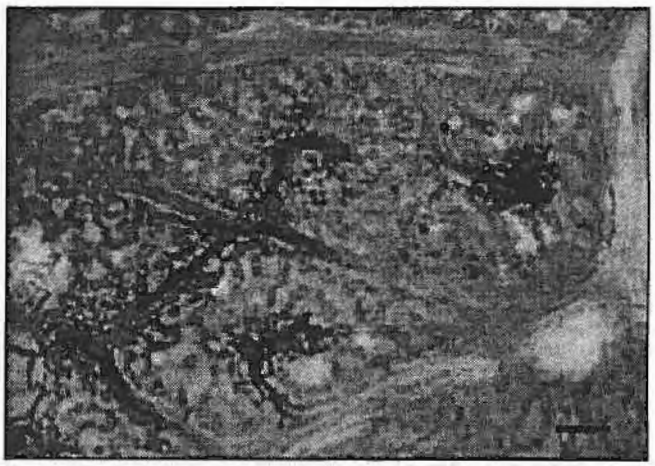


Figure 5.9 Fresh paradermal hand-section of a leaf of *Eucalyptus crenulata* which had been transpiring for 80 min in a solution of sulphorhodamine G. The dye solution is present at low concentration in the vessels of the larger veins, but is not visible at that concentration. In the smallest veins the dye has become so concentrated by loss of water to the symplasm that dye crystals have formed inside vessels (cf. Figure 5.8). Sectioned in oil, bright-field optics. Bar represents 100 μm (see Colour Plate xx)

To what extent do the conclusions about solute movement reached from these experiments apply to the natural solutes? The most abundant cation of the transpiration stream is potassium (K^+), and its behaviour in the vessel network has been worked out in some detail although diffusivities of such small inorganic ions through cell walls have not yet been measured. Potassium does not concentrate in the narrowest vessels as the dyes do, because the xylem parenchyma and bundle sheath cells have carrier systems in their cell membranes which transport K^+ to the symplasm. The tendency for apoplasmic K^+ to become concentrated by water loss is thus counterbalanced by absorption into surrounding cells. High K^+ concentrations recorded in leaf vessels (100–200 mM) probably reflect an abundance of K^+ in living xylem vessels prior to maturation and release of cell contents (Canny 1995).

5.2.7 Solute recycling: phloem export

All solutes in the transpiration stream will be subject to the general rules worked out for dyes or K^+: a tendency to become concentrated as water passes rapidly through the symplasm prior to transpirational loss, entry of some solutes into the symplasm from a slow-moving exchange bed of fine veins, or, for ions not accepted by any living cells, a slow diffusive spread in the cell wall apoplasm. Details for all solutes except K^+ are still largely unknown.

Potassium is known to be re-exported via the phloem (Section 5.1). In leaves, the concentrated sap flowing through the narrow xylem vessels of fine veins (Figures 5.4 and 5.6) is separated from sieve tubes of the phloem by only two or three parenchyma cells (e.g. Figure 5.5). Exchange from xylem to phloem is probably made by this direct path. Solutes travel in the phloem back to stems, either distally to younger developing leaves and the stem apex or proximally towards roots. In dicotyledonous stems, solutes can re-enter the xylem by crossing the cambium and being used on the way as an osmotic engine to swell the young secondary vessel elements. These solutes re-enter the transpiration stream when vessel elements mature and die to form functional xylem vessels (Canny 1995).

5.2.8 Solute recycling: scavenging cells

Recycling of solutes out of leaves can also be fed by a variety of special structures adapted for processing rather larger volumes of sap, and so suited for collecting solutes present in the stream at quite low concentrations. The transfusion tissue of conifer needles is one of these structures. As sap leaves the xylem of a vascular strand it moves through a bed of tracheids mixed intimately with transfusion parenchyma cells (Figure 5.10). The endodermis acts as the ultrafiltration barrier, allowing water to pass through while leaving dilute solutes to accumulate in transfusion tracheids. Transfusion parenchyma cells have very active H^+-ATPases in their cell membranes which accumulate selected solutes (certainly some amino acids) back into the symplasm for return to the phloem and re-export. Such actively accumulating cells are called *scavenging cells*.

Figure 5.10 Higher resolution view of the pine needle in Figure 5.3 showing suberin/lignin deposits in radial walls of the endodermis (arrowheads) that block diffusive movements through the cell wall apoplasm and confine fluxes to the symplasm. Transfusion tissue within the endodermis consists of two cell types, dead transfusion tracheids (dark), which contain the transpiration stream, and living transfusion parenchyma (dull red), which scavenge solutes from the steam and forward them through the endodermal symplasm to the mesophyll cells. The complicated cavities of the recessed stomata are fluorescing blue-white. Rhodamine B stain, fluorescence optics. Bar represents 50 μm. (see Colour Plate xx)
(Courtesy of M. McCully)

A tissue that acts in the same way is a special layer of cells in the central plane of many legume leaves (extended bundle sheath system or paraveinal mesophyll). It consists of scavenging cells with active H^+-ATPases and accumulates amino acids, stores them and forwards them to developing seeds via the phloem.

Jagged 'teeth' on the margins of many leaves also contain scavenging cells. Veins carry large volumes of the xylem sap to these points, where evaporation is especially rapid. Within a 'tooth', xylem strands end in a spray of small vessels among a bed of scavenging cells. Scavenging cells can thereby collect amino acids and load them into the phloem.

5.2.9 Solute excretion

Not all the solutes of the transpiration stream are welcome back in the plant body. Some, such as calcium, are immobilised in insoluble compounds (calcium oxalate crystals) and shed when leaves fall. Others are excreted through the surface of living leaves. A striking excretion system is found along the margin of maize leaves. Here the outermost vein has a single very wide vessel. Rapid evaporation from the exposed leaf

edge cooperates with this low-resistance vessel to draw to the leaf margin all residual solutes that have not been taken out of the stream by other veins. Thus foreign material (like dyes) accumulates in this outermost vessel. The vein sheath is missing from the outer part of this vein so that vessels abut directly the air space at leaf margins (Canny 1990). Solutes are excreted from this marginal vein, dissolved out by rain and dew, and, more actively, at night time by guttation fluid when there is positive pressure in the xylem. A similar accumulation of dye from the transpiration stream is shown along the margin of a eucalyptus leaf in Figure 5.11.

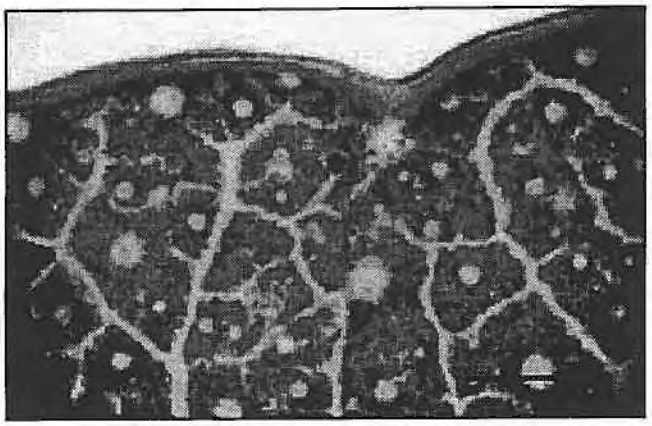


Figure 5.11 Whole mount of the living margin of the eucalypt leaf sectioned in Figure 5.9 prepared after dye had been fed to the cut petiole for 80 min. Dye has spread to the leaf margin in large veins where it accumulates at high concentrations. By analogy with maize leaves (Canny 1990) this is likely to be a system for excreting unwanted solutes. Bright-field optics. Bar represents 1 mm (see Colour Plate xx)

5.3 Distribution of photoassimilates within plants

5.3.1 Introduction

Xylem conduits are responsible for delivery of water, inorganic nutrients and organic forms of nitrogen and phosphate to transpiring leaves (Section 5.2). Phloem conduits are subsequently responsible for redistribution of such resources to organs such as apices and reproductive structures (including developing fruit). These organs have inherently low rates of transpiration and thus sap import via xylem vessels.

5.3.2 Source–path–sink concept

Assimilation of carbon and nutrient resources and subsequent distribution are closely linked events. Resource distribution is analysed by considering a plant as a coordinated network of assimilatory regions (sources) linked to regions of resource utilisation (sinks). The vascular system provides a path for assimilate transport from source to sink.

5.3.3 Source–path–sink transport processes

Activities of the following key transport processes contribute to overall flow of photoassimilates through a source–path–sink system (Figure 5.13).

(a) Source processes

Net export of photoassimilates occurs from fully expanded leaves (Figure 5.12) and long-term storage pools located along the axial transport pathway. Chloroplasts of C_3 plants (Section 2.5) partition photoassimilates between the photo-

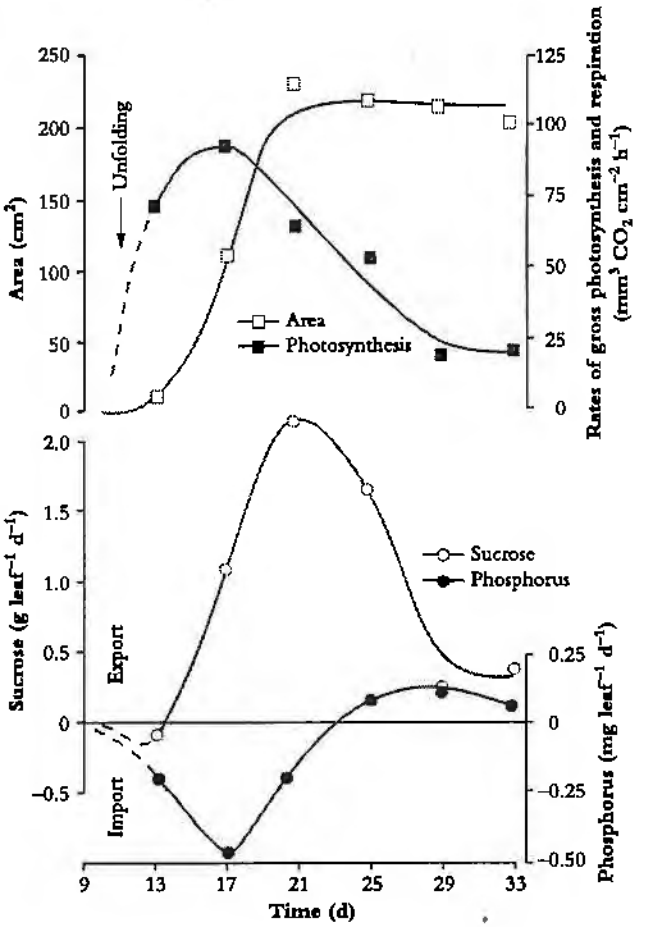


Figure 5.12 Time course of sucrose and phosphorus (P) net import and export from a leaf during its development. As a cucumber leaf expands, net sucrose export coincides with the rise in net leaf photosynthetic rate (O) to meet photoassimilate demands of young leaves. Once a leaf has reached some 30% of its final area, net photosynthesis by the whole leaf exceeds photoassimilate demand by growth processes and so excess sucrose can be exported. Thereafter, the rate of sucrose export closely follows photosynthetic rate, reaching a maximum when the leaf reaches its final size and gradually declining thereafter. Import of P (and other mineral nutrients) continues throughout leaf expansion and P export only starts once the leaf is fully expanded. Sucrose import and export were calculated from the difference between rates of whole leaf photosynthesis and dry matter gain
Based on Hopkinson 1964

cytoplasm through the post-sieve-element transport pathway (see Section 5.6), assimilates are either metabolised to satisfy the energy, maintenance and growth requirements of sink cells or are compartmented into polymer or vacuolar storage. Collectively, metabolism and compartmentation create a demand for assimilates which is ultimately responsible for driving phloem import.

5.3.4 Photoassimilate transport and biomass production

(a) Whole-plant growth

Sink and source strength must be in balance at a whole-plant level. Thus, an increase in whole-plant sink strength must be matched by an equal increase in source strength, either through increases in source activity or source size. Prior to canopy closure in a crop, much of the increase in source strength comes from increased source size, source activity remaining relatively constant. Significantly, until a leaf has reached some 30% of its final size, photoassimilates for leaf production are exclusively imported through the phloem from fully expanded leaves (Figure 5.12).

On canopy closure, self-shading and senescence of the lower leaves result in whole-plant photosynthesis being restricted to the uppermost leaves exposed to direct sunlight. Under these conditions, either genetic or environmentally imposed differences in source activity can influence biomass production.

(b) Photoassimilate transport and crop yield

During domestication of crop plants, plant breeders selected for crop yield via maximum investment into harvested organs (mostly seeds). Total plant biomass production of advanced wheat is the same as its wild progenitors yet grain yield has increased some 30-fold through breeding (Table 5.2). That is, whole-plant source and sink strength have not changed.

Table 5.2 Evolution of grain yield in wheat and the contribution of photoassimilate partitioning. Through domestication, humankind has developed wheat by selective breeding from a diploid (2n) wild plant to the modern hexaploid (6n) crop plant we know today. With this development, grain yield per spike (ear) has increased more than 30-fold. A significant factor contributing to the increased grain yield is a greater diversion of photoassimilates to the ear as shown by the relative distribution pattern of ^{14}C photoassimilates exported from the flag leaf blade.

	Ploidy level	Total grain yield (mg per spike)	% ^{14}C in ear after $^{14}CO_2$ exposure of flag leaf
Wild	2*n*	53	4
Primitive	4*n*	882	29
Advanced	6*n*	1764	52

(After Evans and Dunstone 1970)

Increases in wheat yield (Table 5.2) are associated with a diversion of photoassimilates from vegetative organs to the developing grain, as illustrated by the relative accumulation of ^{14}C photoassimilates exported from the flag leaf (Table 5.2).

Final grain yield is not only determined by partitioning of current photoassimilates, but also depends upon remobilisation of non-structural carbohydrates stored in stems, particularly under conditions where environmental stress impairs leaf photosynthesis (Wardlaw 1990). In fact, remobilisation of reserves affects yield in many food plants. For example, deciduous fruit trees depend entirely on remobilised photoassimilates to support flowering and fruit set as do early stages of pasture regrowth following grazing.

5.3.5 Whole-plant distribution of photoassimilate

Photoassimilate transport to harvestable organs plays a central role in crop yield brought about by greater harvest indices. This raises questions about transport and transfer processes that collectively influence photoassimilate partitioning between competing sinks.

Historically, these questions were elucidated by observing partitioning patterns of photoassimilates exported from specified source leaves labelled with ^{14}C supplied as a pulse of $^{14}CO_2$. Following a chase period, in which ^{14}C photoassimilates are transported to and accumulated by recipient sink organs, the plant is harvested. The pattern of photoassimilate partitioning operating during the pulse is deduced from ^{14}C activity accumulated by sinks (Figure 5.14).

Photoassimilates are partitioned from source leaves to sinks in characteristic and reproducible patterns. For instance, in a

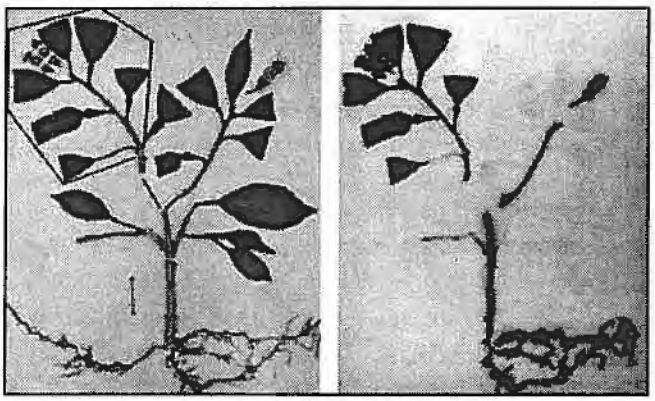


Figure 5.14 Photoassimilate distribution in a rooted cutting of Washington Navel orange. (Mounted specimen shown on left; matching autoradiograph on right.) $^{14}CO_2$ was supplied to source leaves (boxed area top left) for a day, and movement of 14C-labelled assimilate followed by autoradiography of harvested plant material. ^{14}C photosynthates were distributed widely via vascular conduits to sinks including some roots and a fruit on an adjacent shoot (note stem labelling between sources and sinks). Nearby mature leaves failed to import; they were additional sources of photosynthate. Vertical bar = 2 cm (Unpublished material courtesy P.E. Kriedemann (formerly CSIRO, Horticulture Merbein))

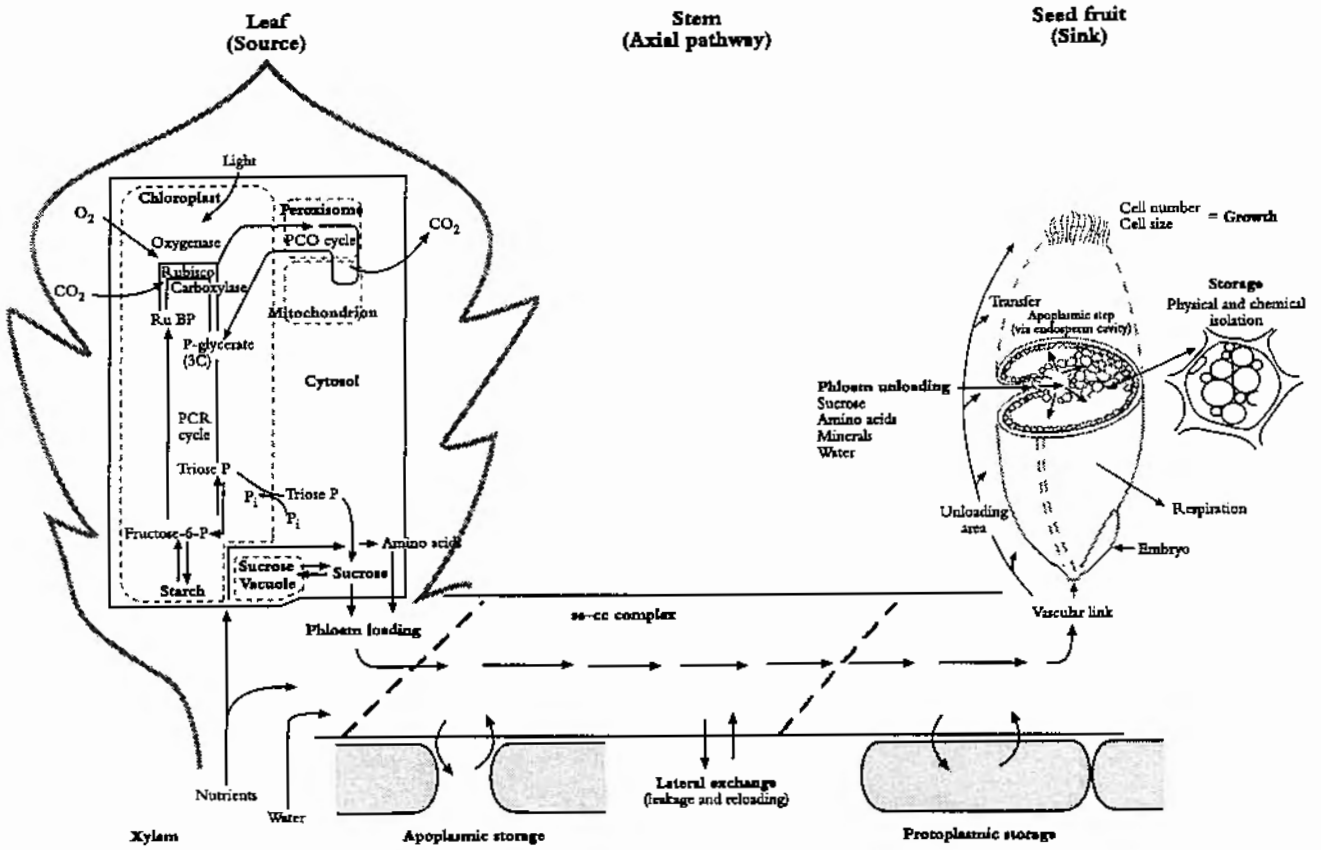


Figure 5.13 Schematic diagram of transfer and transport processes contributing to the flow of assimilates acquired from aerial or soil environments, through the source–path–sink system. CO_2 fixed by photosynthesis in chloroplasts has several possible fates (Chapter 2). It either enters photorespiration or starch biosynthesis or crosses to the cytosol as triose phosphate (triose P), giving rise to sucrose. Some sucrose can be stored in vacuoles of mesophyll cells. Both starch and vacuolar sucrose serve as temporary storage pools from which the cytoplasmic sucrose pool is replenished. Amino acids can be synthesised from reduced carbon and nitrogen delivered in the transpiration stream. Sucrose, amino acids and mineral nutrients are loaded into sieve element–companion cell (se–cc) complexes of leaf phloem for long-distance transport to non-photosynthetic sinks. These solutes are exchanged reversibly between se–cc complexes and short- and long-term storage pools along the axial pathway. Short-term storage pools include phloem apoplasm, whereas the protoplasm of non-transport cells provides a long-term storage pool. Transport of solutes from se–cc complexes to either storage pools or principal sinks is called phloem unloading. On reaching the cytoplasm of principal sink cells, photoassimilates and mineral nutrients support respiration and growth or are stored as solutes in vacuoles or polymers in amyloplasts (starch) or protein bodies (protein).

synthetic oxidative cycle and starch biosynthesis or release them immediately to the cytosol as triose phosphate for sucrose synthesis. In non-starch-forming leaves, high concentrations of sugars can be accumulated in the vacuoles of mesophyll cells or made available for immediate loading into the phloem and export. Leaves also serve as secondary sources for nutrients and amino acids previously delivered in the transpiration stream. Nutrients and amino acids can be exported in the phloem immediately, or after accumulation in short-term storage pools.

