Chapter

10

Translocation in the Phloem

SURVIVAL ON LAND POSES SOME SERIOUS CHALLENGES to terrestrial plants, foremost of which is the need to acquire and retain water. In response to these environmental pressures, plants evolved roots and leaves. Roots anchor the plant and absorb water and nutrients; leaves absorb light and exchange gases. As plants increased in size, the roots and leaves became increasingly separated from each other in space. Thus, systems evolved for long-distance transport that allowed the shoot and the root to efficiently exchange products of absorption and assimilation.

You will recall from Chapters 4 and 6 that the xylem is the tissue that transports water and minerals from the root system to the aerial portions of the plant. The **phloem** is the tissue that translocates the products of photosynthesis from mature leaves to areas of growth and storage, including the roots. As we will see, the phloem also redistributes water and various compounds throughout the plant body. These compounds, some of which initially arrive in the mature leaves via the xylem, can be either transferred out of the leaves without modification or metabolized before redistribution.

The discussion that follows emphasizes translocation in the phloem of angiosperms because most of the research has been conducted on that group of plants. Gymnosperms will be compared briefly to angiosperms in terms of the anatomy of their conducting cells and possible differences in their mechanism of translocation. First we will examine some aspects of translocation in the phloem that have been researched extensively and are thought to be well understood. These include the pathway and patterns of translocation, materials translocated in the phloem, and rates of movement.

In the second part of the chapter we will explore aspects of translocation in the phloem that need further investigation. Some of these areas, such as phloem loading and unloading and the allocation and partitioning of photosynthetic products, are being studied intensively at present.

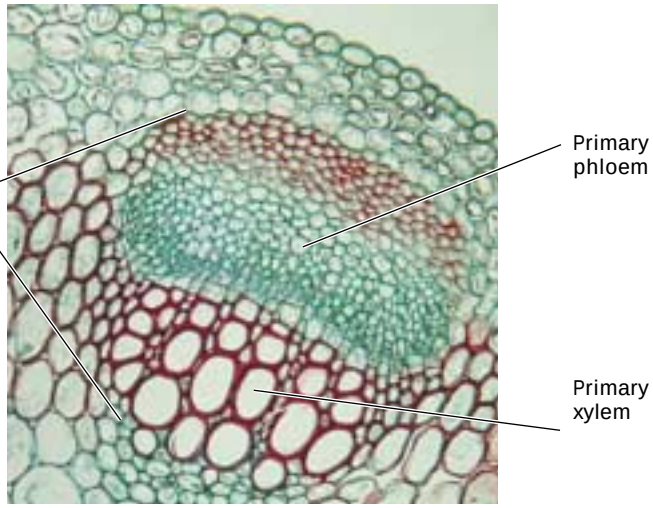


FIGURE 10.1 Transverse section of a vascular bundle of trefoil, a clover (*Trifolium*). (130×) The primary phloem is toward the outside of the stem. Both the primary phloem and the primary xylem are surrounded by a bundle sheath of thick-walled sclerenchyma cells, which isolate the vascular tissue from the ground tissue. (© J. N. A. Lott / Biological Photo Service.)

PATHWAYS OF TRANSLOCATION

The two long-distance transport pathways—the phloem and the xylem—extend throughout the plant body. The phloem is generally found on the outer side of both primary and secondary vascular tissues (Figures 10.1 and 10.2). In plants with secondary growth the phloem constitutes the inner bark.

The cells of the phloem that conduct sugars and other organic materials throughout the plant are called **sieve elements**. *Sieve element* is a comprehensive term that includes both the highly differentiated **sieve tube elements** typical of the angiosperms and the relatively unspecialized **sieve cells** of gymnosperms. In addition to sieve elements, the phloem tissue contains companion cells (discussed below) and parenchyma cells (which store and release food molecules). In some cases the phloem tissue also includes fibers and sclereids (for protection and strengthening of the tissue) and laticifers (latex-containing cells). However, only the sieve elements are directly involved in translocation.

The small veins of leaves and the primary vascular bundles of stems are often surrounded by a **bundle sheath** (see Figure 10.1), which consists of one or more layers of compactly arranged cells. (You will recall the bundle sheath cells involved in C_4 metabolism discussed in Chapter 8.) In the vascular tissue of leaves, the bundle sheath surrounds the small veins all the way to their ends, isolating the veins from the intercellular spaces of the leaf.

We will begin our discussion of translocation pathways with the experimental evidence demonstrating that the sieve elements are the conducting cells in the phloem. Then we will examine the structure and physiology of these unusual plant cells.

Sugar Is Translocated in Phloem Sieve Elements

Early experiments on phloem transport date back to the nineteenth century, indicating the importance of long-distance transport in plants (see **Web Topic 10.1**). These classical experiments demonstrated that removal of a ring of bark around the trunk of a tree, which removes the phloem, effectively stops sugar transport from the leaves to the roots without altering water transport through the xylem. When radioactive compounds became available, radiolabeled $^{14}CO_2$ was used to show that sugars made in the photosynthetic process are translocated through the phloem sieve elements (see **Web Topic 10.1**).

Mature Sieve Elements Are Living Cells Highly Specialized for Translocation

Detailed knowledge of the ultrastructure of sieve elements is critical to any discussion of the mechanism of translocation in the phloem. Mature sieve elements are unique among living plant cells (Figures 10.3 and 10.4). They lack

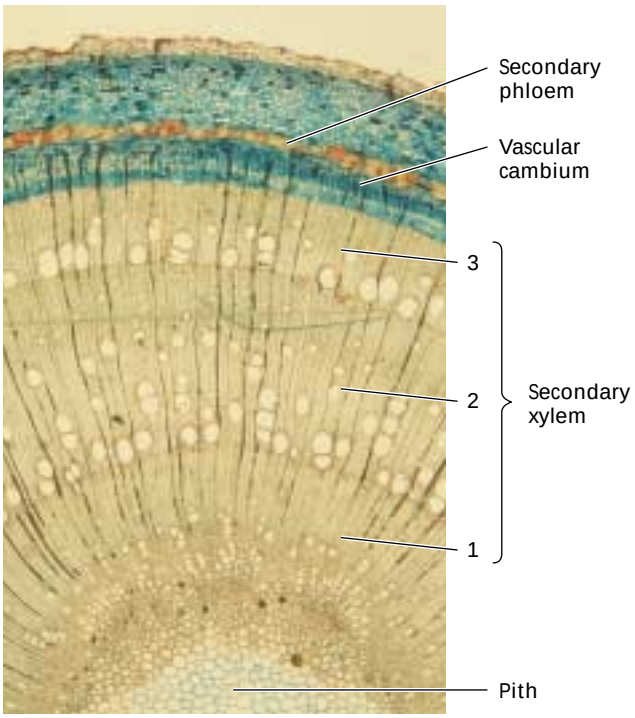


FIGURE 10.2 Transverse section of a 3-year-old stem of an ash (*Fraxinus excelsior*) tree. (27×) The numbers 1, 2, and 3 indicate growth rings in the secondary xylem. The old secondary phloem has been crushed by expansion of the xylem. Only the most recent (innermost) layer of secondary phloem is functional. (© P. Gates / Biological Photo Service.)