An additional source of photoassimilates is located along the axial phloem path (petioles, stems, peduncles, pedicels and roots) as a result of leakage from the vascular tissues. Leaked photoassimilates accumulate in short- or long-term storage pools which serve as secondary sources to buffer photoassimilate supplies to the sinks against shifts in export rates from the primary photoassimilate sources.

(b) Path processes

Assimilates including sucrose, amino acids and nutrients are transferred into sieve elements of fully expanded leaves against significant concentration and electrochemical gradients. This process is referred to as phloem loading. The cellular pathways of phloem loading, and hence transport mechanisms and controls, vary between plant species (Section 5.5). Longitudinal transport of assimilates through sieve elements is achieved by mass flow and is termed phloem translocation. Mass flow is driven by a pressure gradient generated osmotically at either end of the phloem pathway, with a high concentration of solutes at the source end and a lower concentration at the sink end (Section 5.4). At the sink, assimilates exit the sieve elements and move into recipient sink cells where they are used in growth or storage processes. Movement from sieve elements to recipient sink cells is called phloem unloading. The cellular pathway of phloem uuloading, and hence transport mechanisms and controls, vary depending upon sink function (Section 5.6).

(c) Sink processes

Many sink organs are characterised by low rates of transpiration (an exception is a developing leaf) so that most assimilates are delivered by the phloem. Having reached the sink cell

CASE STUDY 5.1 Partitioning of carbon and nitrogen in a legume

J.S. Pate

Plant composition in respect of carbon and nitrogen

Dry matter of a typical green plant contains a large and relatively constant proportion of carbon (35–50%), virtually all of which is derived from photosynthetic fixation of CO_2 by leaves or other green parts. Comparable values for nitrogen in dry matter tend to be more variable, with lowest levels in roots and stems (0.5–1.5%) and considerably higher levels in leaves (3–5%) where much nitrogen is associated with enzymes involved in photosynthesis. Higher levels are encountered in seeds (6–7%) and other storage organs in which large quantities of protein are being stored.

Most plants rely on nitrate (NO_3^-) or ammonium (NH_4^+) in the soil as their prime sources of nitrogen but certain leguminous plants gain nitrogen directly from the atmosphere by engaging in symbiosis with N_2-fixing bacteria in nodules on their roots (Figure 1A and B). White lupin is one such leguminous plant. Accompanying photographs show a flowering shoot (Figure 1B), nodules (Figure 1C) and pods (Figure 1D) of white lupin (*Lupinus albus* L.). Subcellular details of a nodule, with bacterial symbionts clearly visible, are seen in a transmission micrograph (Figure 1A).

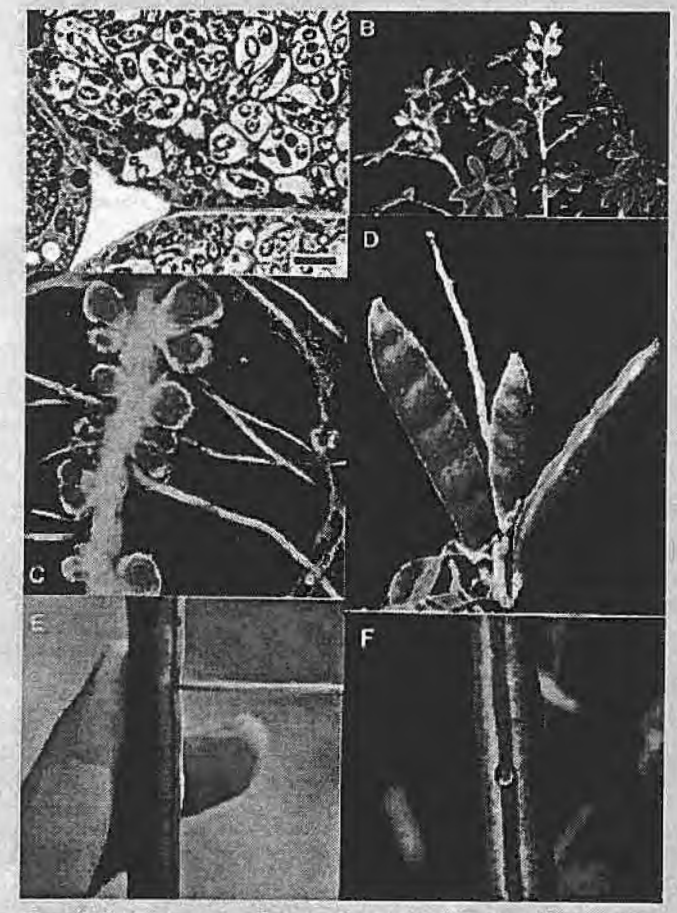


Figure 1(A) Transmission electron micrograph of a root nodule; (B) flowering specimen of white lupin (Lupinus albus); (C) nodules on the root system of a white lupin plant. Some of the nodules are cut open to reveal the pink haemoglobin pigmentation typical of actively N_2-fixing nodules; (D) fruits of lupin with their ends cut off to induce bleeding from the phloem; (E) 'cryopuncturing' of the fruit of cowpea (*Vigna unguiculata*) with a needle cooled in liquid nitrogen; (F) phloem bleeding from a cryopunctured fruit (see Colour Plate xx)

Requirements of plant parts for carbon and nitrogen

Consider lupin plants which are effectively nodulated and growing in a rooting medium to which all nutrients except nitrogen have been supplied. Allocation of carbon and nitrogen within the plant can then be examined in terms of the interrelationships of two mutually dependent, self-supporting (autotrophic) processes — photosynthesis and N_2 fixation. Within such a system, nodules supply further fixed nitrogen only so long as shoots export photosynthetically produced sugars. Shoots provide energy for nitrogen fixation, as well as carbon skeletons for synthesis of amino acids and other nitrogenous solutes (Figure 3.22) which carry fixed nitrogen from nodules to other plant parts. Conversely, increasing demands for photosynthetic products as plants progress through their life cycles can be met only if nitrogen from nodules continues to be available for expansion and functioning of new photosynthetic surfaces.

One complication which pervades experimental study on a plant such as lupin is that the carbon and nitrogen status of differently aged parts of the plant change continuously over a growth cycle. This is principally because new, actively growing tissues continuously come on stream as users of carbon and nitrogen whereas older plant parts progressively lose dry matter and especially nitrogen as they approach senescence. Because of their high protein content and relatively unthickened walls, young tissues require nitrogen rather than carbon. At the same time, nitrogen can be mobilised most effectively back into the plant from older organs prior to their senescence, since this nitrogen is attached mostly to degradable molecules such as protein. Conversely, carbon in dry matter is associated mostly with the non-degradable fabric of cell walls and is accordingly retrieved to a very limited extent prior to organ and tissue death. Thus, large proportions of the already accumulated resources of nitrogen within a plant are continuously recycled from old stem and leaf tissue to new leaves and eventually to fruits. Such recycling occurs to a much smaller extent with respect to carbon.

Before one can understand how carbon and nitrogen are allocated to different organs over a prescribed interval of growth of a plant such as lupin, quantitatively accurate information is required on the following:

1. changes in carbon and nitrogen contents of dry matter in each plant part;

2. assessment of net gaseous exchanges of carbon as CO_2 occurring in photosynthesis or respiration of each plant part;
3. estimates of total nitrogen gain of plants by N_2 fixation, as determined by summation of net increments or losses of nitrogen in plant parts over the study period;
4. estimates of net daytime photosynthetic gain of carbon by whole plants, as determined from measurements of increments or losses of carbon in organ dry matter, respiratory losses of carbon by root and nodules and nighttime respiratory losses of carbon by the shoot system.

It then becomes possible to construct simple models illustrating patterns of consumption of carbon and nitrogen by various plant parts. These are shown in Figure 2 for a specific week in the life of our lupin plant. Each model visualises the proportional amounts of carbon or nitrogen being processed, using appropriately shaped 'cartoons' for each class of organ drawn to an area proportional to the respective amounts they gain (positive values, shapes in full lines) or lose (negative values, shapes in broken lines) during the interval under examination. The model for carbon (Figure 2a), depicting proportional allocation of the 1061 mg carbon produced as net photosynthate, includes a stippled halo around each plant part to represent the respiratory losses accompanying a specified gain or loss of carbon by that part. Overall, we find that the nodulated root (NR) consumes a very large proportion (43%) of carbon generated by the plant in net photosynthesis, and most of the carbon so used is lost as respired CO_2 rather than incorporated into dry matter. The two upper strata of leaflets (L_3 and L_4, Figure 2b) consume the next greatest proportion (24%) followed by the various levels of stem axis plus petioles (SP_1–SP_4) (22%), shoot apex (A) (8%) and older leaves (L_1 and L_2) (4%).

The equivalent allocation 'cartoon' for the 34.8 mg nitrogen fixed in the study period (Figure 2b) is shaped very differently. L4 is the dominant sink for nitrogen (38%) followed by the nodulated root (NR) (29%), shoot apex (A) (19%) and L_1 (9%); extremely modest gains by stem and petiole tissue (SP) and net losses of nitrogen from the lower strata of leaves (L_1 and L_2) are observed.

Exchanges of carbon and nitrogen in phloem and xylem

So far we have not considered the role of long-distance transport conduits of xylem and phloem when carbon and nitrogen are partitioned to fulfil the requirements of plant parts (Figure 2). White lupin bleeds xylem fluid from the top of its root after the shoot has been cut off, thus enabling an inventory to be made of organic solutes passing up from roots and nodules to shoots through the xylem. Importantly, white lupin also bleeds from the sieve tubes of its phloem, if shallow cuts are made into stem, petiole or fruit (Figure 1D shows phloem sap bleeding from pods of lupin). Data from these sap samples are used to assess what carbon and nitrogen solutes are moving in the various streams of translocate being transported from any of the age groups of leaves to each of a number of possible consuming (sink) organs within the system. Another legume, cowpea (*Vigna unguiculata*), also bleeds from phloem and has been used for studies similar to those described here for lupin. Cowpea, however, bleeds only from its fruits and only if these are cryopunctured (Figure 1E and F). To determine which source leaves serve which sink regions within plants, ^{14}C-feeding studies need to be conducted in single source leaves which are fed $^{14}CO_2$ and the fate of ^{14}C-labelled sucrose and other assimilates which they export are subsequently traced within the plant.

Armed with the above information, a transport profile can

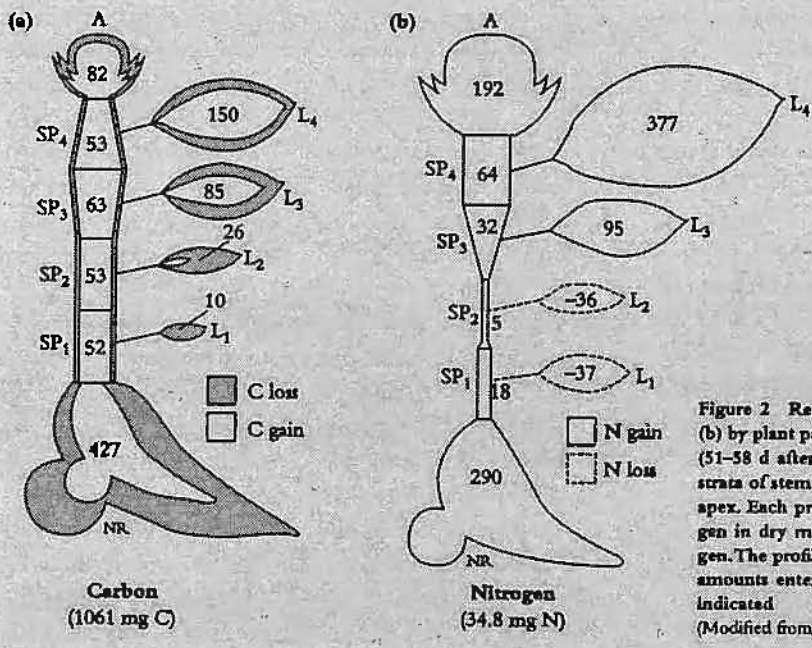


Figure 2 Representations of the consumption of carbon (a) and nitrogen (b) by plant parts of white lupin (*Lupinus albus*) during mid-vegetative growth (51–58 d after sowing). L_1–L_4, four strata of leaflets; SP_1–SP_4, corresponding strata of stem segments and associated petioles; NR, nodulated root; A, shoot apex. Each profile for utilisation depicts net gain or loss of carbon or nitrogen in dry matter, relative to a net input of 1000 units of carbon or nitrogen. The profile for carbon includes losses of carbon as respired CO_2. Absolute amounts entering the plant as carbon of net photosynthate or fixed N, are indicated
(Modified from Pate and Layzell 1981)

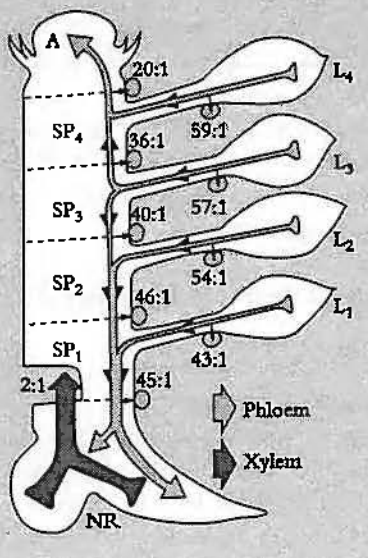


Figure 3 Carbon to nitrogen weight ratios in the xylem and various phloem streams of plants of white lupin during mid-vegetative growth (51–58 d after sowing). Plants were entirely dependent on N_2-fixing nodules for nitrogen. Coding of plant parts as in Figure 2
(From Pate and Layzell 1981)

be gained, a picture of where resources move in a lupin plant (Figure 3). *Directions* of flow between source leaves and consuming parts of the plant, as determined by ^{14}C feeding, are indicated by arrowed pathways in the figure. Local ratios (by weight) of carbon to nitrogen within different plant parts are shown; they reflect outcomes of carbon and nitrogen transport. For example, the xylem (drawn in black) delivers fluid to transpiring parts with solutes of very low C : N ratio, because sugars are virtually absent and the major solute is the amino acid asparagine (C : N of 2 : 1). Conversely, the youngest leaves (L_4) which are most active in exporting sugars, are not yet losing much nitrogen, so they produce phloem translocate (dotted pathways) of relatively high C : N ratio (59 : 1). Downward moving translocate from the shoot shows a C : N ratio which could be predicted from a mixture of the streams of translocate originating in the three strata of leaves which supply the root. One surprising finding is that the apical part of a shoot receives phloem translocate with a relatively low C : N ratio of 20 : 1, despite being fed from the upper leaves (L_3 and L_4) which are generating much less nitrogen rich solute streams (C : N ratios of 57 : 1 and 59 : 1) respectively. Clearly phloem sap must be modified en route from source leaves to shoot apex. How is this differential partitioning of nitrogen towards the apex achieved?

Combining the information described so far the model for partitioning of 1000 units of carbon of net photosynthate is dominated by phloem-mediated transfer of photosynthate from leaves to root and shoot apex. Corresponding xylem flow of carbon is proportionately much less and reflects the extent to which carbon supplied to nodules is returned to the shoot in the form of xylem-exported amino compounds formed in N_2 fixation. Approximately 8% of the total carbon exported from photosynthesising leaves cycles through roots and nodules. This substantial demand by nodules for photosynthate is most striking. During mid-vegetative growth, 24% of the carbon of net photosynthate is translocated to nodules, over half of which is respired, and the remainder incorporated into nodule dry matter or returned to the plant attached to fixation products.

The complementary model for partitioning 1000 units of fixed nitrogen revolves around bulk export of newly fixed nitrogen from nodules. As expected, transpiring leaves act as principal initial destinations for such nitrogen. However, a proportion of nitrogen is transferred from xylem to phloem within veins of lower leaves, becoming available to roots. Similar xylem to phloem transfer in upper leaves provides nitrogen for shoot apices.

Note that the three lower strata of leaves (L_1–L_3), are, in effect, short changed for nitrogen through withdrawal of nitrogen from xylem traces supplying these lower leaves. After withdrawal, this nitrogen passes back into xylem traces, moving further up the stem.

As a result, xylem sap in the body of the stem becomes progressively enriched with nitrogen, and upper leaves thus receive considerably more nitrogen per unit of transpirational activity than nitrogen-deprived older leaves further down the canopy. This subtle shuttle system within a stem results in over 40% of currently fixed nitrogen being targeted towards upper, still expanding leaves — the very site of greatest demand for nitrogen at this stage in plant growth.

Two other features of this model for nitrogen deserve mention:

1. In a legume totally dependent on its nodules for nitrogen, parts of the root system extending below or laterally outwards beyond the nodules cannot receive nitrogen directly from xylem since the sap in this tissue moves upwards. Instead they gain nitrogen indirectly as phloem translocate supplied from the shoot (Section 3.6). Consequently, roots growing in soils deficient in nitrogen will be tightly controlled by nitrogen from shoots.
2. Substantial amounts of nitrogen moving through stem xylem into uppermost parts of the shoot are shuttled laterally across to the phloem stream moving photosynthate from upper leaves to the shoot apex. The apex accordingly acquires much more nitrogen than one would ever expect from its weak transpirational activity or from the phloem streams generated in the upper nurse leaves. Such stem-located processes of xylem to phloem transfer, together with xylem to xylem transfers in lower stems, comprise extremely important elements in differential partitioning of nitrogen and, indeed, of a number of other nutrient elements in herbaceous plants such as barley, castor bean, pea and lupin (Pate and Jeschke 1995). Demonstrations of such transfer activity between xylem and phloem clearly call into dispute previous suppositions that vascular tissues in stems play merely passive roles in straight through-put of solutes.