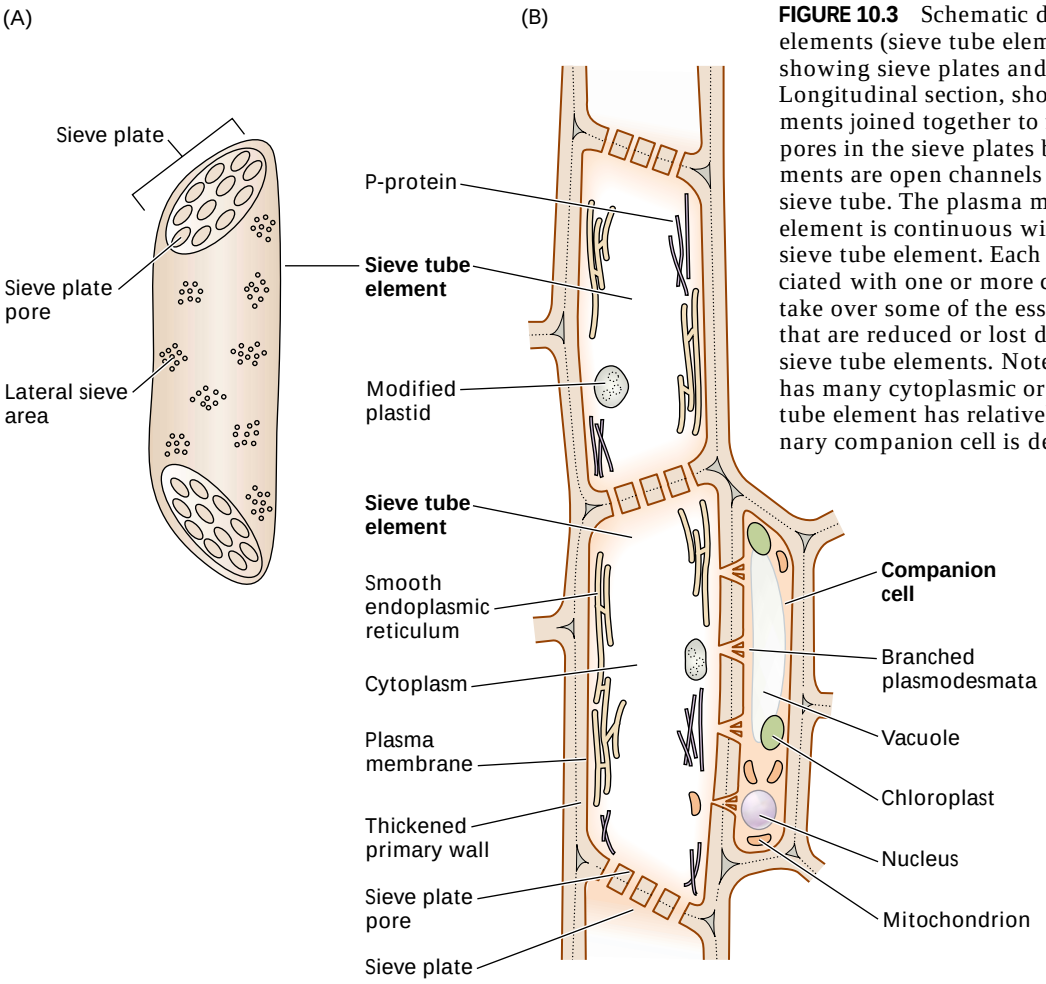


FIGURE 10.3 Schematic drawings of mature sieve elements (sieve tube elements). (A) External view, showing sieve plates and lateral sieve areas. (B) Longitudinal section, showing two sieve tube elements joined together to form a sieve tube. The pores in the sieve plates between the sieve tube elements are open channels for transport through the sieve tube. The plasma membrane of a sieve tube element is continuous with that of its neighboring sieve tube element. Each sieve tube element is associated with one or more companion cells, which take over some of the essential metabolic functions that are reduced or lost during differentiation of the sieve tube elements. Note that the companion cell has many cytoplasmic organelles, whereas the sieve tube element has relatively few organelles. An ordinary companion cell is depicted here.

many structures normally found in living cells, even the undifferentiated cells from which mature sieve elements are formed. For example, sieve elements lose their nuclei and tonoplasts (vacuolar membrane) during development. Microfilaments, microtubules, Golgi bodies, and ribosomes are also absent from the mature cells. In addition to the plasma membrane, organelles that are retained include somewhat modified mitochondria, plastids, and smooth endoplasmic reticulum. The walls are nonlignified, though they are secondarily thickened in some cases.

Thus the sieve elements have a cellular structure different from that of tracheary elements of the xylem (which are dead at maturity), lack a plasma membrane, and have lignified secondary walls. As we will see, living cells are critical to the mechanism of translocation in the phloem.

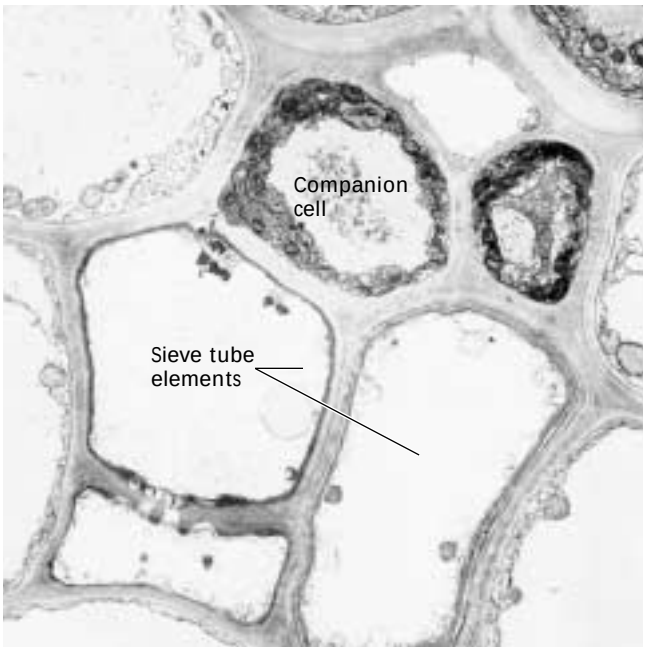


FIGURE 10.4 Electron micrograph of a transverse section of ordinary companion cells and mature sieve tube elements. (3600×) The cellular components are distributed along the walls of the sieve tube elements. (From Warmbrodt 1985.)

Sieve Areas Are the Prominent Feature of Sieve Elements

Sieve elements (sieve cells and sieve tube elements) have characteristic sieve areas in their cell walls, where pores interconnect the conducting cells (see Figure 10.5). The sieve area pores range in diameter from less than 1 µm to approximately 15 µm. Unlike sieve areas of gymnosperms, the sieve areas of angiosperms can differentiate into **sieve plates** (see Figure 10.5 and Table 10.1).

Sieve plates have larger pores than the other sieve areas in the cell and are generally found on the end walls of sieve tube elements, where the individual cells are joined together to form a longitudinal series called a **sieve tube** (see Figure 10.3). Furthermore, the sieve plate pores of sieve tube elements are open channels that allow transport between cells (see Figure 10.5).

In contrast, all of the sieve areas are more or less the same in gymnosperms such as conifers. The pores of gymnosperm sieve areas meet in large median cavities in the middle of the wall. Smooth endoplasmic reticulum (SER) covers the sieve areas (Figure 10.6) and is continuous through the sieve pores and median cavity, as indicated by ER-specific staining. Observation of living material with confocal laser scanning microscopy confirms that the observed distribution of SER is not an artifact of fixation (Schulz 1992).

TABLE 10.1
Characteristics of the two types of sieve elements in seed plants

Sieve tube elements found in angiosperms
1. Some sieve areas are differentiated into sieve plates; individual sieve tube elements are joined together into a sieve tube.
2. Sieve plate pores are open channels.
3. P-protein is present in all dicots and many monocots.
4. Companion cells are sources of ATP and perhaps other compounds and, in some species, are transfer cells or intermediary cells.
Sieve cells found in gymnosperms
1. There are no sieve plates; all sieve areas are similar.
2. Pores in sieve areas appear blocked with membranes
3. There is no P-protein.
4. Albuminous cells sometimes function as companion cells.

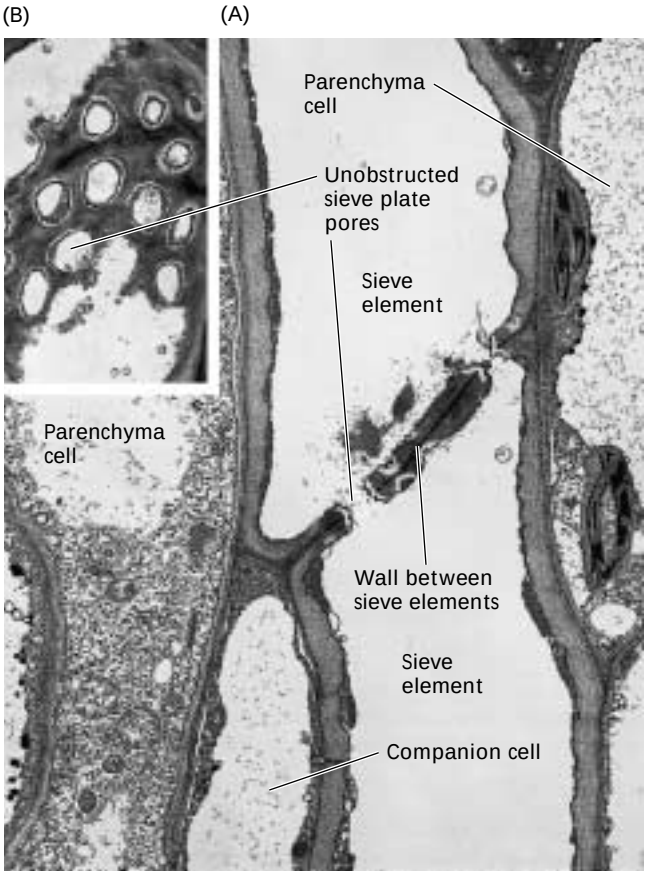


FIGURE 10.5 Sieve elements and open sieve plate pores. (A) Electron micrograph of a longitudinal section of two mature sieve elements (sieve tube elements), showing the wall between the sieve elements (called a sieve plate) in the hypocotyl of winter squash (*Cucurbita maxima*). (3685×) (B) The inset shows sieve plate pores in face view (4280×). In both images A and B, the sieve plate pores are open—that is, unobstructed by P-protein. (From Evert 1982.)