Transport can be regulated at all points along the pathway.

Predictive use of partitioning models

Although our model is 'empirically' based on actual observations and measurements constructed for a particular set of growth circumstances, it can still be used to predict what might happen if the system were to be perturbed in any of a number of ways. For instance, were N_2 fixation suddenly to cease, the model would suggest that xylem export of nitrogen from the nodulated root would cease almost immediately and that those organs first to suffer would be the shoot apex and any young leaves still dependent on xylem to meet the demands of their growing photosynthetic tissues for nitrogen. Then, if nitrogen starvation continued, senescence of lower leaves would commence and, according to the model, nitrogen released would become available mostly to root growth as opposed to rescuing upper shoots from nitrogen deficiency. Indeed, from observation, nitrogen starvation reduces new shoot growth, followed in turn by yellowing of leaves, and increased rather than decreased root growth.

Further study

Empirical models of the kind described in this essay may describe adequately events involved in partitioning of carbon and nitrogen, but they still tell us virtually nothing of the nature of the underlying cellular and molecular processes which modulate and regulate resource distribution in whole plants. To achieve such a picture, much more has to be learned about regulation of solute loading and unloading into both xylem and phloem elements. Particularly interesting in this connection would be a detailed study of how xylem to xylem or xylem to phloem transfers are coordinated along the stem throughout the life of a plant and how these activities change in relation to supply and demand of various donor and receptor regions of a plant during a cycle of growth and development.

References

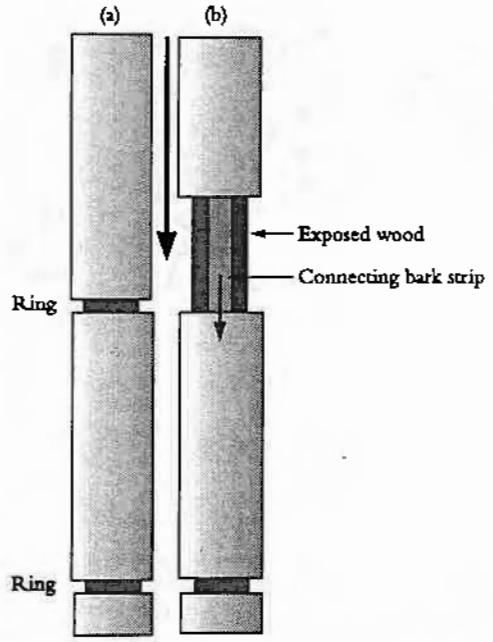
Pate, J.S. (1996). 'Photoassimilate partitioning and consumption in nitrogen-fixing crop legumes', in *Photoassimilate Partitioning in Plants and Crops: Source: Sink Relationships*, eds, E. Zamski, and A.A. Schaffer, 467–477, Marcel Dekker, Inc.: New York.

Pate, J.S. Layzell, D.B. (1981). 'Carbon and nitrogen partitioning in the whole plant — a thesis based on empirical modelling', in *Nitrogen and Carbon Metabolism*, ed. J.D. Bewley, 94–134, Martinus Nijhoff/Dr. W. Junk Publishers: The Hague.

Pate, J.S. and Jeschke, W.D. (1995). 'Role of stems in transport, storage and circulation of ions and metabolites by the whole plant', in *Stems and Trunks: Their Roles in Plant Form and Function*, ed. Barbara L. Gartner, 177–204, Academic Press: New York.

vegetative plant, lower leaves are the principal suppliers of photoassimilate to roots, whereas upper leaves are the principal suppliers to the shoot apex. Leaves in an intermediate position export equal quantities of photoassimilates in either direction. However, the pattern of photoassimilate partitioning is not static, it changes with plant development. In vegetative plants, the direction of flow from a leaf changes as more leaves above it become net exporters. Furthermore, at the onset of reproductive development, growing fruits or seeds become dominant shoot sinks for photoassimilates at the expense of vegetative apices.

Photoassimilate partitioning patterns can be altered experimentally by removal of selected sources (e.g. leaves) or sinks (e.g. fruits). These manipulative experiments demonstrate that photoassimilate partitioning reflects the relative strengths of individual sources and sinks. Properties of the phloem pathway connecting sources with sinks are examined in Section 5.4.



Width of bark strip (% of intact stem)				
Carbohydrate transported to the lower part of the stem in 24 h (mg)	0	10	33	87
	0	437	609	744

Figure 5.15 The role of bark (phloem) in sugar movement in plants. Mason and Maskell (1928) demonstrated that removing a complete ring of bark (a) while leaving the wood (xylem) intact prevented downward movement of sugars. When a strip of bark was retained between upper and lower stem parts (b), sugars flowed downwards in indirect proportion to the width of remaining bark.

5.4 Phloem transport

5.4.1 Introduction

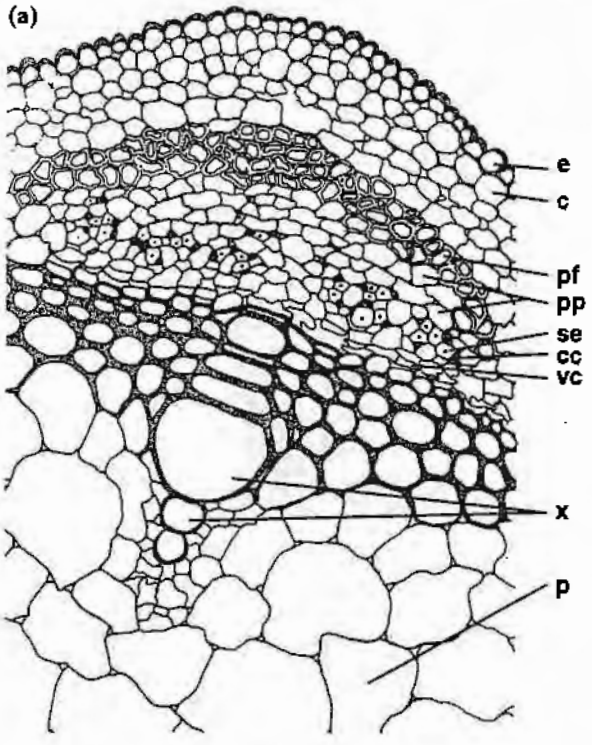
Bark of a woody plant contains phloem but not xylem and the transport function of that phloem can be demonstrated by ring-barking (Figure 5.15). Using this approach, Mason and Maskell (1928) showed unambiguously that sugar transport through stems of cotton plants was dependent on a *continuity* of bark but independent of contact with xylem. Phloem is thus a living tissue, and metabolic energy is needed for phloem to work.

5.4.2 Characteristics of phloem transport

(a) Phloem structure

Phloem is made up of phloem fibres, phloem parenchyma, sieve cells (sieve elements) and their accompanying companion cells (Figure 5.16A). Sieve elements are ideally suited for rapid transport of substances at high rates over long distances. They are elongate and are arranged end to end in files referred to as sieve tubes (Figure 5.16B). Abutting sieve elements are interconnected through membrane-lined pores (sieve pores) with large diameters (1 to 15 μm). These pores collectively form sieve plates (Figure 5.16C). The transport capacity of sieve tubes is dependent on a developmentally programmed degeneration of the sieve element protoplasm (cell contents) leaving an open, membrane-bound tube. In mature conducting sieve elements, the protoplast is limited to a functional plasma membrane enclosing a sparse cytoplasm containing low densities of plastids, mitochondria and smooth endoplasmic reticulum distributed along the lateral walls (Figure 5.16D). These relatively empty sieve tubes provide a longitudinal network which conducts phloem sap (Figure 5.16B).

Sieve elements are closely associated with one or more companion cells, forming a sieve element–companion cell (se-cc) complex (Figure 5.16D) that plays an important role in transport. These distinct cell types result from division of a common procambial mother cell. In mature se–cc complexes, relatively open sieve elements contrast with adjacent companion cells containing dense, ribosome-rich cytoplasm with a prominent nucleus and abundant mitochondria and rough



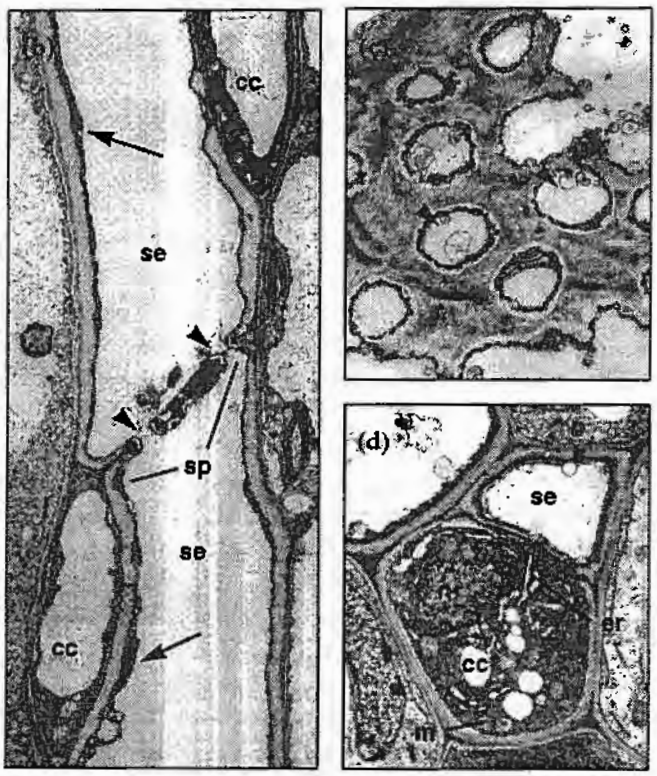


Figure 5.16 (a) Spatial arrangement of cell types in a vascular strand from the primary stem of *Phaseolus vulgaris* (French bean); electron micrographs of stem phloem of *Cucurbita maxima* (b, c) and *P. vulgaris* (d) illustrating significant structural characteristics of sieve elements and companion cells. (a) Conducting cells of the phloem (sieve elements) and accompanying companion cells form groups of cells that are separated by phloem parenchyma cells. This mosaic of cells is located between the cortex and xylem and capped by phloem fibres. Bar represents 7.3 μm. (b) A longitudinal section through two sieve elements arranged end to end to form part of a sieve tube. Companion cells can also be seen. The abutting wall (sieve plates) displays characteristic membrane-lined sieve pores (arrowheads). Cytoplasm of the sieve elements has largely degenerated leaving only endoplasmic reticulum (arrows) and a few plastids around the mature sieve element. Bar represents 5 μm. (c) A face view of part of a sieve plate showing sieve pores (arrowheads). Bar represents 0.5 μm. (d) Transverse section through a sieve element and its accompanying companion cell illustrating the sparse cytoplasm and low density of organelles in the sieve element contrasting with the dense ribosome-rich cytoplasm of the nucleated companion cell. Note the mitochondria and rough endoplasmic reticulum. Bar represents 1.0 μm. c, cortex; cc, companion cell; e, epidermis; er, endoplasmic reticulum; m, mitochondrion; n, nucleus; p, pith; pf, phloem fibres; pp, phloem parenchyma; se, sieve element; sp, sieve plates; vc, vascular cambium; x, xylem.
(b and c based on Raven *et al.* 1992)

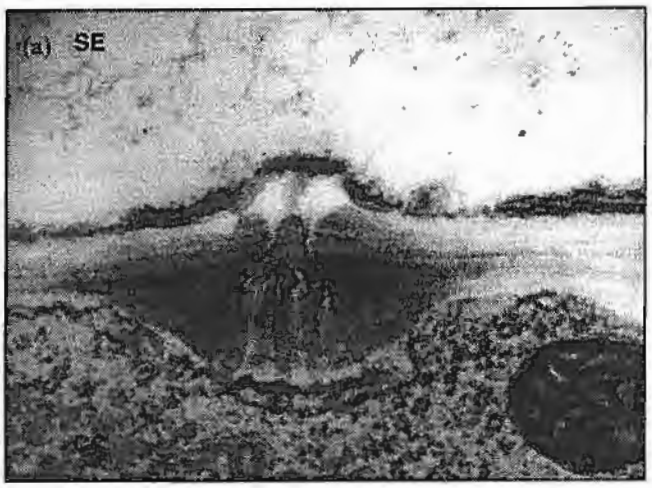
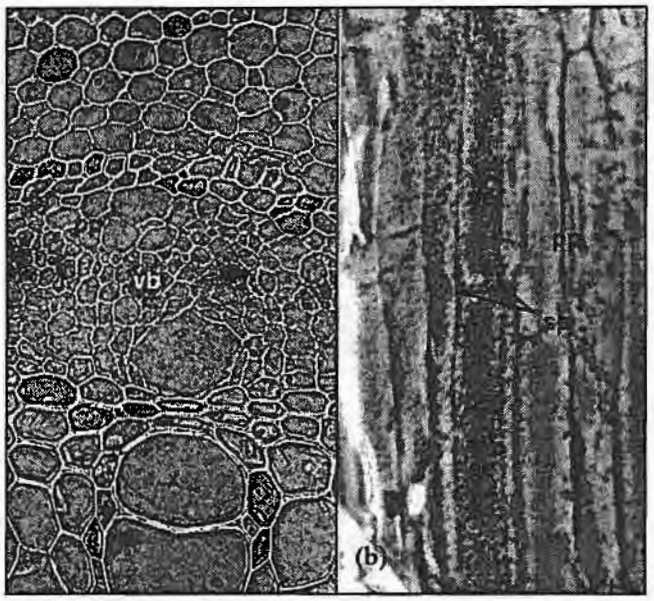


Figure 5.18 Microautoradiographs of (a) transverse and (b) longitudinal sections of *Phaseolus vulgaris* stem tissue illustrating localisation of ^{14}C-labelled photosynthate in sieve tubes. These sections are obtained by snap freezing plant tissue and removing frozen water by sublimation (e.g. freeze-drying or freeze substitution). ^{14}C-labelled compounds do not move during preparation. Tissues are embedded in absolute dryness and thin sections are cut, mounted dry on microscope slides and overlain with a thin film of photographic emulsion. Silver grains are visible in the emulsion where ^{14}C, an ideal radioisotope for these experiments, irradiates the film. Abbreviations: se, sieve element; pp, phloem parenchyma; vb, vascular bundle.



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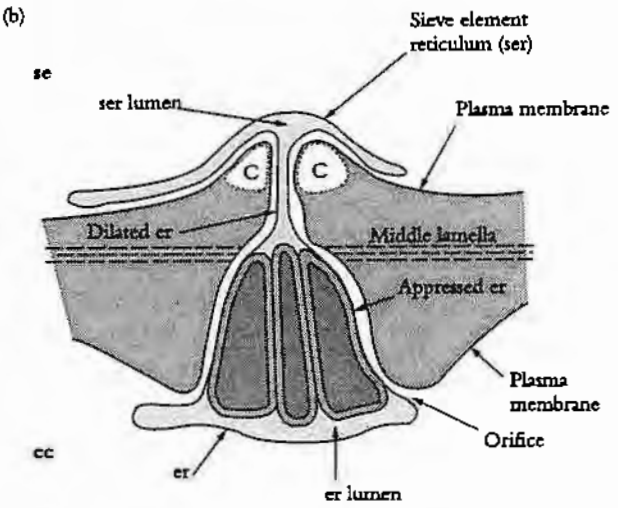
endoplasmic reticulum (Figure 5.16D). High densities of extensively branched plasmodesmata in contiguous walls of sieve elements and companion cells (Figure 5.17) account for intense intercellular coupling in se–cc complexes (van Bel 1993). Thus, companion cells are considered to perform the metabolic functions surrendered by, but required for, maintenance of viable sieve elements. This functional cooperativity has led to the concept of se–cc complexes being responsible for phloem transport.



(b) Visualising the translocation stream

Transport of radioactively labelled substances through phloem has been demonstated using microautoradiography (Figure 5.18), providing irrefutable evidence that sieve elements are conduits for transport of phloem sap. Experimentally, a pulse of $^{14}CO_2$ is fixed photosynthetically and ^{14}C-labelled sugars are given time to reach the stem, which is then excised and processed for microautoradiography. As ^{14}C first moves through the stem, most of the isotope is confined to the transport pathway and very little has had time to move laterally into storage pools. High densities of ^{14}C-labelled sugars are found in sieve elements (Figure 5.18), demonstrating that these cells constitute a transport channel.

(c) Phloem sealing mechanisms

Herbivory or environmental factors causing physical damage could pose a threat to transport through sieve tubes and has undoubtedly imposed strong selection pressure for the evolution of an efficient and rapid sealing mechanism for damaged sieve tubes. Since sieve-tube contents are under a high turgor pressure (P), severing would cause phloem contents to surge from the cut site, incurring excessive assimilate loss in the absence of a sealing mechanism. For dicotyledonous species, an abundant phloem-specific protein (P-protein) provides an almost instantaneous seal. P-protein is swept into sieve pores where it becomes entrapped, thus sealing off the damaged sieve tubes. Production of callose (β-(1→3) glucan) in response to wounding or high temperature stress is another strategy to seal off damaged sieve tubes. Callose also seals off sieve pores during overwintering in deciduous plants. Callose is deposited between the plasma membrane and cell wall, eventually blocking sieve pores. Whether deposited in response to damage or overwintering, callose can be degraded, allowing sieve tubes to regain transport capacity.

5.4.3 Chemical nature of translocated material

(a) Techniques to collect phloem sap

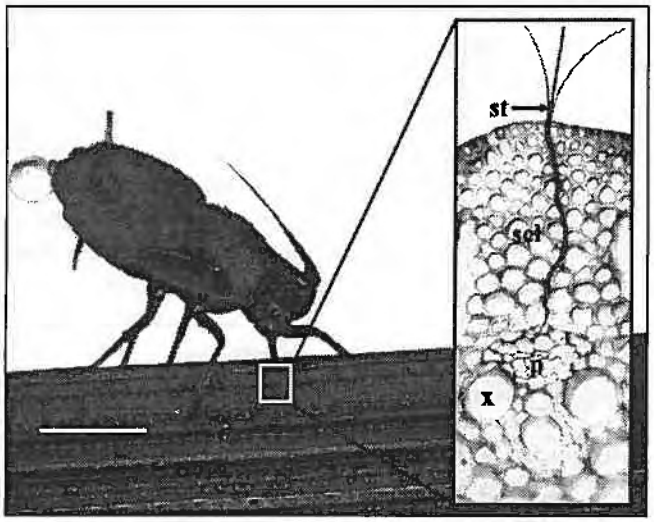
Since phloem translocation is confined to sieve elements embedded within a tissue matrix, it is difficult to obtain uncontaminated samples of translocated sap. The least equivocal approach has been to take advantage of the high P of sieve tube contents. Puncturing or severing sieve-tubes should cause exudation of phloem sap provided a sealing mechanism is not activated.

For some plant species, sieve-pore sealing (Section 5.4.2(c)) develops slowly, or can be experimentally down-regulated by massage or repeated excisions (Milburn and Kallarackal 1989). Carefully placed incisions that do not disturb the underlying xylem of these species permit collection of relatively pure phloem sap exuded through severed sieve tubes (see Case study 5.1). The excision technique has been expanded to plant species that do not readily exude, by chemically inhibiting the sealing mechanism. Callose production is blocked when wounded surfaces are exposed to the chelating agent ethylenediaminetetraacetic acid (EDTA) by complexing with calcium, a cofactor for callose synthetase. Immersing whole, excised organs in EDTA solution, which is essential to inhibit blockage, risks contaminating sap with solutes lost from non-conducting cells. This is not an ideal technique.

Enlisting sap-sucking aphids to sample sap has been more successful. Aphids can guide a long syringe-like mouthpart (a stylet) into conducting sieve elements (Figure 5.19). Pressure normally forces sieve-tube sap through the stylet into the aphid's gut where it becomes food or is excreted as 'honeydew'. By detatching the aphid from its mouthpart pure phloem sap can be collected from the cut end of the implanted stylet.

(b) Chemical analysis of phloem sap

Chemical analyses of phloem sap collected from a wide range of plant species have led to a number of generalisations (e.g. Milburn and Baker 1989). Phloem sap is a concentrated solution (10 to 12% dry matter), generating an osmotic pressure (P) of -1.2 to -1.8 MPa. Sap pH is characteristically alkaline (pH 8.0 to 8.5). The principal organic solutes are non-reducing sugars, amides (glutamine and asparagine), amino acids (glutamic and aspartic acids) and organic acids (malic acid). Of these solutes, non-reducing sugars generally occur in the highest concentrations (300 to 900 mM). Nitrogen is transported through the phloem as amides and amino acids; nitrate is absent and ammonium only occurs in trace amounts. Calcium, sulphur and iron are also absent from phloem sap while all other inorganic nutrients are present, particularly potassium which is commonly in the range of 60



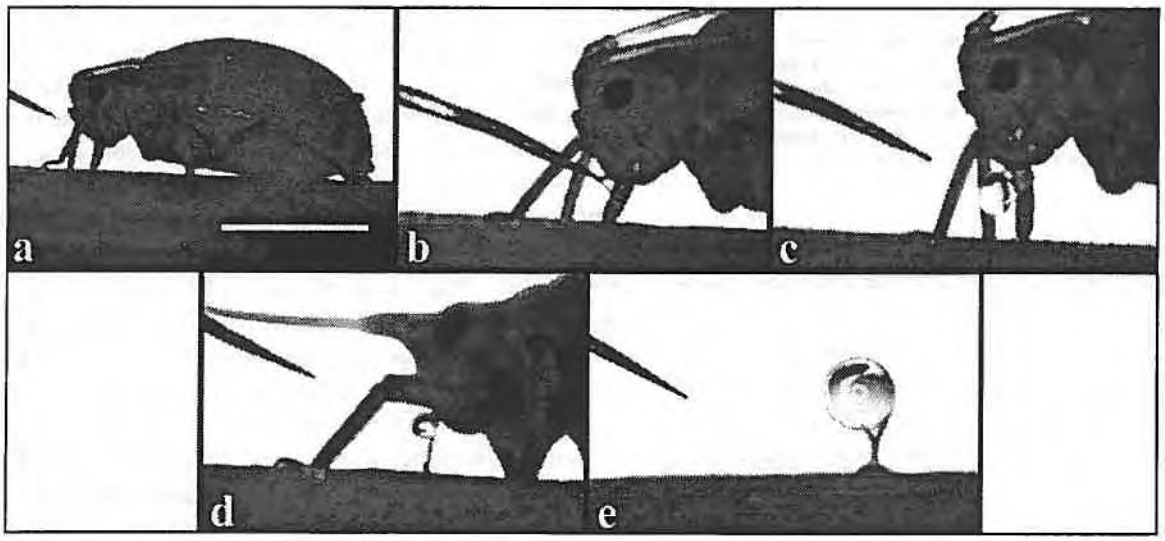


Figure 5.19 Aphids can be used to collect phloem sap. Top photograph: a feeding aphid with its stylet embedded in a sieve tube (see insert). Note the drop of 'honeydew' being excreted from the aphid's body. Plates (a) to (e) show a sequence of stylet cutting with an RF microcautery unit at about 3–5 s intervals (a to d) followed by a two-minute interval (d to e) which allowed exudate to accumulate. The stylet has just been cut in (b); droplets of hemolymph (aphid origin) are visible in (b) and (c); once the aphid moves to one side the first exudate appears (d), and within minutes a droplet (e) is available for microanalysis. Scale bars: top = ??, bottom = ?? (see Colour Plate xx)

to 120 mM. Physiological concentrations of auxins, gibberellins, cytokinins and abscisic acid have been detected in phloem sap along with nucleotide phosphates. The principal macromolecule is protein, comprised largely of P-protein in dicotyledons, but a number of enzymes are also detectable.

(c) Significance of the chemical forms translocated

Phloem sap provides most inorganic and all organic substrates necessary to support plant growth (see Case study 5.1). Non-transpiring tissues are particularly dependent on resources delivered in the phloem (Section 5.3.1). That translocated sugars represent the major chemical fraction of the phloem sap (see Section 5.4.3(b)) is consistent with the bulk of plant dry matter (90%) being composed of carbon, hydrogen and oxygen. Carbon transport is further augmented by transport of nitrogen in organic forms.

Carbohydrate is translocated as non-reducing sugars in which the metabolically reactive aldehyde or ketone group is reduced to an alcohol (mannitol, sorbitol) or combined with a similar group from another sugar to form an oligosaccharide. Apart from sucrose, transported oligosaccharides belong to the raffinose series. In this series, sucrose is bound with increasing numbers of galactose residues to form raffinose, stachyose and verbascose respectively. However, sucrose is the most common sugar species transported. In a small number of plant families, other sugar species predominate. For example, the sugar alcohol sorbitol is the principal transport sugar in the Rosaceae (e.g. apple) and stachyose predominates in the Cucurbitaceae (e.g. pumpkin and squash). Exclusive transport of non-reducing sugars probably reflects packaging of carbohydrate in a chemical form which protects it from being metabolised. Metabolism of these transported sugars requires their conversion to an aldehyde or ketone by enzymes which are absent from sieve-tube sap.

5.4.4 Phloem flux

Phloem flux can be estimated in a number of ways. The simplest is to determine dry weight gain of a discrete organ connected to the remainder of a plant by a clearly definable axis of known phloem cross-sectional area. Developing fruits or tubers meet these criteria. Sequential harvests from a population of growing fruit or tubers provide measures of the organ's net gain of dry matter imported through the phloem. Net gains or losses of dry matter resulting from respiration or photosynthesis are incorporated into calculations to give gross gain in dry matter by the organ. Flux of dry matter through the phloem (specific mass transfer — SMT; Canny 1973) can then be computed on a phloem or preferably on a sieve-tube lumen cross-sectional area basis. Area estimates can be obtained from histological sections of the pedicel or stolon that connects a test organ to its parent plant. Expressed on a *phloem* cross-sectional area basis, SMT estimates are normally in the range of 2.8 to 11.1 g m^{-2} phloem s^{-1} (Canny 1973). Flux on the basis of *sieve-tube lumen* cross-sectional area is preferable but relies on identification of sieve tubes and the assumption that they are equally functional as transport conduits. Sieve tubes account for some 20% of phloem cross-sectional area, suggesting fluxes are about five-fold higher through a sieve-tube lumen.

Speed of phloem translocation can be determined from simultaneous measurements of SMT and phloem sap concentrations as shown in Equation 5.1 below:

$$\text{Speed (m s}^{-1}) = \text{SMT(g m}^{-2}\text{s}^{-1})/\text{concentration (g m}^{-3}) \quad (5.1)$$

Substituting sucrose concentrations and SMT values into Equation 5.1, phloem sap is estimated to move at speeds of up to 56×10^{-5} m s^{-1} or 200 cm h^{-1}. These estimates have been verified by following the movement of radioisotopes introduced into the phloem translocation stream.

These estimates of transport rates and speeds tacitly assume that phloem sap moves through sieve tubes by mass flow (water and dissolved substances travel at the same speed). Independent estimates of transport rate, concentration of phloem sap and translocation speed lend support to, but do not verify, the assumption that movement occurs as a mass flow.

A simple and direct test for mass flow is to determine experimentally whether water and dissolved substances move at the same speed. This test should be relatively easy to apply using radioactively labelled molecules. Unfortunately, in practice it turns out that different molecular species are not loaded into the sieve tubes at the same rates and the plasma membranes lining the sieve tubes are not equally permeable to each substance. Thus, the analysis is complicated by the necessity to use model-based corrections for rates of loading into and losses from the sieve tubes. Nevertheless, the speed estimates obtained from such experiments are found to be similar for dissimilar molecules, supporting the proposition that mass flow accounts for most transport through sieve tubes.

5.4.5 Mechanism of phloem translocation

Phloem translocation is generally believed to be driven by pressure. MÅnch (1930) proposed that a passive mass flow of phloem sap through sieve tubes was driven by the osmotically generated pressure gradient between source and sink regions (Figure 5.20). At source regions, the principal osmotica of phloem sap are actively loaded into sieve tubes, thereby driving water towards the lower water potentials within sieve tubes (Section 4.3). As water enters, P rises. Unloading of solutes from sieve tubes at sink regions reverses water poten-

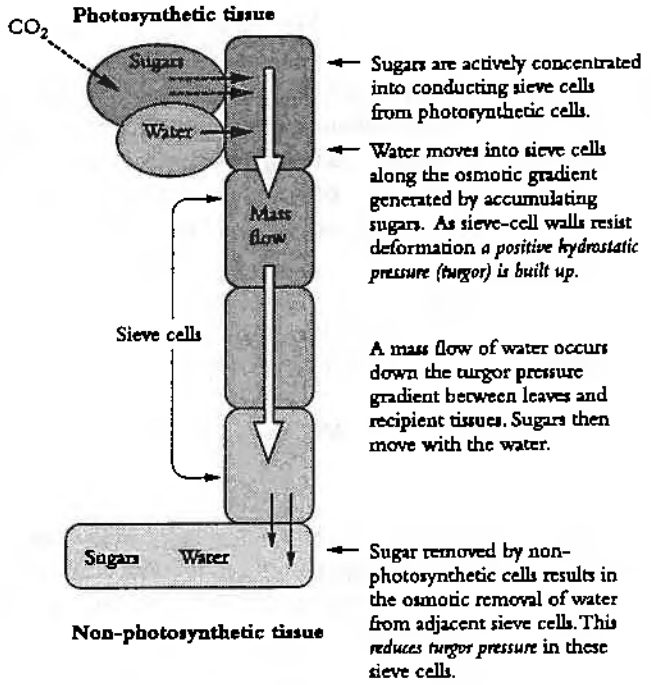


Figure 5.20 Scheme describing the pressure-flow hypothesis of phloem transport
(After Münch 1930)

tials; water flows out of sieve tubes and P falls relative to that of sieve tubes in source regions.

The pressure-flow hypothesis can be modelled using the relationship that rate of mass flow (F_f) of a substance is given by the product of speed (S) of solution flow, path cross-sectional area (A) and its concentration (C). That is:

$$F_f = S.A.C \qquad (5.2)$$

Speed (m s^{-1}) has the same units as volume flux (J_v — m^3 m^{-2} s^{-1}) of solution passing through a transport conduit. Poisseuille's Law describes the volume flux (J_v) of a solution of a known viscosity (η) driven by a pressure difference (ΔP) applied over the length (l) of pathway of radius (r) as:

$$J_v = \pi r^4 \Delta P / 8\eta l \qquad (5.3)$$

The term $\pi r^4/8\eta l$ in Equation 5.3 provides an estimate of hydraulic conductivity (L_p) of the sieve-tube conduit which is set by the radius of the sieve pores. Raised to the fourth power, small changes in the sieve-pore radius will exert profound effects on the hydraulic conductivity of the sieve tubes (Section 5.2). The viscosity of sieve-tube sap is determined by the chemical species (particularly sugars) and their concentrations in the phloem sap.

Key features of the pressure-flow hypothesis are encapsulated in Equation 5.3. The central question is whether a pressure gradient exists in sieve tubes with the expected direction and of sufficient magnitude to support observed rates of sap flow. Indirect estimates of P in sieve tubes made through determination of intra- and extracellular Π support the pressure-flow hypothesis. Direct measurements of sieve-tube P are technically challenging because of the inaccessibility of these small, highly turgid cells. They are, for instance, too small for pressure-probe measurements. However, manometric pressure measurements severed aphid stylets agree with indirect estimates (Wright and Fisher 1980). Experimental manipulation of the pressure gradient between the source and sink also results in alterations in phloem translocation rates consistent with the pressure-flow model.

Whether the pressure gradient is sufficiently steep is a more vexing question. The pressure gradient required to drive phloem translocation at observed rates is determined by the transport resistance of the phloem path, according to Ohm's Law. Dimensions of the sieve pores set a limiting radius for volume flux of transported sap (Equation 5.3) and hence transport resistance. If the sieve pores were open and unoccluded by P-protein, a number of studies have demonstrated that the measured pressure gradients are sufficient to support the observed rates of flow. However, the *in situ* radii of sieve pores remain unknown.

Overall, the pressure-flow hypothesis accounts for many observed features of phloem translocation, including distribution of resources. While conclusive evidence supporting this hypothesis is still sought, less attention is now focused on this issue with a growing appreciation that the phloem pathway has spare transport capacity. Evidence from Kallarackal and Milburn (1984), for example, showed that SMT (Section 5.4.4(a)) to an intact fruit of castor bean could be doubled on removal of competing fruits. Moreover, if P of sieve elements at the sink end of the phloem path was reduced to zero, by severing the pedicel and allowing exudation, SMT rose to an incredible 305 g m^{-2} sieve-tube area s^{-1}! In another experiment, when half the conducting tissue was removed from the peduncle of sorghum or wheat plants, grain growth rate was not impaired (Wardlaw 1990). Together, these observations imply that phloem has excess carrying capacity in both dicotsyledons and monocotsyledons. Particularly in monocotyledonous plants, a strong selection pressure for spare transport capacity must exist because there is no vascular cambial activity to replace damaged sieve elements.

5.4.6 Control of assimilate transport from source to sink

Loading of sugars, potassium and accompanying anions into sieve tubes at sources determines solute concentrations in phloem sap (Table 5.3). The osmotic pressure of these solutes influences P generated in sieve tubes. Thus, source output determines the total amount of assimilate available for phloem transport as well as the pressure head driving transport along the phloem path to recipient sinks. Withdrawal of assimilates from sieve tubes at the sink end of the phloem path, by the combined activities of phloem unloading and metabolism/

compartmentation (Table 5.3), determines Π of phloem sap. Other sink-located membrane transport processes influence Π around sieve tubes. The difference between intra and extracellular Π of sieve tubes is a characteristic property of each sink and determines P in sink sieve tubes.

Table 5.3 Properties and processes of the source, phloem path and sink which influence the driving variables for phloem transport and thereby determine pressure-driven assimilate flow through phloem (see Equations 5.2 and 5.3)

Transport component	Phloem transport variables	Property or process
Source	Assimilate concentration (C) Turgor pressure (P_{source})	Net photosynthetic rate; allocation to storage pools; phloam loading
Phloem path	Assimilate concentration (C) Lumen cross-sectional area (A) Hydraulic conductivity (L_p)	Unloading/reloading; Sieve-element number; Sieve-pore radius
Sink	Turgor pressure (P_{sink}) Compartmentation	Phloem unloading; metabolism

The pressure difference between source and sink ends of the phloem pathway drives sap flow (Equation 5.3) and hence phloem translocation rate (Equation 5.2) from source to sink. The source and sink processes governing the pressure difference (Table 5.3) are metabolically dependent, thus rendering phloem translocation rates susceptible to cellular and environmental influences. The pressure-flow hypothesis predicts that the phloem path contribution to longitudinal transport is determined by the structural properties of sieve tubes (Table 5.3). Variables of particular importance are cross-sectional area (A) of the path (determined by numbers of sieve pores in a sieve plate and sieve-tube numbers) and radius of these pores (sets r in Equation 5.3). These quantities appear in Equations 5.2 and 5.3. Thus, the individual properties of each sink and those of the phloem path connecting that sink to its source will determine the potential rate of assimilate import to the sink (Figure 5.21).

The transport rate (R) of assimilate along each phloem path, linking a source with each respective sink, can be predicted from the pressure-flow hypothesis (see Equations 5.2 and 5.3) as:

$$R = K_{path}\,(P_{source} - P_{sink})C \qquad (5.4)$$

where path conductance (K_{path}) is the product of path hydraulic conductivity (L_p) and cross-sectional area (A). Hence, the relative flows of assimilates between hypothetical sinks (*sink 1* and *sink 2*) shown in Figure 5.21 may be expressed by the following equation:

$$\frac{K_{path\ 1}\,(P_{source} - P_{sink\ 1})C}{K_{path\ 2}\,(P_{source} - P_{sink\ 2})C} \qquad (5.5)$$

Partitioning of assimilates between two competing sinks is thus a function of path conductance and P at the sink end of the phloem path (Equation 5.5). Since phloem has spare capacity, any differences in the conductance of the interconnecting paths (Figure 5.21) would exert little influence on the rate of phloem transport to the competing sinks. Assimilate partitioning between competing sinks would then be determined by the relative capacity of each sink to depress sieve-tube P at the sink end of the respective phloem path. Even when differences in path conductance are experimentally imposed, phloem transport rates are sustained by adjustments to the pressure differences between the sonrce and sink ends of the phloem path (Wardlaw 1990).

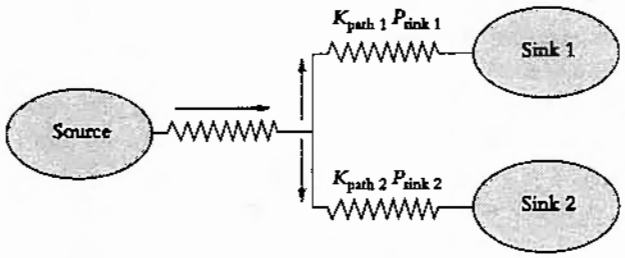


Figure 5.21 Scheme describing photoassimilate flow from a source leaf linked to two competing sinks, Sink 1 and Sink 2. Assimilate flows through alternative phloem paths (Path 1 and Path 2) each with its own conductance (K_{path}) and pressure difference (P) between source and sink. Hence Path 1 is distinguished by $K_{path\ 1}$ and $P_{sink\ 1}$ and Path 2 by $K_{path\ 2}$ and $P_{sink\ 2}$.

These conclusions have led to a shift in focus from phloem transport to phloem loading and unloading, which are iustrumental in determining the amount of assimilate translocated and its partitioning between competing sinks, respectively.

5.5 Phloem loading

5.5.1 Introduction

Phloem loading plays a central role in determining productivity. Photoassimilates are loaded along the entire phloem transport pathway, from photosynthetic leaves to importing sinks. While most photoassimilate loading occurs in photosynthetically active leaves, root-produced metabolites, such as amino acids, move readily from xylem to phloem particularly at the stem nodes (see Case study 5.1). Phloem loading also occurs in storage organs during periods when reserves are remobilised and exported. Indeed, the membrane transport events contributing to phloem loading were first examined using export of sucrose remobilised from the endosperm of germinating castor bean seed as an experimental model (Kriedemann and Beevers 1967).

Events in leaves dominate this account of phloem loading. However, resources supplied from the xylem and during

remobilisation of stored reserves will also be considered. An opening analysis of the cellular pathway for assimilate loading is followed by insights into mechanisms and controls of phloem loading.

5.5.2 Pathway of phloem loading in source leaves

(a) Delineating the transport path

Phloem loading is used variously to describe transport events outside, and inside, phloem tissues of leaves. The broader general application is adopted here — that is, phloem loading describes photoassimilate transport from the cytoplasm of photosynthetic mesophyll cells to se–cc complexes of leaf phloem.

Phloem loading commences in mesophyll cells and ends in the leaf vascular system. The se–cc complexes occur in a wide array of vascular bundle sizes. In dicotyledonous leaves, veins undergo repeated branching, forming the extensive minor vein network described in Section 5.2. For example, sugar beet leaves contain 70 cm of minor vein per cm^2 of leaf blade, while the major veins contribute only 5.5 cm per cm^2 of leaf blade (Geiger 1975). These observations and physiological studies (van Bel 1993) show that the principal site of phloem loading is in the minor vein network of dicotyledonous leaves. In contrast, the major veins transport loaded photoassimilates out of leaves (Section 5.2.2).