Deposition of P-Protein and Callose Seals Off Damaged Sieve Elements

The sieve tube elements of most angiosperms are rich in a phloem protein called **P-protein** (see Figure 10.3B) (Clark et al. 1997). (In classical literature, P-protein was called *slime*.) P-protein is found in all dicots and in many monocots, and it is absent in gymnosperms. It occurs in several different forms (tubular, fibrillar, granular, and crystalline) depending on the species and maturity of the cell.

In immature cells, P-protein is most evident as discrete bodies in the cytosol known as **P-protein bodies**. P-protein bodies may be spheroidal, spindle-shaped, or twisted and coiled. They generally disperse into tubular or fibrillar forms during cell maturation.

P-proteins have been characterized at the molecular level. For example, P-proteins from the genus *Cucurbita* consist of two major proteins: PP1, the phloem filament protein, and PP2, the phloem lectin. The gene that encodes PP1 in pumpkin (*Cucurbita maxima*) has sequence similarity to genes encoding cysteine proteinase inhibitors, suggesting a possible role in defense against phloem-feeding insects. Both PP1 and PP2 are thought to be synthesized in companion cells (discussed in the next section) and transported via the plasmodesmata to the sieve elements, where they associate to form P-protein filaments and P-protein bodies (Clark et al. 1997).

P-protein appears to function in sealing off damaged sieve elements by plugging up the sieve plate pores. Sieve tubes are under very high internal turgor pressure, and the sieve elements in a sieve tube are connected through open sieve plate pores. When a sieve tube is cut or punctured,

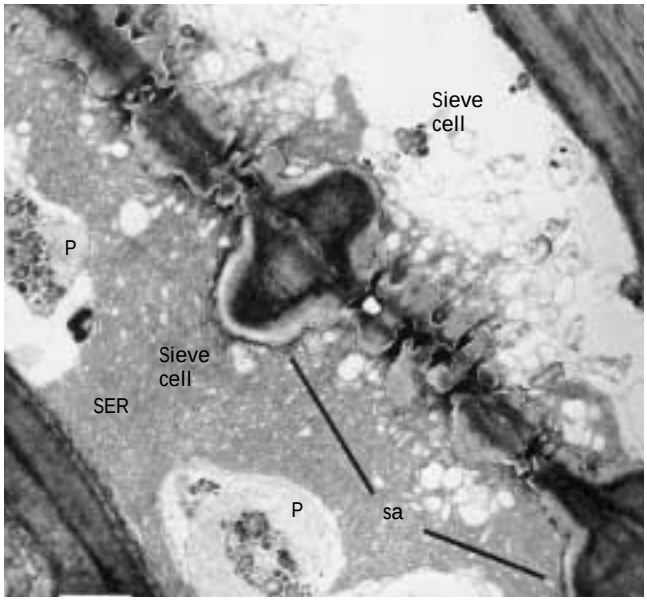


FIGURE 10.6 Electron micrograph showing a sieve area (sa) linking two sieve cells of a conifer (*Pinus resinosa*). Smooth endoplasmic reticulum (SER) covers the sieve area on both sides and is also found within the pores and the extended median cavity. Plastids (P) are enclosed by the SER. (From Schulz 1990.)

the release of pressure causes the contents of the sieve elements to surge toward the cut end, from which the plant could lose much sugar-rich phloem sap if there were no sealing mechanism. (*Sap* is a general term used to refer to the fluid contents of plant cells.) When surging occurs, however, P-protein and other cellular inclusions are trapped on the sieve plate pores, helping to seal the sieve element and to prevent further loss of sap.

A longer-term solution to sieve tube damage is the production of **callose** in the sieve pores. Callose, a β-1,3-glucan, is synthesized by an enzyme in the plasma membrane and is deposited between the plasma membrane and the cell wall. Callose is synthesized in functioning sieve elements in response to damage and other stresses, such as mechanical stimulation and high temperatures, or in preparation for normal developmental events, such as dormancy. The deposition of **wound callose** in the sieve pores efficiently seals off damaged sieve elements from surrounding intact tissue. As the sieve elements recover from damage, the callose disappears from these pores.

Companion Cells Aid the Highly Specialized Sieve Elements

Each sieve tube element is associated with one or more **companion cells** (see Figures 10.3B, 10.4, and 10.5). The division of a single mother cell forms the sieve tube element and the companion cell. Numerous plasmodesmata (see Chapter 1) penetrate the walls between sieve tube elements and their companion cells, suggesting a close functional relationship and a ready exchange of solutes between the two cells. The plasmodesmata are often complex and branched on the companion cell side.

Companion cells play a role in the transport of photosynthetic products from producing cells in mature leaves to the sieve elements in the minor (small) veins of the leaf. They are also thought to take over some of the critical metabolic functions, such as protein synthesis, that are reduced or lost during differentiation of the sieve elements (Bostwick et al. 1992). In addition, the numerous mitochondria in companion cells may supply energy as ATP to the sieve elements.

There are at least three different types of companion cells in the minor veins of mature, exporting leaves: "ordinary" companion cells, transfer cells, and intermediary cells. All three cell types have dense cytoplasm and abundant mitochondria.

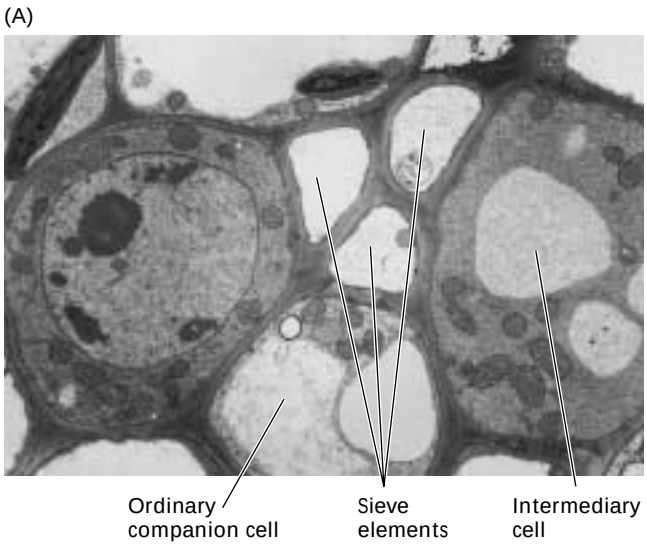
Ordinary companion cells (Figure 10.7A) have chloroplasts with well-developed thylakoids and a cell wall with a smooth inner surface. Of most significance, relatively few plasmodesmata connect this type of companion cell to any of the surrounding cells except its own sieve element. As a result, the symplast of the sieve element and its companion cell is relatively, if not entirely, symplastically isolated from that of surrounding cells.

Transfer cells are similar to ordinary companion cells, except for the development of fingerlike wall ingrowths, particularly on the cell walls that face away from the sieve element (Figure 10.7B). These wall ingrowths greatly increase the surface area of the plasma membrane, thus increasing the potential for solute transfer across the membrane.

Because of the scarcity of cytoplasmic connections to surrounding cells and the wall ingrowths in transfer cells, the ordinary companion cell and the transfer cell are thought to be specialized for taking up solutes from the apoplast or cell wall space. Xylem parenchyma cells can also be modified as transfer cells, probably serving to retrieve and reroute solutes moving in the xylem, which is also part of the apoplast.

Though ordinary companion cells and transfer cells are relatively isolated symplastically from surrounding cells, there are some plasmodesmata in the walls of these cells. The function of these plasmodesmata is not known. The fact that they are present indicates that they must have a function, and an important one, since the cost of having them is high: They are the avenues by which viruses become systemic in the plant. They are, however, difficult to study because they are so inaccessible.