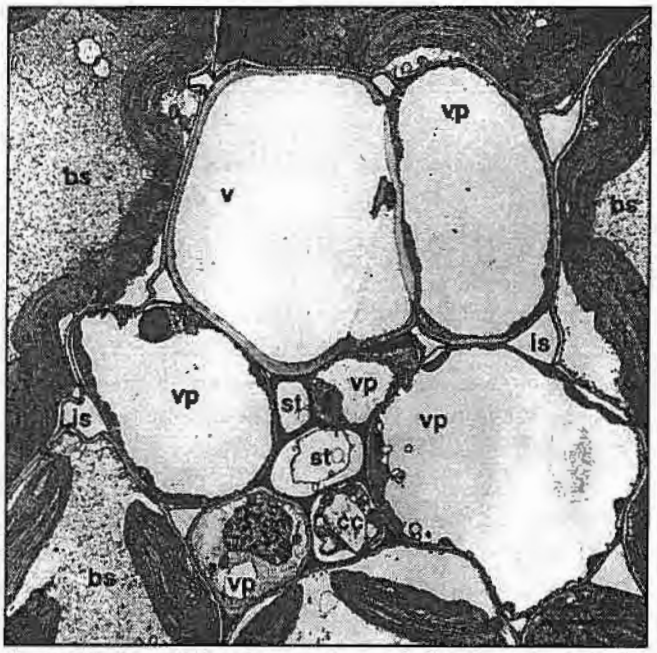


Figure 5.22 Transmission electron micrograph through a minor vein of a source leaf of maize (*Zea mays* L.). This vascular bundle consists of two sieve elements (st), one xylem vessel (v) and five vascular parenchyma cells (vp). These sieve elements are of two types, one thin walled and accompanied by a companion cell, the other thick walled and adjacent to the xylem vessel. Other symbols are: bs, bundle sheath; cc, companion cell; is, intercellular space; st, sieve tube. Bar represents 4.2 μm
(Based on Evert *et al.* 1978)

Minor veins usually comprise a single xylem element, vascular parenchyma cells and one to two sieve elements surrounded by one to four companion cells (Figure 5.22). The se–cc complex in minor veins bears similarities to that of stems (Figure 5.16). Companion cells have dense cytoplasm containing many mitochondria and are often considerably larger than the sieve elements they accompany. Companion cells are symplasmically connected to the associated sieve elements by branched plasmodesmata (Figure 5.17; Section 10.1.2).

Cross-sectional areas of veins in monocotyledonous leaves reveal large and small parallel veins (Figure 5.4). Photoassimilates are loaded into the small veins and conducted through large veins. Fine transverse veins carry photoassimilates loaded into small veins across to large veins for export.

(b) Cellular pathways — symplasmic versus apoplasmic

Photoassimilates could move intercellularly through interconnecting plasmodesmata from chloroplasts in mesophyll cells to the lumena of sieve elements (*symplasmic* phloem loading) or cross plasma membranes, travelling part of the route through the cell wall continuum (*apoplasmic* phloem loading). These fundamentally different pathways are shown schematically in Figure 5.23. Debate persists over which cellular pathway of

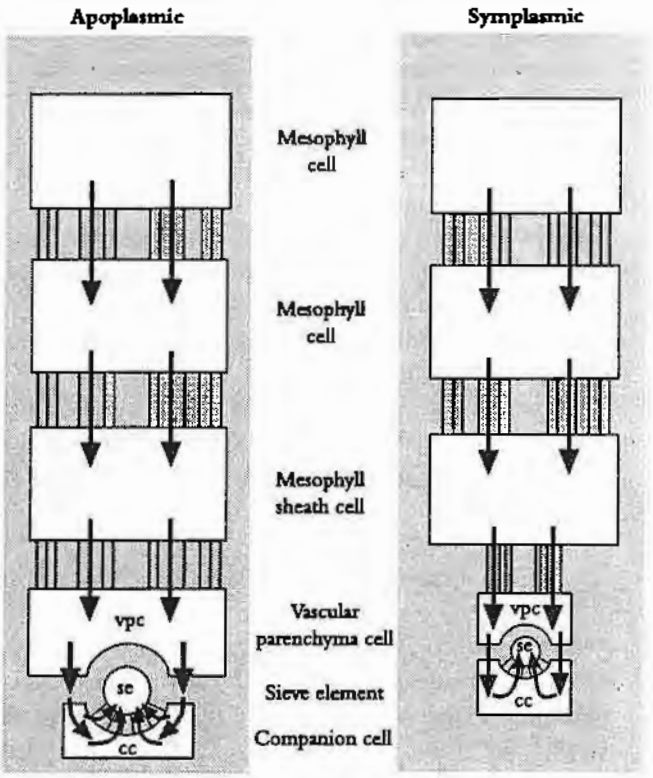


Figure 5.23 Scheme describing symplasmic and apoplasmic pathways of phloem loading. Lines without arrows joining boxes represent symplasmic continuity (i.e. plasmodesmata). Dark arrows indicate symplasmic transport; light arrows indicate apoplasmic transport. (vpc, vascular parenchyma cell; se, sieve element; cc, companion cell
(Based on van Bel 1992)

phloem loading prevails because experiments on transport from mesophyll cells to sieve elements are difficult — photoassimilates travel over short distances (50 to 500 μm) deep within leaf tissues.

Extraordinarily, the cellular pathway of phloem loading reflects evolutionary relationships. Species from ancient plant groups display symplasmic loading, while species of more modern plant groups exhibit apoplasmic phloem loading (van Bel 1993). Evidence for respective routes of loading follows.

A symplasmic pathway depends upon development of extensive plasmodesmal interconnections (Section 10.1.2) between adjoining cells, forming a cytoplasmic continuum from mesophyll to se–cc complexes (Figure 5.23). Such symplastic continuity is found in leaves of plant families containing trees and shrubs as well as cucurbits such as squash (van Bel 1993). An abundance of plasmodesma interconnections demonstrates potential for symplasmic transport but does not establish whether such transport actually occurs. Membrane-impermeant fluorescent dyes microinjected into mesophyll cells are transported to se–cc complexes, demonstrating that plasmodesmata can provide a route for photoassimilate transport. Furthermore, when leaves were fed $^{14}CO_2$ and treated with inhibitors that block sugar transport across plasma membranes, transport of ^{14}C-labelled photoassimilates continued unaffected along the enforced symplasmic unloading route (Figure 5.24; van Bel 1993).

Plant species that load phloem from the leaf apoplasm are characterised by a low abundance of plasmodesmata between se–cc complexes and abutting vascular cells. However, as for symplasmic loaders, mesophyll cells of these species are interconnected by abundant plasmodesmata (Figure 5.23). Herbaceous and many crop species belong to this group of phloem loaders, including grasses (van Bel 1993). Conventional physiological observations are consistent with phloem loading in leaves of these species including a membrane transport event located somewhere between mesophyll cells and the se–cc complexes of minor veins (Figure 5.24).

Molecular biology has brought new insights to phloem loading (Frommer *et al.* 1996). For instance, existence of an apoplasmic step demonstrated with PCMBS (Figure 5.24) has been elegantly confirmed using molecular biology to control activity of the sucrose/proton symporter responsible for sucrose uptake from phloem apoplasm into se–cc complexes. Specifically, potato plants were transformed with an antisense copy of the gene encoding the sucrose/proton symporter, producing a phenotype with low levels of the symporter in plasma membranes of se–cc complexes. Excised leaves of transformed plants exported significantly less photoassimilates than wild-type plants, corroborating the inhibitory effect of PCMBS on apoplasmic phloem loading (Figure 5.24). This provides compelling evidence that passage of photoassimilates from mesophyll cells to se–cc complexes in potato leaves includes an apoplasmic step.

Vascular parenchyma cells are the most probable site for photoassimilate exchange to phloem apoplasm (van Bel 1993), ensuring direct delivery for loading into se–cc complexes. Furthermore, plasma membranes of se–cc complexes in minor veins have increased surface areas to support photoassimilate transfer from phloem apoplasm. Notably, the surface area of se–cc complexes in sugar beet leaves is a surprisingly 0.88 cm^2 per cm^2 of leaf blade surface. By implication, these large membrane surfaces are involved in phloem loading. Further

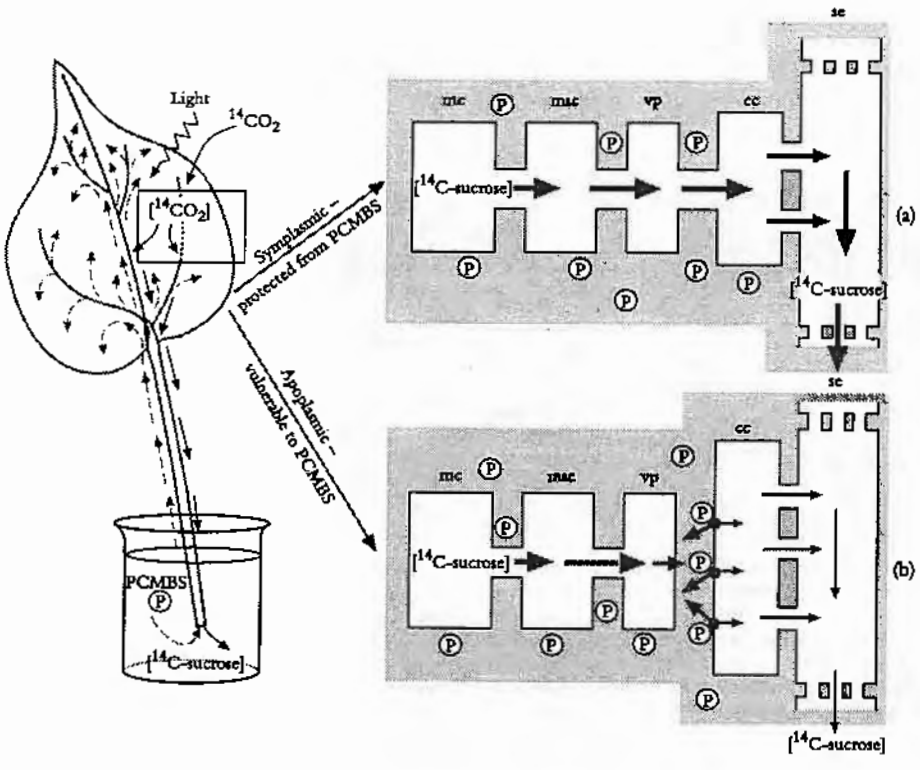


Figure 5.24 Testing whether photoassimilates move from mesophyll cells to se–cc complexes through (a) an entirely symplasmic route or (b) a route with an apoplasmic step. The approach is to use PCMBS as an inhibitor of membrane transport. PCMBS does not cross membranes but binds to the apoplasmic face of plasma membranes. Therefore, it blocks apoplasmic transport while symplasmic phloem loading is unaffected. PCMBS was introduced into the leaf apoplasm through the transpiration stream of excised leaves. Leaves were then exposed in a closed illuminated chamber to $^{14}CO_2$. The ^{14}C photoassimilate exported from labelled leaf blades was used to monitor phloem loading. PCMBS only reduced photoassimilate export (i.e. phloem loading) from those leaves with few plasmodesmata interconnecting se–cc complexes with surrounding cells. Thus, photoassimilate flow included a membrane transport step from the leaf apoplasm in certain plant species while others loaded via a symplasmic route. cc, companion cell; mc, mesophyll cell; msc, mesophyll sheath cell; PCMBS (para-chloromercuribenzenesulphonic acid, also abbreviated to p); se, sieve element; vp, vascular parenchyma
(Based on van Bel 1992)

support comes from cytochemical studies, demonstrating a great abundance of proteins associated with energy-coupled sucrose transport (Section 5.5.3(b)).

Leaf anatomies in some plant species suggest a potential for simultaneous phloem loading through apoplasmic and symplasmic pathways (van Bel 1993). Whether these pathways connect the same sieve element, different sieve elements in the same minor vein order or sieve elements in different vein orders is still unknown.

Table 5.4 Estimated sap osmolalities of various cell types in exporting leaves of Beta vulgaris *(apoplasmic phloem loader) and* Curcurbita pepo *(symplasmic phloem loader). Sap osmolalities were estimated from the molar concentration of mannitol at which 50% of each cell type plasmolysed when leaf discs were equilibrated at a range of mannitol concentrations*

Cell type	*Beta vulgaris*[a]	*Curcurbita pepo*[b]
Mesophyll	1.3	0.8
Mesophyll sheath	—	0.8
Vascular parenchyma	0.7	—
Sieve element	3.0	4.4

(After [a]Geiger *et al.* (1973); [b]Turgeon and Hepler 1989)

5.5.3 Mechanisms of phloem loading

(a) General characteristics

Any hypothesis of phloem loading must account for the following characteristics:

1. *Elevated solute concentration in se–cc complexes.* Estimated solute concentrations in sap of se–cc complexes is much higher than concentrations in sap of surrounding cell types, irrespective of whether phloem loading is by an apoplasmic or a symplasmic route (Table 5.4). These estimates of sap osmolality are derived from incipient plasmolysis of leaf tissue responsible for phloem loading.
2. *Selective loading of solutes into se–cc complexes.* Chemical analysis of phloem sap by techniques discussed in Section 5.4.3(a) reveals *relative solute concentrations* different from those in surrounding cells. Phloem loading is therefore a selective process (Section 5.4.3(c)).

(b) Symplasmic loading

These characteristics have been used to argue against loading of se–cc complexes through a symplasmic route on the grounds that plasmodesmata lack mechanisms for concentrating and selecting solutes. However, a contribution of plasmodesmata to concentrating and selecting solutes cannot be precluded from our current knowledge of plasmodesmal structure and function (Section 10.1.2).

Plants that load se–cc complexes through a symplasmic route translocate 20–80% of sugars in the form of raffinose-related compounds such as raffinose, stachyose and verbascose (Section 5.4.3(c)). Grusak *et al.* (1996) proposed a model for symplasmic phloem loading that accounts for the general characteristics stated in Section 5.5.3(a). According to this model (Figure 5.25), sucrose diffuses from mesophyll and bundle sheath cells into intermediary (companion) cells through plasmodesmata. Within companion cells, sucrose is thought to be enzymatically converted to oligosaccharides (raffinose or stachyose) maintaining a diffusion gradient for sucrose from mesophyll cells into se–cc complexes. The molecular size-exclusion limit of plasmodesmata interconnecting mesophyll and companion cells is such that it prevents back diffusion of stachyose and raffinose molecules, which are larger than sucrose. These oligosaccharides are able to diffuse through plasmodesmata with larger diameters linking companion cells with sieve elements (van Bel 1993). This model

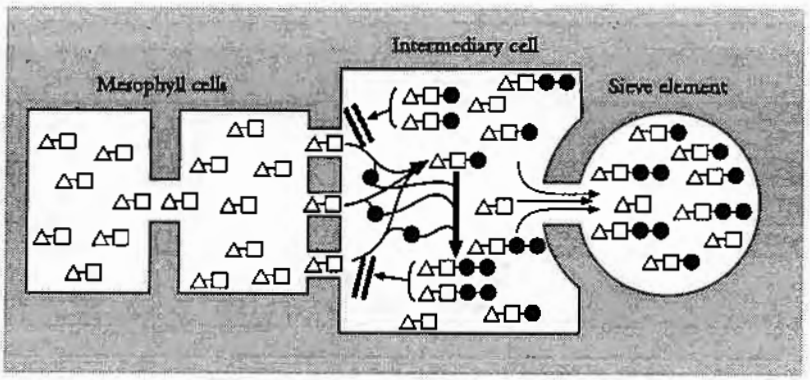


Figure 5.25 Model of the 'polymerisation trap mechanism' to explain symplasmic phloem loading against a solute concentration gradient. Sucrose moves through a symplasmic path from photosynthetic cells into intermediary (companion) cells of the minor veins. Sucrose movement is by diffusion down a concentration gradient maintained by the polymerisation of sucrose into oligosaccharides (raffinose and stachyose) in intermediary cells. Diffusion of these oligosaccharides into mesophyll cells is prevented, as their size exceeds the molecular exclusion limit of plasmodesmata joining mesophyll and intermediary cells. However, the larger-diametered plasmodesmata linking intermediary cells with sieve elements permit oligosaccharides to be loaded into sieve elements for export from the leaf. □, glucose; Δ, fructose; ●, galactinol
(After Grusak *et al.* 1996)

accounts for selective loading of sugars to achieve high photoassimilate concentrations in phloem elements.

(c) Apoplasmic loading

Phloem loading with an apoplasmic step is an attractive model, explaining both how solutes become concentrated in se–cc complexes (energy-coupled membrane transport) and how they could be selected by specific membrane transporters (see van Bel 1993). Identifying transport mechanisms responsible for photoassimilate transport to and from the leaf apoplasm has proved challenging.

Based on estimates of sucrose fluxes and high sucrose concentrations in phloem sap, there is little doubt that sucrose loading into phloem is energy dependent. The demonstration that PCMBS blocks loading of photoassimilates in whole leaves of certain species (Section 5.5.2(b)) points to carrier-mediated transport across plasma membranes. Genes encoding sucrose porters have been cloned from leaf tissue (Frommer *et al.* 1996) and shown to be specifically expressed in leaf phloem. Complementation studies in yeast defective in sucrose transport suggest that the phloem-located sucrose porter catalyses sucrose/proton symport in a similar was to that illustrated in Figure 5.32. Antisense transformants of potato with low abundance of this symporter have impaired sucrose transport (Section 5.5.2(b)).

In contrast to photoassimilate *uptake* from phloem apoplasm, very little is known about the mechanism of sugar efflux into the apoplasm. Estimates of photoassimilate flux to phloem apoplasm, based on rates of sucrose export from leaves, suggest that this transport event must be facilitated by other transport processes (van Bel 1993). The precise mechanism of this membrane step remains unknown.

5.5.4 Regulation of phloem loading

Phloem loading forms an interface between photoassimilate production by leaves and photoassimilate use by importing sink regions. Rates of phloem loading are likely to be regulated by both source and sink processes to ensure efficient matching of photoassimilate production with demand. Understanding control of phloem loading requires information on location of source photoassimilate pools within leaves.

(a) Cellular locations of photoassimilate destined for export

Reduced carbon from photosynthesis is not necessarily immediately available for export from source leaves. Photoassimilate flow through carbohydrate pools can be followed by exposing a source leaf to a two-minute pulse of $^{14}CO_2$ and monitoring ^{14}C activity remaining in the leaf over time

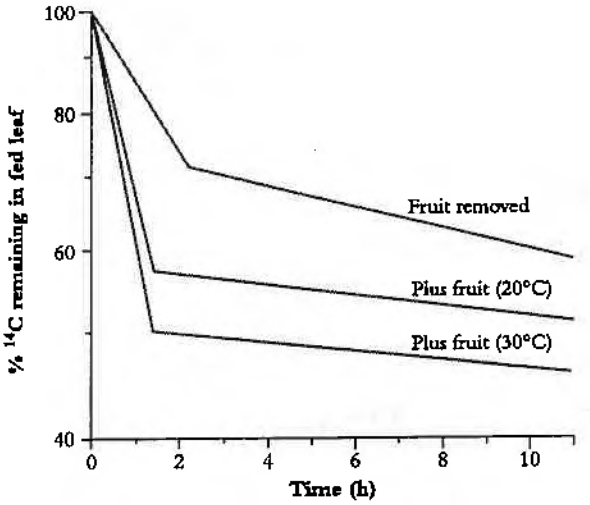


Figure 5.26 Time-course of photoassimilate export from source leaves of tomato plants. Control plants, in which fruits were a major sink for photoassimilates, were maintained at 20°C. Treatments involved (1) removing fruit or (2) exposing plants with fruits to 30°C. The proportion of ^{14}C label remaining in source leaves after a radioactive pulse was monitored through time to show that (1) presence of major sinks or (2) more rapid metabolism accelerated ^{14}C export from source leaves
(Based on Moorby and Jarman 1975)

(Figure 5.26). Typically, the initial rapid loss of ^{14}C gives way to a slower phase of loss after about two hours. Taking into account ^{14}C losses through leaf respiration and differences in photoassimilate pool sizes (Wardlaw 1990), kinetics of ^{14}C loss have been used to interpret turnover of photoassimilate pools in source leaves. In general, rapid loss of ^{14}C is considered to reflect loading of the phloem from a labile transport pool. Loss from the more slowly exchanging pool (after two hours) is interpreted as remobilisation of ^{14}C photoassimilates from starch within the chloroplasts or from sucrose stored in vacuoles of the mesophyll cells.