Intermediary cells appear well suited for taking up solutes via cytoplasmic connections (Figure 10.7C). Intermediary cells have numerous plasmodesmata connecting them to surrounding cells, particularly to the bundle sheath cells. Although the presence of many plasmodesmatal connections to surrounding cells is their most characteristic feature, intermediary cells are also distinctive in having



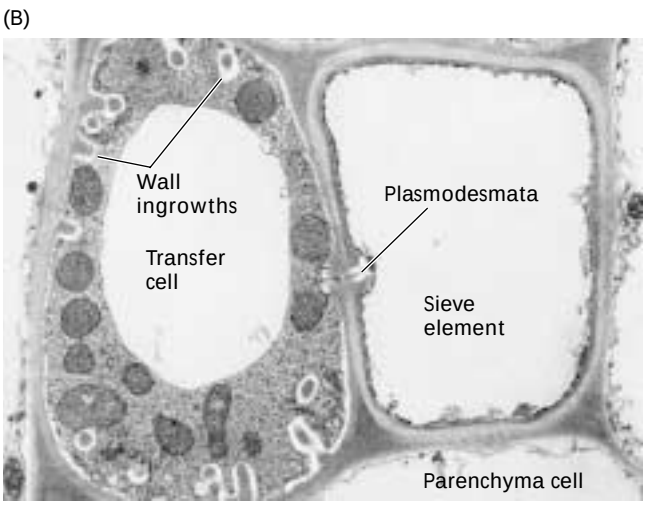


FIGURE 10.7 Electron micrographs of companion cells in minor veins of mature leaves. (A) Three sieve elements abut two intermediary cells and a more lightly stained ordinary companion cell in a minor vein from *Mimulus cardinalis*. (6585×) (B) A sieve element adjacent to a transfer cell with numerous wall ingrowths in pea (*Pisum sativum*). (8020×) Such ingrowths greatly increase the surface area of the transfer cell's plasma membrane, thus increasing the transfer of materials from the mesophyll to the sieve elements. (C) A typical intermediary cell with numerous fields of plasmodesmata (arrows) connecting it to neighboring bundle sheath cells. These plasmodesmata are branched on both sides, but the branches are longer and narrower on the intermediary cell side. Minor-vein phloem was taken from heartleaf maskflower (*Alonsoa warscewiczii*). (4700×) (A and C from Turgeon et al. 1993, courtesy of R. Turgeon; B from Brentwood 1978.)

numerous small vacuoles, as well as poorly developed thylakoids and a lack of starch grains in the chloroplasts.

In general, ordinary companion cells and transfer cells are found in plants that feature an apoplastic step in the transfer of sugars from mesophyll cells to sieve elements. Companion cells and transfer cells transfer sugars from the apoplast to the symplast of the sieve elements and companion cells in the source. Intermediary cells, on the other hand, function in symplastic transport of sugars from mesophyll cells to sieve elements in plants where no apoplastic step appears to occur in the source leaf.

PATTERNS OF TRANSLOCATION: SOURCE TO SINK

Sap in the phloem is not translocated exclusively in either an upward or a downward direction, and translocation in the phloem is not defined with respect to gravity. Rather, sap is translocated from areas of supply, called *sources*, to areas of metabolism or storage, called *sinks*.

Sources include any exporting organs, typically mature leaves, that are capable of producing photosynthate in excess of their own needs. The term *photosynthate* refers to products of photosynthesis. Another type of source is a storage organ during the exporting phase of its development. For example, the storage root of the biennial wild beet (*Beta maritima*) is a sink during the growing season of the first year, when it accumulates sugars received from the source leaves. During the second growing season the same root becomes a source; the sugars are remobilized and utilized to produce a new shoot, which ultimately becomes reproductive.

It is noteworthy that cultivated varieties of beets have been selected for the capacity of their roots to act as sinks during all phases of development. Thus, roots of the cultivated sugar beet (*Beta vulgaris*) can increase in dry mass during both the first and the second growing seasons, so

the leaves serve as sources during both flowering and fruiting stages.

Sinks include any nonphotosynthetic organs of the plant and organs that do not produce enough photosynthetic products to support their own growth or storage needs. Roots, tubers, developing fruits, and immature leaves, which must import carbohydrate for normal development, are all examples of sink tissues. Both girdling and labeling studies support the source-to-sink pattern of translocation in the phloem.

Source-to-Sink Pathways Follow Anatomic and Developmental Patterns

Although the overall pattern of transport in the phloem can be stated simply as source-to-sink movement, the specific pathways involved are often more complex. Not all sources supply all sinks on a plant; rather, certain sources preferentially supply specific sinks. In the case of herbaceous plants, such as sugar beet and soybean, the following generalizations can be made.

Proximity. The proximity of the source to the sink is a significant factor. The upper mature leaves on a plant usually provide photosynthates to the growing shoot tip and young, immature leaves; the lower leaves supply predominantly the root system. Intermediate leaves export in both directions, bypassing the intervening mature leaves.

Development. The importance of various sinks may shift during plant development. Whereas the root and shoot apices are usually the major sinks during vegetative growth, fruits generally become the dominant sinks during reproductive development, particularly for adjacent and other nearby leaves.

Vascular connections. Source leaves preferentially supply sinks with which they have direct vascular connections. In the shoot system, for example, a given leaf is generally connected via the vascular system to other leaves directly above or below it on the stem. Such a vertical row of leaves is called an **orthostichy**. The number of internodes between leaves on the same orthostichy varies with the species. Figure 10.8A shows the three-dimensional structure of the phloem in an internode of dahlia (*Dahlia pinnata*).

Modification of translocation pathways. Interference with a translocation pathway by wounding or pruning can alter the patterns established by proximity and vascular

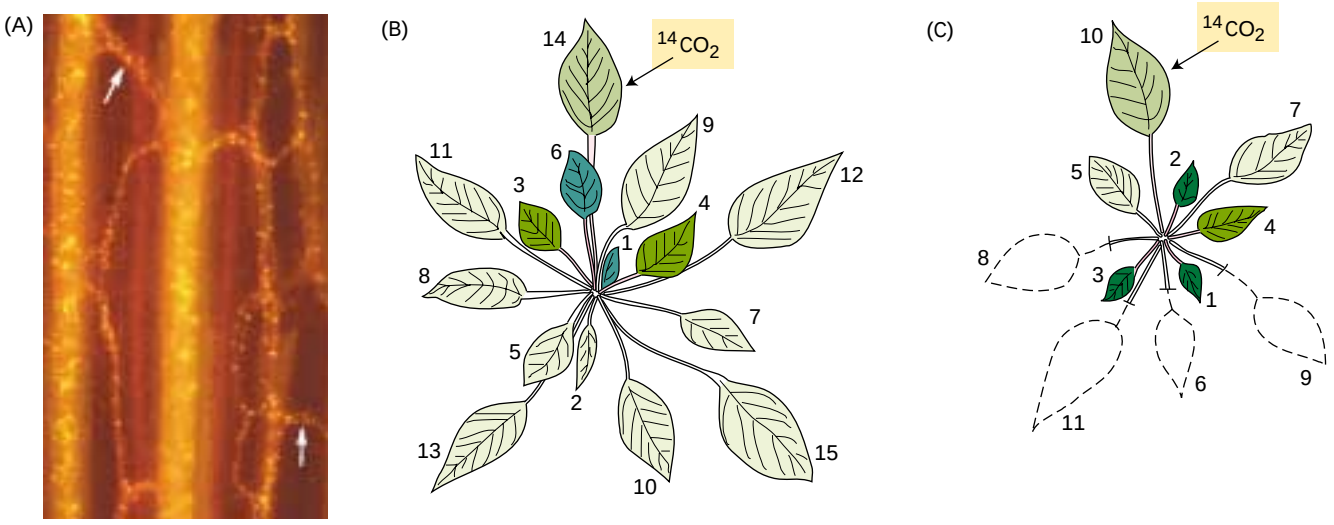


FIGURE 10.8 (A) Longitudinal view of a typical three-dimensional structure of the phloem in a thick section (from an internode of dahlia [*Dahlia pinnata*]). View here after clearing, staining with aniline blue, and observing under an epifluorescent microscope; the sieve plates are seen as numerous small dots because of the yellow staining of callosa in the sieve areas. Two large longitudinal vascular bundles are prominent. This staining reveals the delicate sieve tubes forming the phloem network; two phloem anastomoses are marked by arrows. (B) Distribution of radioactivity from a single labeled source leaf in an intact plant. The distribution of radioactivity in leaves of a sugar beet plant (*Beta vulgaris*) was determined 1 week after $^{14}CO_2$ was supplied for 4 hours to a single source leaf (arrow). The degree of radioactive labeling is indicated by the intensity of shading of the leaves. Leaves are numbered according to their age; the youngest, newly emerged leaf is designated 1. The ^{14}C label was translocated mainly to the sink leaves directly above the source leaf (that is, sink leaves on the same orthostichy as the source; for example, leaves 1 and 6 are sink leaves directly above source leaf 14). (C) Same as B, except all source leaves on the side of the plant opposite the labeled leaf were removed 24 hours before labeling. Sink leaves on both sides of the plant now receive ^{14}C-labeled assimilates from the source. (A courtesy of R. Aloni; B and C based on data from Joy 1964.)

connections that have been outlined here. In the absence of direct connections between source and sink, vascular interconnections, called **anastomoses** (singular *anastomosis*) (see Figure 10.8A), can provide an alternative pathway. In sugar beet, for example, removing source leaves from one side of the plant can bring about cross-transfer of photosynthates to young leaves (sink leaves) on the pruned side (Figure 10.8C). Removal of the lower source leaves on a plant can force the upper source leaves to translocate materials to the roots, and removal of the upper source leaves can force lower source leaves to translocate materials to the upper parts of the plant.

The plasticity of the translocation pathway depends on the extent of the interconnections between vascular bundles and thus on the species and organs studied. In some species the leaves on a branch with no fruits cannot transport photosynthate to the fruits on an adjacent defoliated branch. But in other plants, such as soybean (*Glycine max*), photosynthate is transferred readily from a partly defruited side to a partly defoliated side.

MATERIALS TRANSLOCATED IN THE PHLOEM: SUCROSE, AMINO ACIDS, HORMONES, AND SOME INORGANIC IONS

Water is the most abundant substance transported in the phloem. Dissolved in the water are the translocated solutes, mainly carbohydrates (Table 10.2). Sucrose is the sugar most commonly transported in sieve elements. There is always some sucrose in sieve element sap, and it can reach concentrations of 0.3 to 0.9 *M*.

Nitrogen is found in the phloem largely in amino acids and amides, especially glutamate and aspartate and their respective amides, glutamine and asparagine. Reported levels of amino acids and organic acids vary widely, even for the same species, but they are usually low compared with carbohydrates.

Almost all the endogenous plant hormones, including auxin, gibberellins, cytokinins, and abscisic acid (see Chapters 19, 20, 21, and 23), have been found in sieve elements. The long-distance transport of hormones is thought to occur at least partly in the sieve elements. Nucleotide phosphates and proteins have also been found in phloem sap.

Proteins found in the phloem include filamentous P-proteins (which are involved in the sealing of wounded sieve elements), protein kinases (protein phosphorylation), thioredoxin (disulfide reduction), ubiquitin (protein turnover), chaperones (protein folding), and protease inhibitors (protection of phloem proteins from degradation and defense against phloem-feeding insects) (Schobert et al. 1995; Yoo et al. 2000).

Inorganic solutes that move in the phloem include potassium, magnesium, phosphate, and chloride (see Table 10.2). In contrast, nitrate, calcium, sulfur, and iron are relatively immobile in the phloem.

TABLE 10.2
The composition of phloem sap from castor bean (*Ricinus communis*), collected as an exudate from cuts in the phloem

Component	Concentration (mg mL^{-1})
Sugars	80.0–106.0
Amino acids	5.2
Organic acids	2.0–3.2
Protein	1.45–2.20
Potassium	2.3–4.4
Chloride	0.355–0.675
Phosphate	0.350–0.550
Magnesium	0.109–0.122

Source: Hall and Baker 1972.

We will begin the discussion of phloem content with a look at the methods used to identify materials translocated in the phloem. We will then examine the translocated sugars and the complexities of nitrogen transport in the plant.

Phloem Sap Can Be Collected and Analyzed

The collection of phloem sap has been experimentally challenging (see **Web Topic 10.2**). A few species exude phloem sap from wounds that sever sieve elements, making it possible to collect relatively pure samples of phloem sap. Another approach is to use the stylet of an aphid as a "natural syringe."

Aphids are small insects that feed by inserting their mouthparts, consisting of four tubular stylets, into a single sieve element of a leaf or stem. Sap can be collected from aphid stylets cut from the body of the insect, usually with a laser, after the aphid has been anesthetized with CO_2. The high turgor pressure in the sieve element forces the cell contents through the stylet to the cut end, where they can be collected. Exudate from severed stylets provides a fairly accurate picture of the composition of phloem sap (see **Web Topic 10.2**). Exudation from severed stylets can continue for hours, suggesting that the aphid prevents the plant's normal sealing mechanisms from operating.

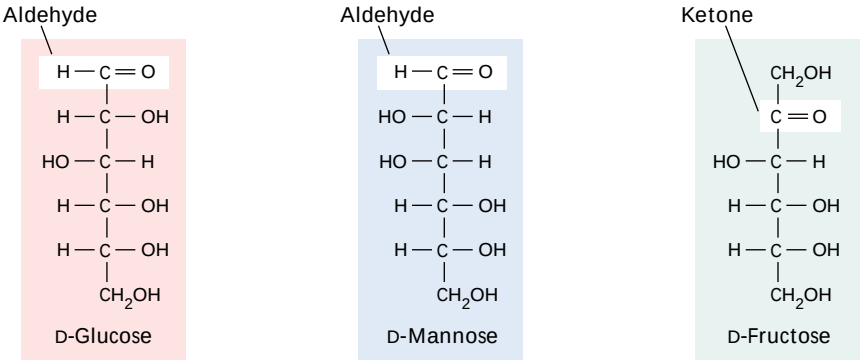
Sugars Are Translocated in Nonreducing Form

Results from analyses of collected sap indicate that the translocated carbohydrates are all nonreducing sugars. Reducing sugars, such as glucose and fructose, contain an exposed aldehyde or ketone group (Figure 10.9A). In a nonreducing sugar, such as sucrose, the ketone or aldehyde group is reduced to an alcohol or combined with a similar group on another sugar (Figure 10.9B). Most researchers believe that the nonreducing sugars are the major compounds translocated in the phloem because they are less reactive than their reducing counterparts.

Sucrose is the most commonly translocated sugar; many of the other mobile carbohydrates contain sucrose bound to varying numbers of galactose molecules. Raffinose consists

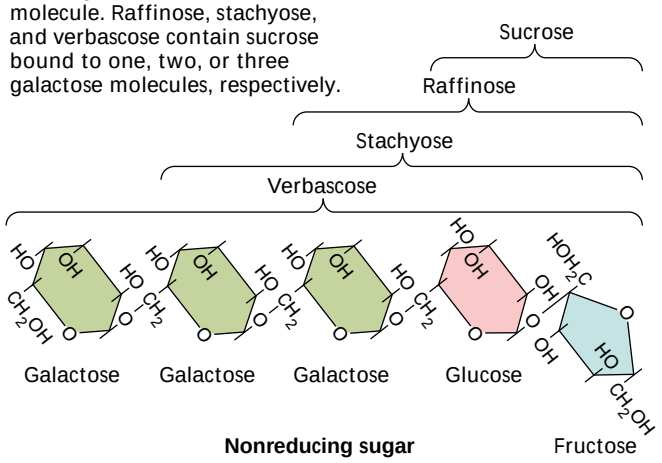
(A) **Reducing sugars, which are not generally translocated in the phloem**

The reducing groups are aldehyde (glucose and mannose) and ketone (fructose) groups.

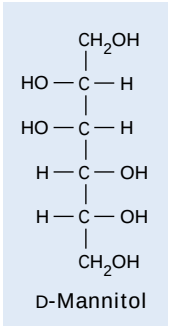


(B) **Compounds commonly translocated in the phloem**

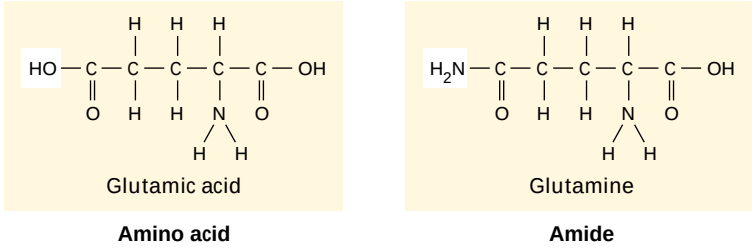
Sucrose is a disaccharide made up of one glucose and one fructose molecule. Raffinose, stachyose, and verbascose contain sucrose bound to one, two, or three galactose molecules, respectively.