As irradiance decreases towards sunset and limits photosynthesis, current photoassimilate production becomes inadequate to maintain translocation. Under these conditions, photoassimilate stored in starch and/or as vacuolar sucrose can be mobilised and exported (Figure 5.26). The rate of export at night is generally much slower than during daylight hours.

(b) Source regulation

Under conditions of source limitation, increases in net photosynthetic rate can lead to proportionate changes in photoassimilate export from leaves (Figure 5.27). Increased flow of triose phosphates from chloroplasts induces feed-forward upregulation of sucrose biosynthesis with a consequent amplification of the sucrose transport pool. The relationship breaks down when net photosynthesis is low and reserves are mobilised or when net photosynthesis suddenly exceeds the levels to which the leaf is acclimated. An upper limit to photoassimilate export appears to be set by activity of sucrose phosphate synthetase (SPS), an enzyme which determines the size of the sucrose transport pool (Figure 5.28). The activity of SPS

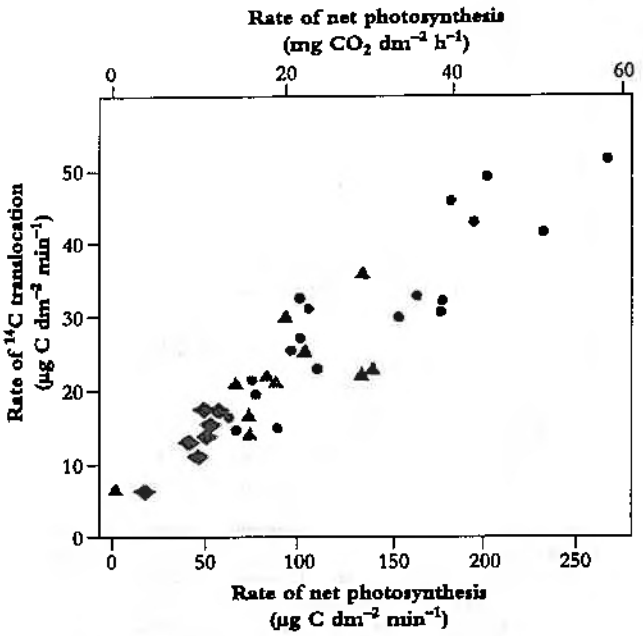


Figure 5.27 Effect of varying net photosynthesis on rates of photoassimilate translocation in sugar beet plants. Photosynthetic rate was varied by exposing leaves to three different light levels and ambient CO_2 concentrations (hence different symbols). After a $^{14}CO_2$ pulse, simultaneous estimates of export (translocation) were made by determining (1) arrival rate of ^{14}C label in sink tissues and (2) export of ^{14}C from source leaves
(Based on Servaites and Geiger 1974)

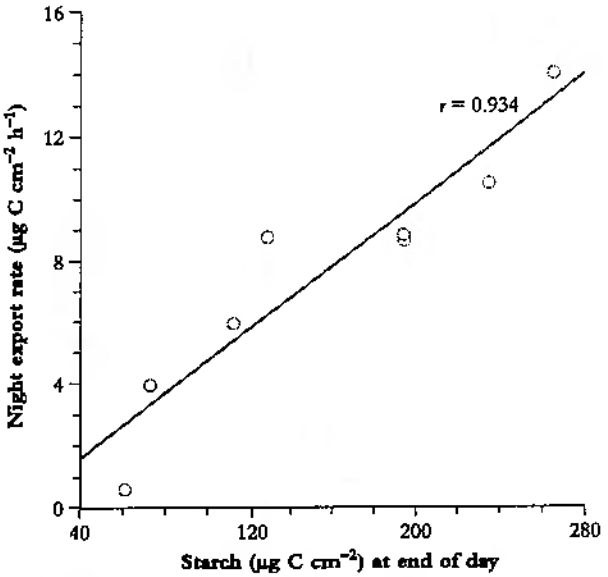


Figure 5.29 Relationship between cotton leaf starch content at the end of a light period and subsequent export rates in the dark. Starch was measured at the end of the light period. To measure export, leaves were exposed to atmospheres containing $^{14}CO_2$ throughout the light period. Therefore, when the dark period began, all photoassimilate pools were labelled to a known specific activity so carbon export would relate closely to ^{14}C export. Discs were simply cut from leaf margins and ^{14}C measured to estimate carbon export at night
(Based on Hendrix and Grange 1991)

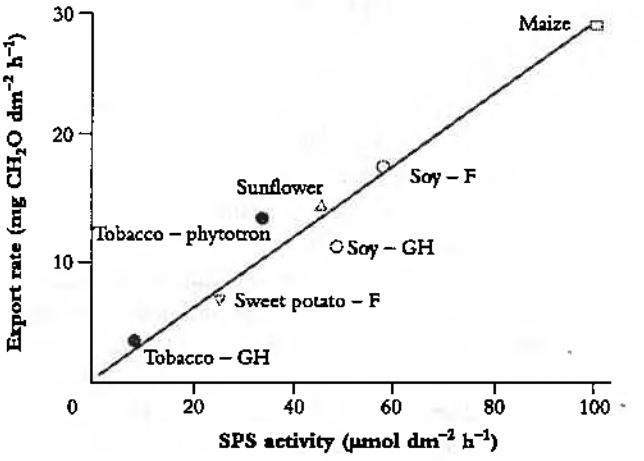


Figure 5.28 A strong relationship exists between photoassimilate export rate and activity of sucrose phosphate synthase (SPS) in fully expanded leaves of several species. Export rates from leaves was measured over 4 h during the middle of the light period. Leaves were then harvested for *in vitro* analysis of the activity of SPS. F, field-grown plants; GH, glasshouse-grown plants; phytotron, plants grown under controlled climates
(Based on Huber *et al.* 1985)

has been shown to be under sink (Section 5.5.5) as well as environmental control. For instance, daylength has a profound effect on partitioning of photoassimilate between sucrose and starch. Under short-day conditions starch accumulation increases up to five-fold and SPS activity declines. Diversion into starch during the day enables maintenance of photoassimilate export during the long night period. Consistent with this remobilisation, the rate of export is a function of starch levels in leaves at the end of day (Figure 5.29).

5.5.5 Sink regulation

(a) Sink effects on export

The response of photoassimilate export to changes in sink demand depends upon whether photoassimilate flow is source or sink limited (Wardlaw 1990). A source-limited system does not respond rapidly to an increase in sink demand, depending more on the capacity of leaves to increase the size of the transport pool. In contrast, alterations in sink demand in a sink-limited system elicit immediate effects on photoassimilate export. Figure 5.26 shows how the presence of fruits accelerates ^{14}C export, especially at high temperatures. For leaves that load the se–cc complexes from apoplasmic pools, changes in sink demand probably influence photoassimilate export by altering membrane transport properties. These changes in membrane transport entrain a flow of adjustments in biochemical partitioning within the leaf through substrate feedback (see below).

(b) Sink effects on membrane transport

Changes in the turgor pressure of phloem sap or altered phytohormone levels could serve as signals for sink demand.

Changes in the pressure of sink phloem sap are rapidly transmitted through sieve tubes to sources. Phloem loading in source tissues responds to this pressure signal by changes in solute transport rates mediated by membrane-associated porters (van Bel 1993). This is a proposed mechanism for phloem loading which would respond rapidly (within minutes) to changes in sink demand.

Phytohormone levels in leaves respond to changes in the source/sink ratio. For instance, gibberellin levels in leaves proximal to developing inflorescences increase at fruit set. In contrast, abscisic acid levels in soybean and grape leaves are inversely related to alterations in sink demand (Brenner 1987). Therefore, changes in leaf phytohormone levels could serve to signal shifts in sink demand for photoassimilates. In this context, direct application of auxin and gibberellic acid to source leaves results in a rapid enhancement of photoassimilate export (Table 5.5). Gibberellic acid did not stimulate leaf photosynthesis or alter photoassimilate partitioning, appearing instead to upregulate phloem loading. This was confirmed by faster ^{14}C loading into isolated phloem strands (Table 5.5).

Table 5.5 Effect of the phytohormones, gibberellic acid (GA3) and indole-3-acetic acid (IAA) on rates of photoassimilate export and phloem loading of sucrose. Leaves of celery were pre-treated for 60 minutes with 10^{-6} M GA_3 or IAA. Subsequent rates of ^{14}C export from intact leaves or in vitro ^{14}C-sucrose uptake by phloem strands isolated from treated leaves were determined. Sucrose concentration in the bathing medium was 5 mol m^{-3}

Hormone addition	^{14}C export	[^{14}C]-sucrose influx (μmol g^{-1} fresh wt h^{-1})
None	13	2.2
GA3	22	3.0
IAA	27	4.1

(After Daie *et al.* 1986)

(c) Sink influences on biochemical partitioning within source leaves

A substrate feedback response is elicited if the rate of photoassimilate export from chloroplasts is limited by sink demand. If sucrose export from source pools is accelerated by phloem loading, substrate feedback inhibition of photoassimilate delivery is alleviated. A cascade of adjustments in the activity of key regulatory enzymes follows (see Section 2.5) with the final outcome an increased flow of sucrose into transport pools. Conversely, if photoassimilate flow is limited by photosynthetic rate, the activity of enzymes responsible for sucrose biosynthesis is not subject to feedback inhibition by substrates. As a consequence, responses to increased sink demand can only be mediated by increases in photosynthetic enzyme activity.

5.6 Phloem unloading and sink utilisation

5.6.1 Introduction

Photoassimilate removal from phloem and delivery to recipient sink cells (phloem unloading) is the final step in photoassimilate transport from source to sink. Within sink cells, cellular metabolism and compartmentation are the end-users of phloem-imported photoassimilates. Combined activities of these sink-located transport and transfer events determine the pattern of photoassimilate partitioning between competing sinks and hence contribute to crop yield (Sections 5.3 and 5.4).

Phloem unloading describes transport events responsible for assimilate movement from se–cc complexes to recipient sink cells. A distinction must be made between transport across the se–cc complex boundary and subsequent movement to recipient sink cells. The former transport event is termed *sieve-element unloading* and the latter, *post-sieve-element transport*. On reaching the cytoplasm of recipient sink cells, imported photoassimilates can enter metabolic pathways or be compartmented into organelles (e.g. amyloplasts, protein bodies and vacuoles). Metabolic fates for photoassimilates include catabolism in respiratory pathways, biosynthesis (maintenance and growth) and storage as macromolecules (starch and fructans).

Compared with phloem loading, phloem unloading and subsequent sink utilisation of imported photoassimilates operate within a much broader range of configurations:

1. *morphological* (e.g. apices, stems, roots, vegetative storage organs, reproductive structures);
2. *anatomical* (e.g. provascular differentiating sieve elements, protophloem sieve elements lacking companion cells, metaphloem se–cc complexes);
3. *developmental* (e.g. cell division, cell expansion);
4. *metabolic* (e.g. storage of soluble compounds/polymers, growth sinks).

A correspondingly large range of strategies for phloem uuloading and sink utilisation must be anticipated.

5.6.2 Cellular pathways of phloem unloading

Most photoassimilates travel along one of three cellular pathways: apoplasmic, symplasmic or a combination of both with symplasmic transport interrupted by an apoplasmic step (Figure 5.30).

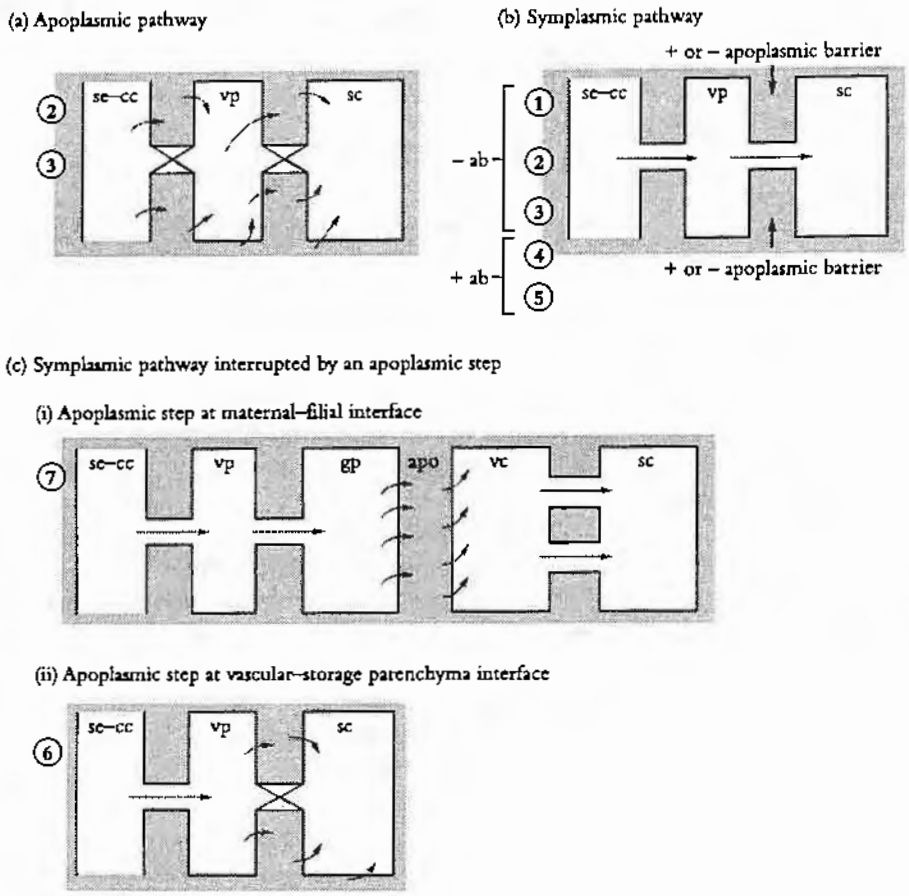


Figure 5.30 Scheme describing the cellular pathways of phloem unloading and their relationship with sink types. (a) Apoplasmic unloading showing direct transport of photoassimilates from se–cc complexes to the phloem apoplasm. (b) Symplasmic unloading pathway which may or may not have an apoplasmic barrier between sieve elements and recipient sink cells. (c) Symplasmic unloading with the intervention of an apoplasmic step at (i) the phloem parenchyma–ground parenchyma boundary and (ii) the maternal–filial interface of developing seeds. Circled numbers denote different sink types assigned to each pathway. 1, vegetative apex; 2, elongating axis of a dicotyledonous stem; 3, mature axis of a primary dicotyledonous stem ± permanent storage; 4, mature axis of a primary monocotyledonous stem ± permanent storage; 5, mature primary root; 6, fleshy fruit; 7, developing seed. ab, apoplasmic barrier; apo, apoplasm; gp, ground parenchyma; sc, sink cell; se–cc, sieve element–companion cell complex; vp = vascular parenchyma.

(a) Apoplasmic pathways

Photoassimilates can move directly across plasma membranes of se–cc complexes to the surrounding apoplasm (Figure 5.30a). Apoplasmic unloading is important along the axial transport pathway of roots and stems where vascular parenchyma and ground tissues serve as reversible storage sinks.

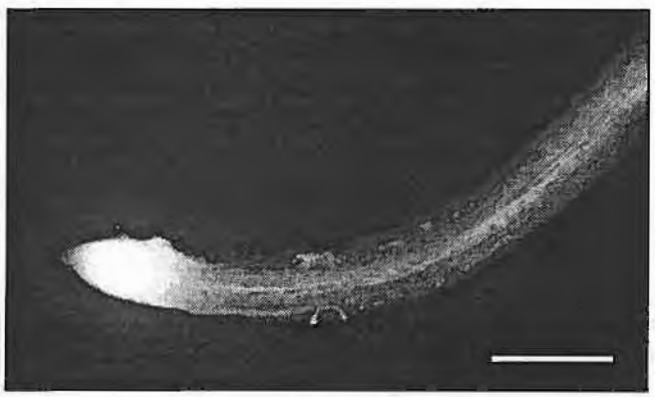


Figure 5.31 Fluorescent micrograph of the distribution of a membrane impermeant fluorescent dye, carboxyfluorescein (CF), imported through the phloem into roots of French bean (*Phaseolus vulgaris* L.). The green/yellow fluorescence of CF is confined to the se–cc complexes of mature portions of roots as seen by the thin central band of fluorescence away from the root apex. In contrast, dye spreads through the apex apparently via the symplasm of young cells. Bar represents 2 mm

(b) Symplasmic pathways

An entirely symplasmic path of photoassimilate transport from sieve elements to recipient sink cells (Figure 5.30b) operates in a wide range of morphological and metabolic sink types. Terminal growth sinks such as root (Figure 5.31) and shoot apices, as well as vegetative storage sinks such as stems, roots and potato tubers, demonstrate symplasmic unloading.

In most sinks that exhibit symplasmic unloading, photoassimilates are metabolised into polymeric forms within the recipient sink cells. Sugar cane is a notable exception because it stores sucrose unloaded symplasmically from sieve elements in parenchyma cells of stems. Stem sucrose reaches molar concentrations by this unloading route (Section 5.6.3(b)).

(c) Symplasmic pathway interrupted by an apoplasmic step

Symplasmic discontinuities exist at interfaces between tissues of differing genomes including biotrophic associations (e.g. mycorrhizas and misdetoes) and developing seeds (Figure 5.30c). In addition, within tissues of the same genome, plasmodesmata can close permanently or reversibly at points along the post-sieve-element pathway. This necessitates photoassimilate exchange between symplasmic and apoplasmic compartments (Figure 5.30c). For instance, photoassimilate exchange between apoplasm and symplasm has been detected

in sinks that store high solute concentrations and have unrestricted apoplasmic transport between vascular and storage tissues. Developing seeds, particularly of cereals and large-seeded grain legumes (Patrick and Offler 1995), are another model for symplasmic/apoplasmic pathways.

The apoplasmic space between maternal (seed coat) and filial (embryo plus endosperm) tissues in seeds prevents symplasmic continuity in the unloading pathway (Patrick and Offler 1995). In these organs, photoassimilates are effluxed across membranes of maternal tissues and subsequently taken up across the membranes of filial tissues (Figure 5.30c). Photoassimilates are unloaded from sieve elements and transported symplasmically to effluxing cells where they are released to the seed apoplasm. Influx from the seed apoplasm by the filial generation is restricted to specialised cells located at the maternal–filial interface. The final transport of photoassimilates to the filial storage cells largely follows a symplasmic route.