Mannitol is a sugar alcohol formed by the reduction of the aldehyde group of mannose.



Glutamic acid, an amino acid, and glutamine, its amide, are important nitrogenous compounds in the phloem, in addition to aspartate and asparagine.



Species with nitrogen-fixing nodules also utilize ureides as transport forms of nitrogen.

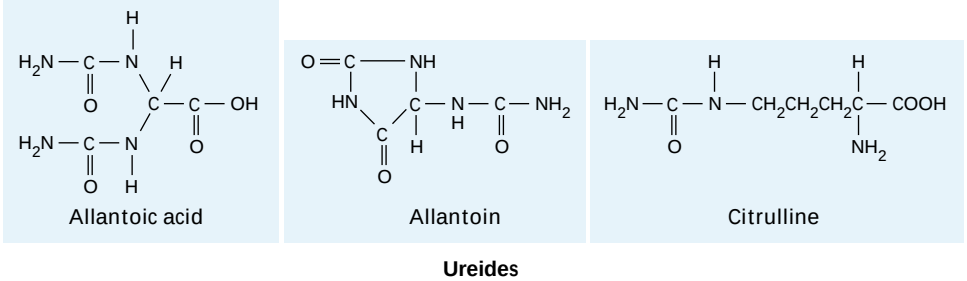


FIGURE 10.9 Structures of compounds not normally translocated in the phloem (A) and of compounds commonly translocated in the phloem (B).

of sucrose and one galactose molecule, stachyose consists of sucrose and two galactose molecules, and verbascose consists of sucrose and three galactose molecules (see Figure 10.9B). Translocated sugar alcohols include mannitol and sorbitol.

Phloem and Xylem Interact to Transport Nitrogenous Compounds

Nitrogen is transported throughout the plant in either inorganic or organic form, with the predominant form depending on several factors, including the transport pathway. Whereas nitrogen is transported in the phloem almost entirely in organic form, in the xylem it can be transported either as nitrate or as part of an organic molecule. (see Chapter 12). Usually the same group of organic molecules carries nitrogen in both the xylem and the phloem.

The form in which nitrogen is transported in the xylem depends on the species studied. Species that do not form a symbiotic association with nitrogen-fixing microorganisms depend on soil nitrate as their major nitrogen source (see Chapter 12). In the xylem of these species, nitrogen is usually present in the form of both nitrate and nitrogen-rich organic molecules, particularly the amides asparagine and glutamine (see Figure 10.9B).

Species with nitrogen-fixing nodules on their roots (see Chapter 12) depend on atmospheric nitrogen, rather than on soil nitrate, as their major nitrogen source. After being converted to an organic form, this nitrogen is transported in the xylem to the shoot, usually in the form of amides or ureides such as allantoin, allantoic acid, or citrulline (see Figure 10.9B).

Whenever nitrogen is assimilated into organic compounds in the roots, both the energy and the carbon skeletons required for assimilation are derived from photosynthates transported to the roots via the phloem. Nitrogen levels in mature leaves are quite stable, indicating that at least some of the excess nitrogen continuously arriving via the xylem is redistributed via the phloem to fruits or younger leaves. (See **Web Topic 10.3** for information on nitrogen transport in the soybean.)

Finally, levels of nitrogenous compounds in the phloem are quite high during leaf senescence. In woody species, senescing leaves mobilize and export nitrogenous compounds to the woody tissues for storage; in herbaceous plants nitrogen is exported generally to the seeds. Other solutes, such as mineral ions, are redistributed from senescing leaves in the same manner.

RATES OF MOVEMENT

The rate of movement of materials in the sieve elements can be expressed in two ways: as **velocity**, the linear distance traveled per unit time, or as **mass transfer rate**, the quantity of material passing through a given cross section of phloem or sieve elements per unit time. Mass transfer rates based on the cross-sectional area of the sieve elements are preferred because the sieve elements are the conducting cells of the phloem. Values for mass transfer rate range from 1 to 15 g h^{-1} cm^{-2} of sieve elements (see **Web Topic 10.4**).

In early publications reporting on rates of transport in the phloem, the units of velocity were centimeters per hour (cm h^{-1}), and the units of mass transfer were grams per hour per square centimeter (g h^{-1} cm^{-2}) of phloem or sieve elements. The currently preferred units (SI units) are meters (m) or millimeters (mm) for length, seconds (s) for time, and kilograms (kg) for mass.

Velocities of Phloem Transport Far Exceed the Rate of Diffusion

Both velocities and mass transfer rates can be measured with radioactive tracers. (Methods of measuring mass transfer rates are described in **Web Topic 10.4**.) In the simplest type of experiment for measuring velocity, ^{11}C- or ^{14}C-labeled CO_2 is applied for a brief period of time to a source leaf (pulse labeling), and the arrival of label at a sink tissue or at a particular point along the pathway is monitored with an appropriate detector.

The length of the translocation pathway divided by the time interval required for label to be first detected at the sink yields a measure of velocity. A more accurate measurement of velocity is obtained from monitoring the arrival of label at two points along the pathway. This method excludes from the measurement the time required for fixation of labeled carbon by photosynthesis, for its incorporation into transport sugar, and for accumulation of sugar in the sieve elements of the source leaf.

In general, velocities measured by a variety of techniques average about 1 m h^{-1} and range from 0.3 to 1.5 m h^{-1} (30–150 cm h^{-1}). Transport velocities in the phloem are clearly quite high, well in excess of the rate of diffusion over long distances. Any proposed mechanism of phloem translocation must account for these high velocities.

THE MECHANISM OF TRANSLOCATION IN THE PHLOEM: THE PRESSURE-FLOW MODEL

The mechanism of phloem translocation in angiosperms is best explained by the pressure-flow model, which accounts for most of the experimental and structural data currently available. We will see in this discussion that the pressure-flow model explains phloem translocation as a flow of solution (bulk flow) driven by an osmotically generated pressure gradient between source and sink.

In early research on phloem translocation, both active and passive mechanisms were considered. All theories, both active and passive, assume an energy requirement in both sources and sinks. In sources, energy is necessary to move photosynthate from producing cells into the sieve elements. This movement of photosynthate is called *phloem loading*, and it is discussed in detail later in the chapter. In sinks, energy is

essential for some aspects of movement from sieve elements to sink cells, which store or metabolize the sugar. This movement of photosynthate from sieve elements to sink cells is called *phloem unloading* and will also be discussed later.

The passive mechanisms of phloem transport further assume that energy is required in the sieve elements of the path between sources and sinks simply to maintain structures such as the cell plasma membrane and to recover sugars lost from the phloem by leakage. The pressure-flow model is an example of a passive mechanism. The active theories, on the other hand, postulate an additional expenditure of energy by path sieve elements in order to drive translocation itself (Zimmermann and Milburn 1975).

A Pressure Gradient Drives Translocation

Diffusion is far too slow to account for the velocities of solute movement observed in the phloem. Translocation velocities average 1 m h^{-1}; the rate of diffusion is 1 m per 32 years! (See Chapter 3 for a discussion of diffusion velocities and the distances over which diffusion is an effective transport mechanism.)

The **pressure-flow model**, first proposed by Ernst Münch in 1930, states that a flow of solution in the sieve elements is driven by an osmotically generated *pressure gradient* between source and sink ($\Delta\Psi_p$). The pressure gradient is established as a consequence of phloem loading at the source and phloem unloading at the sink.

Recall from Chapter 3 (Equation 3.6) that $\Psi_w = \Psi_s + \Psi_p$; that is, $\Psi_p = \Psi_w - \Psi_s$. In source tissues, energy-driven phloem loading leads to an accumulation of sugars in the sieve elements, generating a low (negative) solute potential ($\Delta\Psi_s$) and causing a steep drop in the water potential ($\Delta\Psi_w$). In response to the water potential gradient, water enters the sieve elements and causes the turgor pressure (Ψ_p) to increase.

At the receiving end of the translocation pathway, phloem unloading leads to a lower sugar concentration in the sieve elements, generating a higher (more positive) solute potential in the sieve elements of sink tissues. As the water potential of the phloem rises above that of the xylem, water tends to leave the phloem in response to the water potential gradient, causing a decrease in turgor pressure in the sieve elements of the sink. Figure 10.10 illustrates the pressure-flow hypothesis.

If no cross-walls were present in the translocation pathway—that is, if the entire pathway were a single membrane-enclosed compartment—the different pressures at the source and sink would rapidly equilibrate. The presence of sieve plates greatly increases the resistance along the pathway and results in the generation and maintenance of a substantial pressure gradient in the sieve elements between source and sink. The sieve element contents are physically pushed along the translocation pathway as a bulk flow, much like water flowing through a garden hose.