(d) Pathway linkage with sink function and pathway switching

The symplasm is the most frequently engaged cellular pathway of phloem unloading. Even where an apoplasmic step intervenes (e.g. developing seeds), photoassimilates travel predominantly through the sink symplasm (Figure 5.30c). Symplastic routes do not involve membrane transport and therefore offer lower resistances than apoplasmic routes.

Apoplasmic pathways are restricted to circumstances where (1) symplasmic transport compromises phloem translocation and (2) photoassimilate transport is between genetically distinct (e.g. maternal–filial) tissues. Phloem translocation would be compromised when solutes accumulate to high concentrations in sink cells were it not avoided by symplasmic isolation of phloem from sinks. This is exemplified by the switch to an apoplasmic step during development of tomato fruit. In young fruit, imported sugars are interconverted into polymeric forms to support cell division and excess photoassimilate is accumulated as starch. At this stage, phloem unloading of photoassimilates follows a symplasmic route (Figure 5.30b). However, once sugars commence accumulating during cell expansion, apoplasmic transport is engaged (Figure 5.30c). The apoplasmic path isolates pressure-driven phloem import from rising osmotic pressures (P) occurring in fruit storage parenchyma cells (Patrick and Offler 1996).

Radial photoassimilate unloading in mature roots and stems may switch between apoplasmic or symplasmic routes depending upon the prevailing source/sink ratio of the plant. At low source/sink ratios, photoassimilates remobilised from axial stores are loaded into the phloem for transport to growth sinks (Wardlaw 1990). Under these conditions, symplasmic unloading into axial stores might be blocked by plasmodesmal closure while photoassimilates are absorbed by se–cc complexes from the surrounding apoplasm. This would prevent futile unloading while stores are drawn upon. In contrast, net flow of photoassimilates into axial storage pools at high source/sink ratios would be facilitated by plasmodesma opening.

5.6.3 Mechanisms of phloem unloading

(a) Apoplasmic transport

Se–cc complexes contain high sugar concentrations (Section 5.4.3(b)). Thus, a considerable transmembrane concentration gradient exists to drive a passive leakage of sugars to phloem apoplasm. Sugars leaked to phloem apoplasm are often

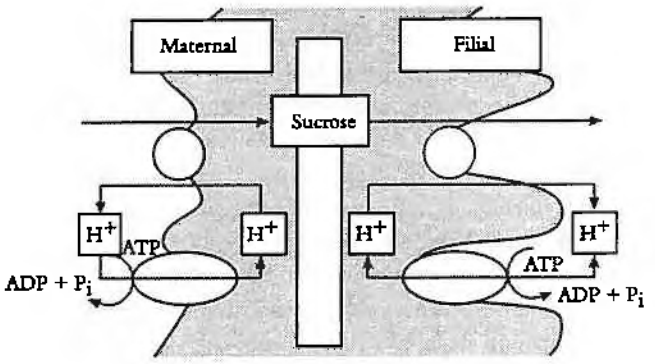


Figure 5.32 Mechanistic model for plasma membrane transport of sucrose from the coat and into the cotyledons of a developing legume seed. Plasma membrane ATPases vectorially pump protons to the seed apoplasm from both the opposing seed coat and cotyledon cells. The proton gradient is coupled to drive sucrose efflux from the seed coats through a sucrose/proton antiporter and sucrose influx into the cotyledons by a sucrose/proton symporter

retrieved by an active sucrose/proton symport mechanism (Figure 5.32). Thus, net efflux of sugars from se–cc complexes is determined by the balance between a passive leakage and sucrose/proton retrieval.

Passive *unloading* (E_p) of sucrose from se–cc complexes to the phloem apoplasm (Equation 5.6) is determined by the permeability coefficient (P) of se–cc complex plasma membranes and the transmembrane sucrose concentration (C) gradient between sieve element lumena (*se*) and surrounding phloem apoplasm (*apo*).

$$E_p = P(C_{se} - C_{apo}) \qquad (5.6)$$

Sinks containing extracellular invertase (e.g. developing tomato fruit, sugar beet tap roots, maize seeds) can hydrolyse sucrose, lowering C_{apo} thereby enhancing sucrose unloading from se–cc complexes. Furthermore, hydrolysis of sucrose renders it unavailable for se–cc complex retrieval by sucrose/proton symport. However, there is no direct experimental evidence showing that alterations in extracellular invertase activity influence unloading from the se–cc complexes. Many sinks in which phloem unloading follows an apoplasmic path

do not exhibit detectable extracellular invertase activity (e.g. mature stems and roots).

(b) Symplasmic transport

Symplasmic transport is mediated by cytoplasmic streaming in series with intercellular transport via plasmodesmata. Plasmodesma transport is usually the overriding resistance determining transport rates between cells.

Root tips offer a useful experimental model to explore post-sieve-element symplasmic transport because of morphological simplicity and accessibility. Exposing pea root tips to *low* sucrose concentrations (< 100 mM) slowed photoassimilate accumulation (Figure 5.33a) by raising intracellular sucrose concentrations. This response to concentration gradients is consistent with a diffusion component to phloem unloading (Equation 5.7). When roots were bathed in much higher concentrations of either sucrose (Figure 5.33a) or a slowly permeating solute, mannitol (Figure 5.33b), turgor pressure (P) of sieve elements and surrounding tissues decreased and ^{14}C import rose. This is consistent with a hydraulically driven (bulk) flow of photoassimilates into the root apex. Thus, photoassimilate movement from phloem through a symplasmic path can be mediated by diffusion and/or bulk flow. The relative contribution of each transport mechanism depends on the magnitude of concentration and pressure gradients (Equations 5.6 and 5.8).

Physical laws can be used to model diffusion and bulk flow of sucrose through a symplasmic route. Sucrose *diffuses* through symplasm at a rate (R_d) defined by the product of plasmodesmal number in the path (n), plasmodesmal conductivity to diffusion (K_d) and sucrose concentration difference (ΔC) between sieve elements and sink cell cytoplasm. That is:

$$R_d = n.K_d.\Delta C \qquad (5.7)$$

Transport by *bulk flow* (R_f) is determined by the product of flow speed (S), cross-sectional area of the plasmodesma flow path (A) and concentration (C) of sucrose transported (Equation 5.2). Flow speed (S), in turn, is a product of hydraulic conductivity (L_p) of a plasmodesma and turgor pressure difference (ΔP) between se–cc complexes and recipient sink cells (Equation 5.8). Flow over the entire pathway considers the number of interconnecting plasmodesmata (n). Thus, bulk flow rate (R_f) is given by:

$$R_f = n.L_p.\Delta P.A.C \qquad (5.8)$$

Equations 5.7 and 5.8 predict that sink control of symplasmic photoassimilate transport resides in plasmodesma conductivity and/or sucrose metabolism/compartmentation.

Sucrose metabolism within sink cells influences cytoplasmic sucrose concentration and Π_{sink}. The difference between

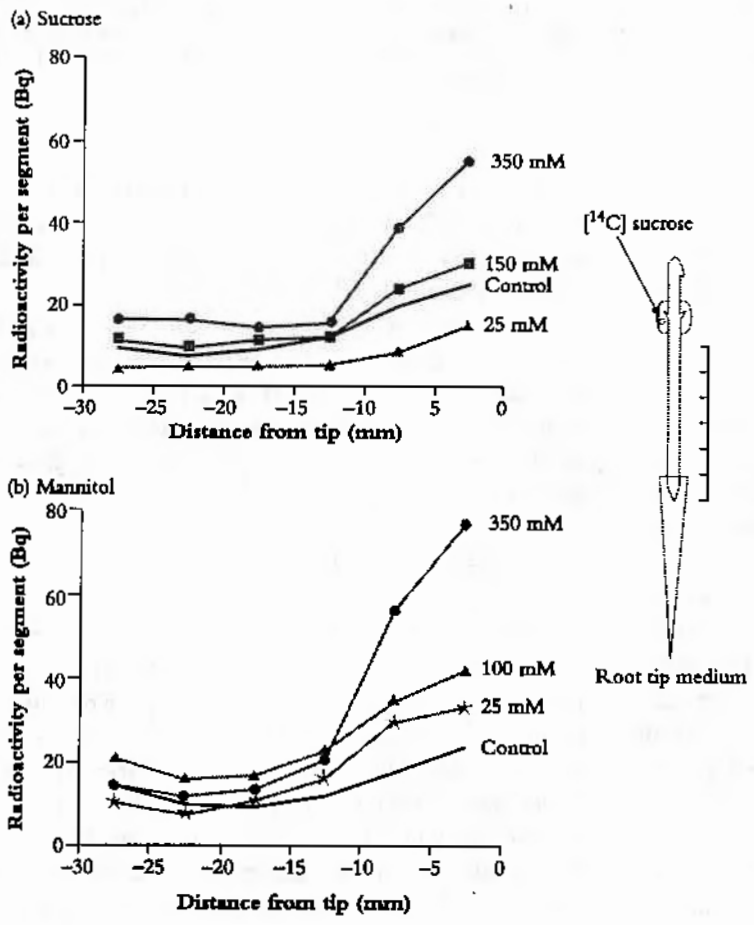


Figure 5.33 Externally supplied solutes have a marked effect on sucrose import into root tips of hydroponically grown pea seedlings. This was tested by immersing root tips in (a) sucrose or (b) mannitol solutions ranging up to 350 mM. Cotyledons, which supply these young roots with carbon, were fed ^{14}C sucrose and ^{14}C arriving in different root parts was measured (Bq per root segment). (a) Import of ^{14}C into root tips was diminished when they were exposed to external sucrose concentrations of less than 100 mol m^{-3} but promoted by sucrose concentrations of 150 to 350 mM. Two effects operate. At low concentrations, sucrose might enter root tip cells and suppress phloem import by a feedback mechanism. At higher concentrations, sucrose might act mainly as an osmoticum (see (b)). Mannitol is not taken up or metabolised quickly and can therefore help answer these questions. (b) Import through the phloem was stimulated by exposing root tips to the slowly permeable sugar mannitol, at concentrations of from 13 to 350 mol m^{-3}. This demonstrates an osmotic dependence of import through the phloem pathway, presumably through progressively decreasing P of root tip cells as external solute concentrations rise
(Based on Schultz 1994)

Π_{sink} and Π_{apo} determines P (Section 4.3). Sucrose metabolism and compartmentation can affect sucrose concentration gradients and ΔP, both driving forces for symplasmic transport from se–cc complexes to sink cells (Equations 5.6 and 5.8).

Transgenic plants which under- or overexpress key sugar metabolising enzymes have allowed definitive experiments to be carried out on the role of sucrose metabolism in symplasmic phloem unloading. A similar approach to phloem loading is discussed in Section 5.5.3(b). For example, reduction of sucrose synthase activity (Section 5.6.4) in tubers of transformed potato to 5–30% of wild-type levels depressed dry weight of tubers and starch biosynthesis (Table 5.6). Tubers of transformed plants had very high hexose levels (hence high Π) which might contribute to down regulation of photoassimilate import. As a corollary, plants with enhanced starch biosynthesis through overexpression of the key starch synthesising enzyme, ADP-glucose pyrrophosphorylase (Section 5.6.4), also had higher rates of photoassimilate import.

For sinks that store sugars to high concentrations (e.g. sugar cane stems), gradients in Π, and hence Π, between se–cc complexes and sink storage cells could become too small to sustain transport. Instead, Π in the apoplasm of storage tissues increases as sucrose (hence Π) in the storage cell sap rises. This maintains a lower P in storage cells than sieve elements and sustains transport. High sucrose concentrations in the apoplasm of storage cells is achieved through an apoplasmic barrier which isolates storage parenchyma cells from sieve elements (Figure 5.34).

Table 5.6 Effect of a decrease in the activity of sucrose synthase in potato plants on final tuber dry weight and sugar content. Sucrose synthase activity was depressed selectively in tubers of transgenic potato plants by transformation with an antisense tuber-specific construct of sucrose synthase. These data are for one transgenic plant that exhibited the lowest activities of sucrose synthase (i.e. 4% of the control wild-type activity). Growth and sucrose synthase activities were unaffected in shoots of transgenic plants

Parameter measured	Control (wild type	Transgenic tuber
Dry weight (g)	4.8	2.4
% tuber dry weight composed of:		
Starch	90	30
Sucrose	1.5	2.5
Hexoses	0.1	9.3

(After Zrenner *et al.* 1995)

(c) Symplasmic transport interrupted by an apoplasmic step

Phloem unloading in legume seed pods is one case of symplasmic and apoplasmic transport operating in series: the pathway is described in Section 5.6.2(c). Whether sucrose efflux requires energy remains unknown since concentration gradients between seed coats and apoplasm might be steep enough to drive facilitated diffusion. Indeed, using an elegant infusion technique (Figure 5.35a), Wang and Fisher (1994) concluded that efflux from the nucellar projection cells of wheat grain was unlikely to be energy dependent. In contrast, sucrose efflux from coats (maternal tissue) of surgically modified legume seeds (Figure 5.35a) is inhibited by about 50% in the presence of PCMBS, a membrane transport inhibitor. Efflux from legume seed coat cells exhibits characteristics of a sucrose/proton antiport (Figure 5.32). Sucrose uptake by filial tissues is mediated by sucrose/proton symport (Figure 5.32).

A fascinating aspect of phloem unloading in legume seed pods is how photoassimilate demand by filial tissues is integrated with supply from maternal tissues, itself an integration of photoassimilate efflux and import from phloem. One variable that could regulate rates of photoassimilate transport through seed coat symplasm and efflux into apoplasm of the maternal–filial interface is *P of seed coat cells* (P_{sc}): this would sense depletion of apoplasmic sucrose through uptake by cotyledons, producing a signal in the form of a ΔP_{sc} (Figure 5.36). Specifically, P_{sc} is determined by DP between

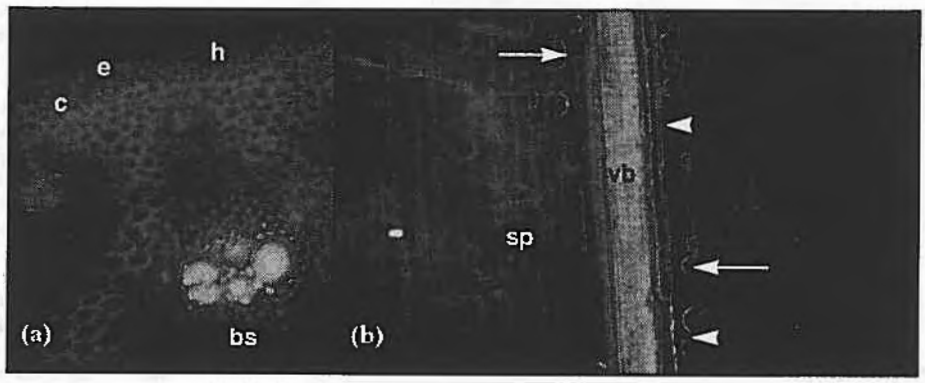


Figure 5.34 Cellular distribution of the apoplasmic tracer fluorescent dye, 3-hydroxy-5,8,10-pyrenetrisulphonate (PTS) imported through the xylem in stem explants of sugar cane. (a) Fluorescent micrograph of a longitudinal section of a stem with PTS (green fluorescence) localised to the vascular bundle. (b) Fluorescent micrograph of a transverse section showing PTS confined to the vascular bundle. Retention of PTS in vascular bundles demonstrates that a barrier to lateral dye movement must be located in the walls of bundle sheath cells. Cell types are: c, chlorenchyma; e, epidermis; h, hypodermis; sp, storage parenchyma; vb, vascular bundle (see Colour Plate xx)
(Based on Jacobsen *et al.* 1992)

the seed coat (Π_{sc}) and seed apoplasm (Π_{apo}), which fluctuates according to photoassimilate withdrawal by cotyledons.

A pressure difference (ΔP) between the points of photoassimilate arrival (sieve tubes) and efflux (seed coats) drives bulk flow of photoassimilates through the seed coat symplasm. Turgor pressure of seed coat efflux cells is maintained homeostatically at a set point (P_{set}) by *P-dependent efflux* into the seed apoplasm. Changes in apoplasmic assimilate concentrations and hence Π are sensed immediately as deviations of P_{sc} from P_{set}. A rise in P_{sc} produced by photoassimilate depletion around filial tissues elicits an error signal, activating *P*-dependent solute efflux (Figure 5.36b) and thereby raising photoassimilate concentrations in the apoplasm to meet demand by cotyledons (Figure 5.36c). Long-term increases of sucrose influx by cotyledons, for example over hours, are accompanied by adjustments in P_{set} (light to dark arrows in Figure 5.36b) which elicit commensurate increases in phloem import rates (light to dark arrows in Figure 5.36a).

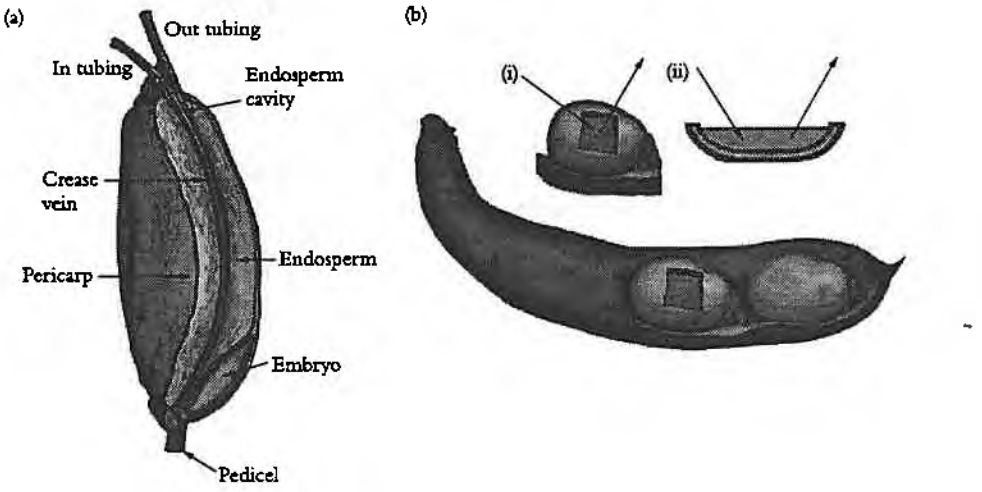


Figure 5.35 Experimental systems used to determine sucrose fluxes in (a) attached caryopses of wheat and (b) coats of developing legume seed. In (a), sucrose effluxed from the maternal tissues was collected by infusing the endosperm cavity of an attached wheat grain with solutions delivered and retrieved through microcapillaries (Wang and Fisher 1994). In (b), embryos are surgically removed from the coats which may be (i) attached to or (ii) detached from the pod wall. The space vacated by the embryo is filled with a wash solution that is changed at frequent intervals. The wash solution is used to deliver treatments to the seed coat and as a trap to collect the effluxed sucrose

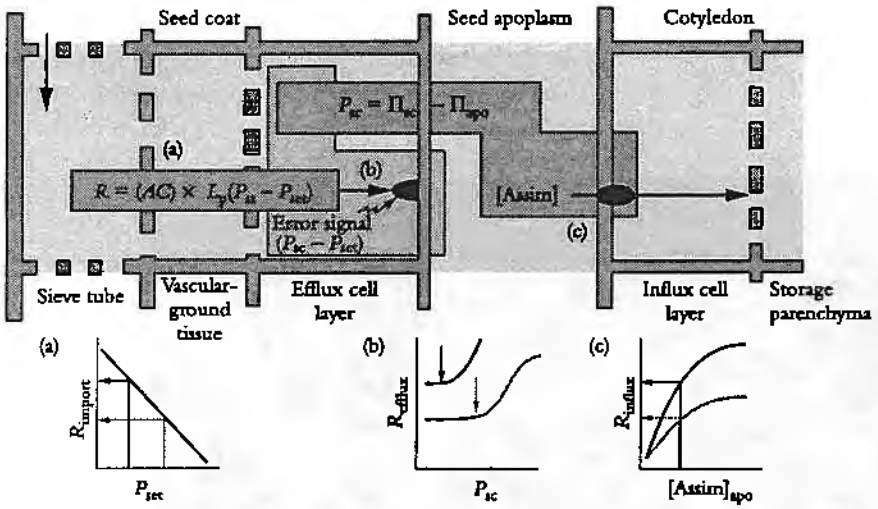


Figure 5.36 A turgor-homeostat model describing the integration of photoassimilate transport to developing legume seeds. Photoassimilate import through phloem (a) and efflux from seed coat to seed apoplasm (b) is mediated by a turgor (*P*)-dependent efflux mechanism and uptake of sugars by cotyledons (c). Metabolic activity in growing seeds influences sucrose concentrations within cotyledon cells, possibly feeding back on activities of symporters located in plasma membranes of the cotyledon dermal cell complex. Graph (c) denotes increased influx (*R*) from light to dark curve as sugar demand increases. The apoplasmic solute pool size is small and turns over in less than one hour (Patrick and Offler 1995). Thus, faster assimilate withdrawal from the seed apoplasm by cotyledons will rapidly lower apoplasmic osmotic concentration. Since the osmotic difference between seed apoplasm (Π_{apo}) and seed coat (Π_{sc}) is only 0.1–0.2 MPa, a small decrease in osmolality of the apoplasm will elicit a significant increase in seed coat *P* (P_{sc}). A shift in Psc above the turgor set point (P_{set}) results in an error signal (see model) which in turn induces an immediate compensatory increase in assimilate efflux to the apoplasm (light curve in graph b). Increased assimilate efflux acts to maintain a constant Π_{apo} in spite of enhanced *flux* through the apoplasm (graph c). Consequently, the increased potential for assimilate uptake by cotyledons can be fully realised (dark curve in graph c). In the short term (minutes), the turgor-homeostat ensures that P_{sc} is maintained and hence phloem import, which is driven by the turgor difference between sieve tubes and unloading cells (P_{st}–P_{sc}) is also maintained. Under conditions where cotyledon demand is *sustained*, P_{set} in the seed coat adjusts downwardr. This results from decreases in Π_{sc}, while Π_{apo} is homeostatically maintained. The decrease in P_{sc} of efflux cells serves to enhance the pressure difference between these cells and the importing sieve elements. As a result, the rate of phloem import into seed coats ($R_{transport}$) is increased (graph a, light to dark curve). This new rate of import is commensurate with accelerated sucrose efflux from seed coats to the apoplasm (graph b, light to dark curve) and, ultimately, cotyledons.