Close inspection of the water potential values shown in Figure 10.10 shows *that water in the phloem is moving against a water potential gradient from source to sink*. Such water movement does not violate the laws of thermodynamics because the water is moving by bulk flow rather than by osmosis. That is, no membranes are crossed during transport from one sieve tube to another, and solutes are moving at the same rate as the water molecules.

Under these conditions, the solute potential, Ψ_s, cannot contribute to the driving force for water movement, although it still influences the water potential. Water movement in the translocation pathway is therefore driven by the pressure gradient rather than by the water potential gradient. Of course, the passive, pressure-driven, long-distance translocation in the sieve tubes ultimately depends on the active, short-distance transport mechanisms involved in phloem loading and unloading. These active mechanisms are responsible for setting up the pressure gradient.

The Predictions of the Pressure-Flow Model Have Been Confirmed

Some important predictions emerge from the pressure-flow model:

- The sieve plate pores must be unobstructed. If P-protein or other materials blocked the pores, the resistance to flow of the sieve element sap would be too great.
- True *bidirectional transport* (i.e., simultaneous transport in both directions) in a single sieve element cannot occur. A mass flow of solution precludes such bidirectional movement because a solution can flow in only one direction in a pipe at any one time. Solutes within the phloem can move bidirectionally, but in different vascular bundles or in different sieve elements.
- Great expenditures of energy are not required in order to drive translocation in the tissues along the path, although energy is required to maintain the structure of the sieve elements and to reload any sugars lost to the apoplast by leakage. Therefore, treatments that restrict the supply of ATP in the path, such as low temperature, anoxia, and metabolic inhibitors, should not stop translocation.
- The pressure-flow hypothesis demands the presence of a positive pressure gradient. Turgor pressure must be higher in sieve elements of sources than in sieve elements of sinks, and the pressure difference must be large enough to overcome the resistance of the pathway and to maintain flow at the observed velocities.

The available evidence testing these predictions supports the pressure-flow hypothesis.

Sieve Plate Pores Are Open Channels

Ultrastructural studies of sieve elements are challenging because of the high internal pressure in these cells. When

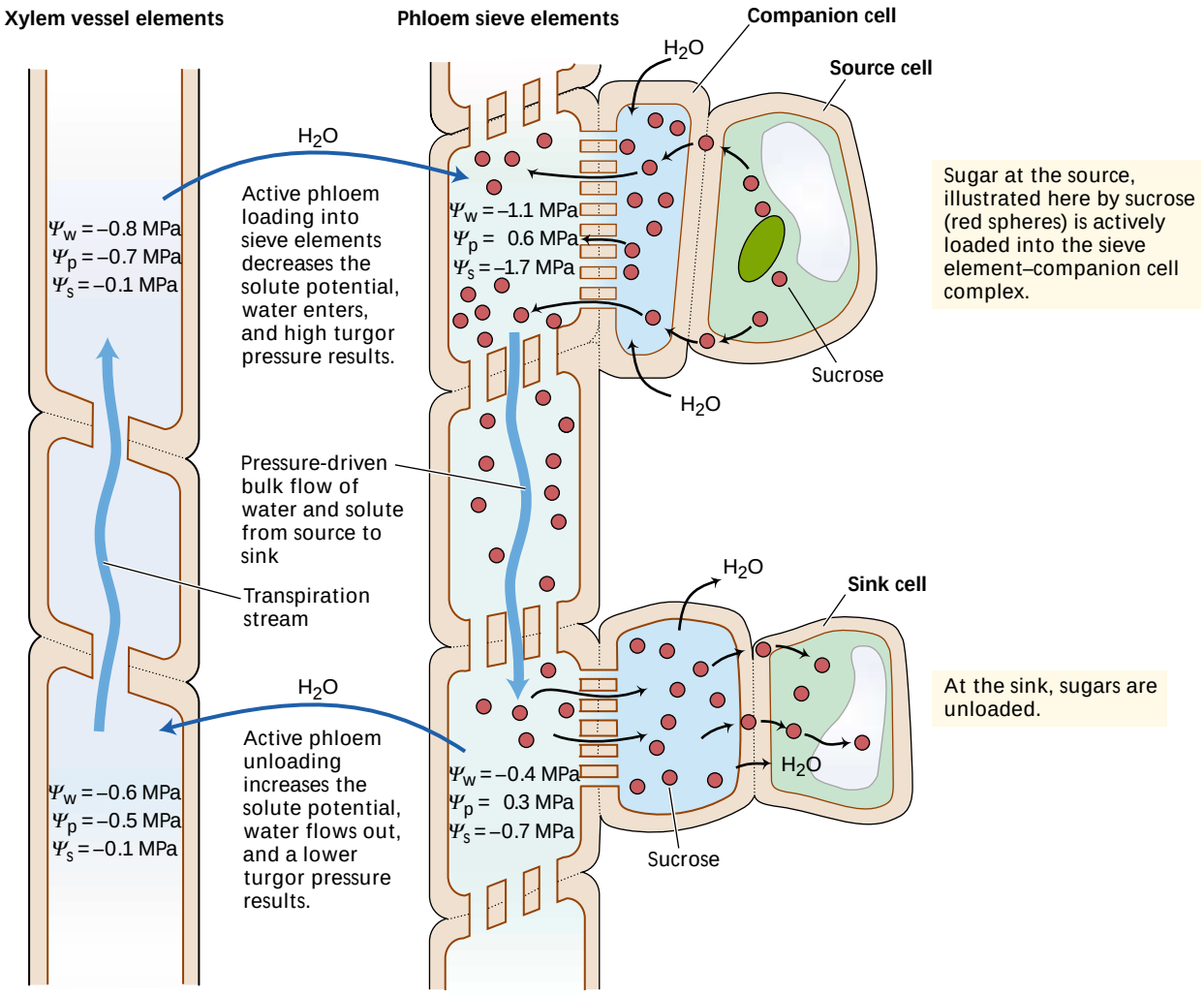


FIGURE 10.10 Pressure-flow model of translocation in the phloem. Possible values for Ψ_w, Ψ_p, and Ψ_s in the xylem and phloem are illustrated. (After Nobel 1991.)

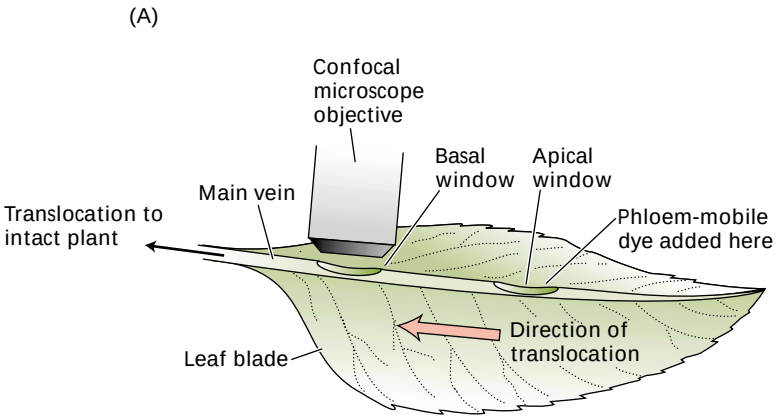
the phloem is excised or killed slowly with chemical fixatives, the turgor pressure in the sieve elements is released. The contents of the cell, including P-protein, surge toward the point of pressure release and, in the case of sieve tube elements, accumulate on the sieve plates. This accumulation is probably the reason that many earlier electron micrographs show sieve plates that are obstructed.