5.6.4 Sugar metabolism/ compartmentation in sinks

The fate of imported photoassimilates depends on sink cell function. In broad terms, imported photoassimilates are primarily used to provide carbon skeletons for *growth* or *storage*. Some photoassimilates provide energy for *maintenance*. Relative flows of photoassimilates to these fates change during cell development and sometimes over shorter time scales depending upon a plant's physiological state.

(a) Cell maintenance

Irrespective of sink function, a portion of imported sugars is respired to provide energy for maintenance of cell function and structure. Most of this energy is required for continual turnover of cellular constituents such as enzymes and membranes. Rates of synthesis and degradation of individual macromolecules vary widely, as does the energy invested in different molecular configurations, so sugar demand for maintenance respiration could differ substantially between tissues.

(b) Cell growth

In growing organs, photoassimilates become substrates for synthesis of new cell material either directly or after biochemical transformations. Other fates for sugars include catabolism in energy-generating pathways which support growth (growth respiration) and storage in vacuolar pools. Stored sugars make an osmotic contribution to growing cells and can act as energy stores in species such as sugar cane. In roots of young barley plants, 40% and 55% of imported sugars are respired and used in structural growth, respectively. Stored sugars turn over each 30 min but account for only 1% of root weight.

(c) Reserve storage in cells

In mature cells, imported sugars enter physical (e.g. vacuoles) and chemical (e.g. starch) storage pools with lesser amounts diverted to respiration (15–20%) and structural components. In contrast to growth sinks, stored carbohydrates are ultimately retrieved from storage pools and used by other storage sinks (e.g. germinating seeds) or translocated to support growth and storage processes elsewhere in the plant. Carbohydrate storage can be brief (hours, days) or extend over considerable periods (months to years). Short-term storage of carbohydrates in stems and roots buffers phloem sap sugar concentrations against changes in photoassimilate export from photosynthetic leaves.

Sugars can also be stored in soluble forms by compartmentation into vacuoles. In this case, the tonoplast provides a physical barrier to protect stored sugars from molecular interconversion by cytoplasmic sugar-metabolising enzymes. Vacuolar sugars are accumulated as sucrose, hexoses or fructans (short-chain polymers of fructose). Sucrose and hexoses can accumulate to molar concentrations (0.1–1.5 M) in storage parenchyma cells of roots, stems and fruits. For instance, tap roots of sugar beet and stems of sugar cane accumulate 1 M sucrose thereby providing 90% of the world's sucrose. Hexoses are a common form of sugar storage in fruit, contributing to sweetness of edible fruits such as tomato, grape, orange and cucumber. The wine industry depends upon hexoses accumulating to high concentrations (1.5 M) in grape berries to fuel fermentation of 'must' in wine making. Fructans are stored in significant quantities in leaf sheaths and stems of temperate grasses and cereals. In pasture species, they contribute to forage quality, and in cereals constitute an assimilate pool that is mobilised to support grain filling.

Alternatively, imported sugars may be stored as starch along the axial transport pathway (available for remobilisation to buffer phloem sap sugar concentrations) or in more long term storage pools of terminal sink organs such as tubers, fruits and seeds. The proportion of photoassimilates diverted into starch differs widely between species and organs. Starch accounts for some 90% of dry weight of potato tubers and cereal grains.

The chemistry of storage products can change during organ development. For instance, starch is the principal storage carbohydrate in young tomato fruit. Later in fruit development, stored starch is hydrolysed and contributes to hexose accumulation in vacuoles of fruit storage parenchyma cells. In other fruits, significant switches between hexose and sucrose accumulation occur during development. All these changes are brought about by ontogenetic shifts in activities of sugar-metabolising enzymes.

5.6.5 Key transfer events in sugar metabolism and compartmentation

Phloem-imported sucrose can reach the cytoplasm of recipient sink cells chemically unaltered or be hydrolysed en route by extracellular invertase into its hexose moieties. These sugars may then enter a number of metabolic pathways or be compartmented to vacuolar storage (Figure 5.37).

(a) Hexose metabolism

Hexoses transported to the sink cytoplasm are rapidly phosphorylated to hexose-6-phosphate esters by glucose- and fructose-specific kinases. In these forms, hexoses can be used as substrates for respiration or for synthesis of new cell constituents. Alternatively, sucrose phosphate synthetase can convert them to sucrose, as in leaves (Chapter 2). Sucrose synthesised by this reaction can be accumulated in vacuoles (e.g. sugar beet tap roots, sugar cane stems) or be rehydrolysed into hexoses by a vacuolar acid invertase (e.g. grape berries).

(b) Sucrose metabolism

Sucrose is metabolically inert and, in order to be metabolised, must be hydrolysed to glucose and fructose. Only two

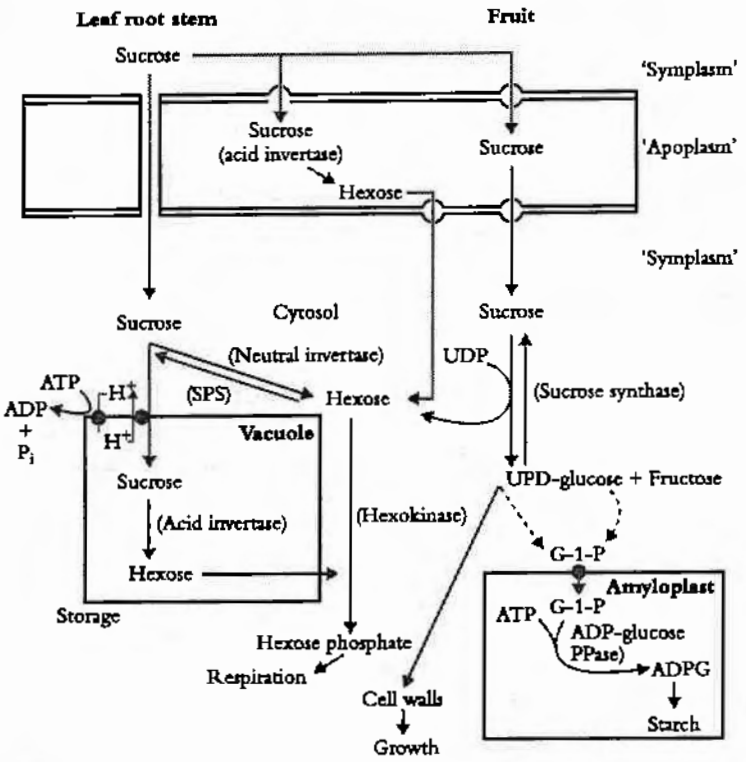


Figure 5.37 Pathways of sugar metabolism and compartmentation within sink cells. Sugars can be delivered to sink cells through either apoplasmic or symplasmic pathways. Within the sink apoplasm, sucrose can be hydrolysed to hexoses by an extracellular invertase. Apoplasmic sugars are transported across plasma membranes of sink cells by proton/sugar symporters. Alternatively, sucrose enters the sink cytoplasm through a symplasmic path. Within the sink cytoplasm, sucrose can be hydrolysed or compartmented into vacuolar storage. Sucrose hydrolysis provides substrates for energy metabolism or for synthesis of macromolecules. Invertase activity is important to sustain hexose supply for glycolysis. *Sucrose synthesis* from the hexose pool is catalysed by sucrose phosphate synthase (SPS). *Hydrolysis of sucrose* by sucrose synthase generates fructose (F) and uridine diphosphate glucose (UDP-glucose) which enters various biosynthetic pathways including cellulose and starch synthesis. In the case of starch biosynthesis, UDP-glucose and fructose generate glucose-1-phosphate (G-1-P) which is transported across the amyloplas membrane. Accumulated G-1-P is interconverted into adenine diphosphate glucose (ADP-glucose) by the enzyme adenine diphosphate glucose pyrophosphorylase (ADP-glucose PPase). ADP-glucose is the substrate for starch polymer formation. Sucrose compartmentation into the vacuole is mediated by a sucrose/proton antiporter located on the tonoplast. Within the vacuole, sucrose can be exposed to invertase hydrolysis with the hexose products accumulating or leaking back to the cytoplasm.

enzymes are capable of metabolising sucrose in green plants. These are invertase and sucrose synthase (Section 2.4.1; Figure 5.37) and they are paramount in sugar metabolism after phloem unloading.

Invertase catalyses irreversible hydrolysis of sucrose to its hexose moieties, glucose and fructose. Both acid and neutral invertases occur in plants, with pH optima of about 5 and 7.5, respectively (Section 2.4.1). The activity of invertases varies with plant species, organ type and stage of development. Acid invertases are usually active in rapidly growing leaves and stems (Wardlaw 1990), making hexoses available for respiration and biosynthesis. Invertases from vacuoles hydrolyse sucrose, producing hexoses which accumulate in vacuoles or enter the cytoplasm. Reduced acid invertase activity in vacuoles during development of sugar cane stems, and its absence from sucrose-accumulating tomato fruit, is a major factor in sucrose accumulation in vacuoles of these tissues. Less is known about the physiological role of neutral invertases which might play a part in sucrose metabolism of mature tissues such as storage parenchyma of sugar cane stems.

Sucrose synthase is exclusively located in the cytoplasm and catalyses sucrose hydrolysis to fructose and UDP-glucose, a high-energy ester of glucose. UDP-glucose is a substrate for synthetic pathways, notably cellulose biosynthesis.

High activities of sucrose synthase are found in both growing and starch storage tissues. In the cytoplasm of starchy tissues, UDP-glucose is converted by UDP-glucose pyrophosphorylase to glucose-1-phosphate, which is transported across amyloplast membranes. In amyloplasts, glucose-1-phosphate provides glucose moieties for starch synthesis in a pathway comparable to starch formation in chloroplasts of photosynthetic leaves. The critical role of sucrose synthase in starch synthesis is demonstrated graphically with potatoes transformed with an antisense construct of the gene encoding tuber-specific sucrose synthase. Tuber sucrose synthase activity in transformed plants was depressed significantly while the

activities of key starch biosynthetic enzymes were unaltered. Low sucrose synthase activity was directly responsible for a proportional decrease in starch accumulation (Zrenner *et al.* 1995).

Sinks that accumulate soluble sugars have predictably low sucrose synthase activities. Contrastingly high sucose synthase activities in phloem vessels that deliver sucrose to these sinks are still to be explained.

5.6.6 Sink control of photoassimilate partitioning

The pressure-flow hypothesis (Section 5.4.5(a)) provides a compelling model to explain sink strength in plants. Evidence such as accelerated import of photoassimilate into roots with artificially lowered P lends empirical support to the model (Figure 5.33).

Knowledge of cellular and molecular events in phloem uuloading (Section 5.6.3) and photoassimilate use (Section 5.6.4) begins to reveal the array of control steps which underlie photoassimilate unloading and relative sink strength. Specifically, photoassimilate import into sinks by apoplasmic pathways (Figure 5.30a) or by diffusion through symplasmic pathways (Figure 5.30) is controlled by P in se–cc complexes. In contrast, when phloem unloading is by bulk flow through a symplasmic route (e.g. legume seed coats), P in cells responsible for photoassimilate efflux to the apoplasm controls unloading. Unloading into storage tissues is controlled by P in sink (storage) cells.

These processes at the cell and tissue level must now be related to a whole-plant perspective of sink control of photoassimilate partitioning, taking into account influences of plant development and environmental factors. How plants use photoassimilates (e.g. switch from growth to storage) is accompanied by alterations in the cellular pathway of import. Phytohormones also play a role in photoassimilate partitioning through their influence on development and intercellular signalling.

Plant development impacts on sink potential of meristemic tissues characterised by active cell division as well as on storage sinks where cell enlargement predominates.

(a) Meristematic sinks

Potential sink size is set largely during the meristematic phase of development through determination of total cell number per organ. Photoassimilate supply has been implicated as a limiting factor in initiation of leaf primordia at the apical dome and subsequent early development by cell division. Substrate supply for developing seeds (endosperm, embryo) and root and floral apices might also be restricted.

Agricultural yields might therefore increase if plants could be modified to enhance the supply of photoassimilates to meristems. Which factors regulate photoassimilate supply to meristematic sinks? Rate equations describing mass flow of phloem sap (Section 5.4.5(a)) predict that photoassimilate supply reaching a sink will be determined by source output (setting photoassimilate concentration in sap and P in sieve elements at the source) and modulated by L_p of the transport pathway. Increased photoassimilate output from source leaves increases growth activity of primary meristems. Even during reduced source output, photoassimilate import and meristematic sink strength can be maintained by remobilisation of storage reserves. Manipulating competition for photoassimilates by more established sinks also suggests that source output influences sink behaviour.

Cultivated plants demonstrate these principles. For example, flushing CO_2 into glasshouses increases flower set and hence yield of floral and fruit crops. Similarly, applying growth regulators to induce abscission of some floral apices lessens the number of sinks competing for photoassimilates at fruit set and leads to larger and more uniform fruit at harvest. Alternatively, breeding programs have reduced sink strength of non-harvestable portions of crops and hence the severity of competition. For instance, breeding dwarf varieties of cereals has reduced photoassimilate demand by stems with a consequent increase in floret numbers set and grain size.

These observations imply that increases in net leaf photosynthesis and phloem loading should set higher yield potentials. Yet meristems import only a small proportion of total plant photoassimilate. It may be that phloem conductance limits photoassimilate delivery to meristems; increases in source output would amplify the driving force for transport and hence bulk flow through a low-conductance pathway.

Given that *mature* phloem pathways have spare transport capacity (Section 5.4.5(b)), any transport limitation imposed by low path conductance might be expected within immature sinks. Photoassimilate import into meristematic sinks involves transport through partially differentiated provascular strands that might extend up to 400 μm. Movement through this partially differentiated path is symplasmic (Section 5.6.2(b)). Hence, plasmodesmal numbers and transport properties of plasmodesmata could play a critical role in photoassimilate supplies to sinks and determination of sink size (Equation 5.7).

(b) Expansion/storage sinks

As cells expand and approach cell maturity, photoassimilates are increasingly diverted into storage products. Towards maturity, fully differentiated phloem pathways with spare transport capacity link expansion/storage sinks with photosynthetic leaves. Photoassimilate import by these sinks depends on duration of the storage phase. This can be short term for sinks located along the axial transport pathway and long term for sinks sited at the ends of transport pathways (e.g. tubers, fruits and seeds).

Storage along the axial pathway occurs mainly when photoassimilate production exceeds photoassimilate demands by terminal sinks. However, storage is not necessarily a passive

response to excess photoassimilate supply. Stems of sugar cane store large quantities of photoassimilates (50% of dry weight is sucrose) even during rapid growth of terminal sinks. Photoassimilates might be stored as simple sugars (e.g. sugar cane stems) or as polymers (fructans in stems of temperate grasses; starch in stems and roots of subtropical cereals, herbaceous annuals and woody perennials). Photoassimilates stored along axial pathways buffer against diurnal and more long term fluctuations in photoassimilate supply to terminal sinks. In woody deciduous species, axially stored photoassimilates also provide a long-term seasonal storage pool that is drawn on to support bud growth following bud-burst. Remobilised photoassimilates can contribute substantially to biomass gain of terminal sinks. For instance, in some mature trees, over half the photoassimilates for new growth come from remobilised reserves; similar proportions of stem-stored fructans contribute to grain growth in cereals when photoassimilate production is reduced (e.g. by drought). Physiological switching between net storage and remobilisation is an intriguing regulatory question.

Growth and development of meristems is determined by phloem unloading events and metabolic interconversion of photoassimilates within recipient sink cells. These transport and transfer processes vary between sinks and can alter during sink development. Techniques now exist to alter expression of membrane porter proteins and possibly enhance photoassimilate import by sinks such as seeds which have an apoplasmic step in the phloem unloading pathway. Prospects of altering plasmodesma conductivity will improve once plasmodesma proteins are isolated.

Further reading

Canny, M.J. (1995). 'Apoplastic water and solute movement: new rules for an old space', *Annual Reviews of Plant Physiology and Plant Molecular Biology*, **46**, 215–236.

Fisher, D.B. and Frame, J.M. (1984). 'A guide to the use of the exuding stylet technique in phloem physiology', *Planta*, **161**, 385–393.

Hall, J.L., Baker, D.A. and Oparka, K.L. (eds) (1996). 'Transport of photoassimilates', papers presented at the International Conference on the Transport of Photoassimilates, University of Kent, Canterbury, UK, 13–17 August 1995, *Journal of Experimental Botany* (Special Issue), **47**, 1119–1333.

Milburn, J.A. and Williamson, V.G. (1996). 'The loss of hydraulic conductance through vascular xylem conduits', *Recent Advances in Wood Anatomy*, 423–429.

Milburn, J.A. (1996). 'Sap ascent in plants: challenges to the cohesion theory ignore the significance of immature xylem and the recycling of Münch water', *Annals of Botany*, **78**, 399–407.

Patrick, J.W. (1997). 'Phloem unloading: sieve element unloading and post-sieve element transport', *Annual Review of Plant Physiology and Molecular Biology*, **48**, 191–222.