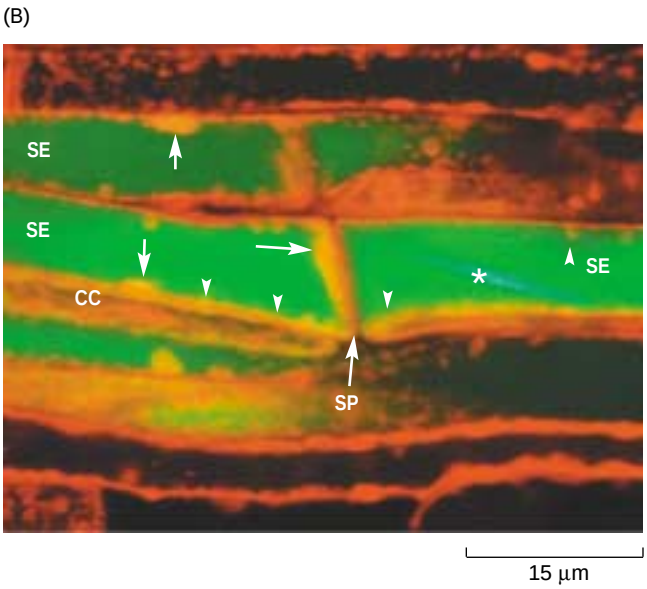
Newer, rapid freezing and fixation techniques provide reliable pictures of undisturbed sieve elements. Electron micrographs of sieve tube elements prepared by such techniques show that P-protein is usually found along the periphery of the sieve tube elements (see Figures 10.3, 10.4, and 10.5), or it is evenly distributed throughout the lumen of the cell. Furthermore, the pores contain P-protein in similar positions, lining the pore or in a loose network. The open condition of the pores, seen in many species, such as cucurbits, sugar beet, and bean (e.g., see Figure 10.5), supports the pressure-flow model.

In addition to obtaining the structural evidence provided by electron microscopy, it is important to determine whether the sieve plate pores are open in the intact tissue. The use of confocal laser scanning microscopy, which allows for the direct observation of translocation through living sieve elements, addresses this question (Knoblauch and van Bel 1998). Such experiments show that the sieve plate pores of living, translocating sieve elements are open (Figure 10.11).

Bidirectional Transport Cannot Be Seen in Single Sieve Elements

Researchers have investigated bidirectional transport by applying two different radiotracers to two source leaves, one above the other (Eschrich 1975). Each leaf receives one





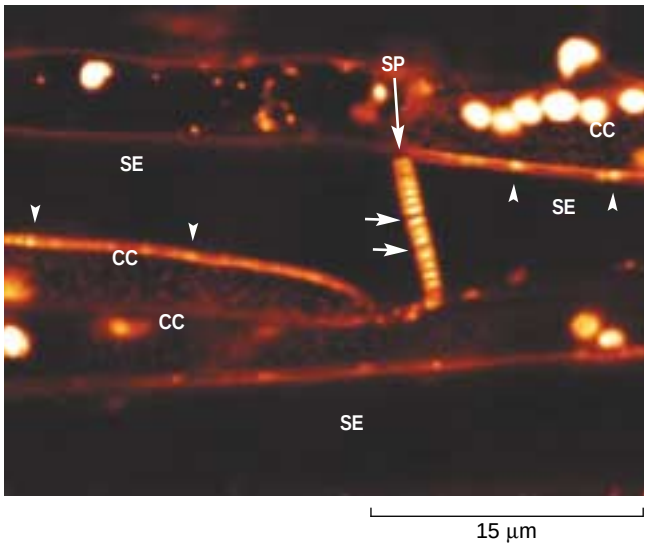


FIGURE 10.11 Translocation in living, functional sieve elements of a leaf attached to an intact broad bean (*Vicia faba*) plant. (A) Two windows were sliced parallel to the epidermis on the lower side of the main vein of a mature leaf, exposing the phloem tissue. The objective of the laser confocal microscope was positioned over the basal window. A phloem-mobile fluorescent dye was added at the apical window. If translocation occurred, the dye would become visible in the microscope at the basal window of the leaf. In this way it could be demonstrated that the sieve elements being observed were alive and functional. (B) Phloem tissue of bean doubly stained with a locally applied fluorescent dye (red) that primarily stains membranes, and a translocated fluorescent dye (green). Protein (arrows) deposited against the plasma membrane and the sieve plate does not impede translocation. A crystalline P-protein body (asterisk) is stained by the green dye. Plastids (arrowheads) are evenly distributed around the periphery of the sieve element. CC = companion cell, SP = sieve plate. See also **Web Topic 10.8**. (From Knoblauch and van Bel 1998; courtesy of A. van Bel.)

of the tracers, and a point between the two sources is monitored for the presence of both tracers.

Transport in two directions has often been detected in sieve elements of different vascular bundles in stems. Transport in two directions has also been seen in adjacent sieve elements of the same bundle in petioles. Bidirectional transport in adjacent sieve elements can occur in the petiole of a leaf that is undergoing the transition from sink to source and simultaneously importing and exporting photosynthates through its petiole. However, simultaneous bidirectional transport in a single sieve element has never been demonstrated.

Translocation Rate Is Typically Insensitive to the Energy Supply of the Path Tissues

In plants that can survive periods of low temperature, such as sugar beet, rapidly chilling a short segment of the petiole of a source leaf to approximately 1°C does not cause sustained inhibition of mass transport out of the leaf (Figure 10.12). Rather, there is a brief period of inhibition, after which transport slowly returns to the control rate. Chilling reduces respiration rate and both the synthesis and the consumption of ATP in the petiole by about 90%, at a time when translocation has recovered and is proceeding normally. These experiments show that the energy requirement for transport through the pathway of these plants is small, consistent with the pressure-flow hypothesis.

Extreme treatments that inhibit all energy metabolism do inhibit translocation. For example, in bean (*Phaseolus vulgaris*), treating the petiole of a source leaf with a metabolic inhibitor (cyanide) inhibited translocation out of the

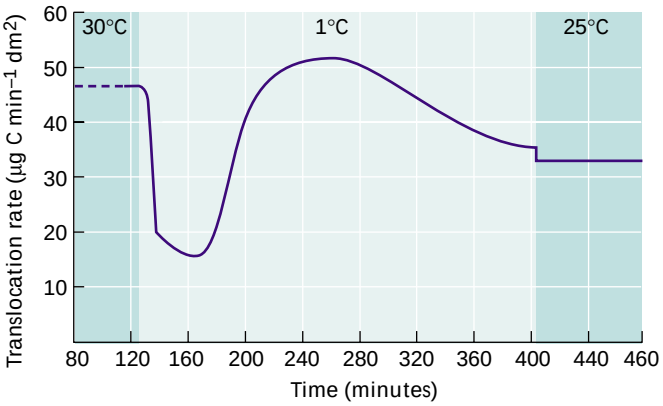


FIGURE 10.12 Loss of metabolic energy resulting from the chilling of the leaf petiole partially reduces the rate of translocation in sugar beet (*Beta vulgaris*), although translocation rates recover with time. The fact that translocation recovers when ATP production and utilization are largely inhibited by chilling indicates that the energy requirement for translocation is small. $^{14}CO_2$ was supplied to a source leaf, and a 2 cm portion of its petiole was chilled to 1°C. Translocation was monitored by the arrival of ^{14}C at a sink leaf. (dm [decimeter] = 0.1 meter) (Data from Geiger and Sovonick 1975.)

leaf. However, examination of the treated tissue by electron microscopy revealed blockage of the sieve plate pores by cellular debris (Giaquinta and Geiger 1977). Clearly, these results do not bear on the question of whether energy is required for translocation along the pathway.

Pressure Gradients Are Sufficient to Drive a Mass Flow of Solution

Turgor pressure in sieve elements can be either calculated from the water potential and solute potential ($\Psi_p = \Psi_w - \Psi_s$) or measured directly. The most effective technique uses micromanometers or pressure transducers sealed over exuding aphid stylets (see Figure 10.2.A in **Web Topic 10.2**) (Wright and Fisher 1980). The data obtained are accurate because aphids pierce only a single sieve element, and the plasma membrane apparently seals well around the aphid stylet. When the turgor pressure of sieve elements is measured by this technique, the pressure at the source is higher than that at the sink.

In soybean, the observed pressure difference between source and sink has been shown to be sufficient to drive a mass flow of solution through the pathway, taking into account the path resistance (caused mainly by the sieve plate pores), the path length, and the velocity of translocation (Fisher 1978). The actual pressure difference between source and sink was calculated from the water potential and solute potential to be 0.41 MPa, and the pressure difference required for translocation by pressure flow was calculated to be 0.12 to 0.46 MPa. Thus the observed pressure difference appears to be sufficient to drive mass flow through the phloem.

We can therefore conclude that all the experiments and data described here support the operation of pressure flow in angiosperm phloem. The lack of an energy requirement in the pathway and the presence of open sieve plate pores provide definitive evidence for a mechanism in which the path phloem is relatively passive. The failure to detect bidirectional transport or motility proteins, as well as the positive data on pressure gradients, is in accord with the pressure-flow hypothesis.

The Mechanism of Phloem Transport in Gymnosperms May Be Different

Although pressure flow explains translocation in angiosperms, it may not be sufficient for gymnosperms. Very little physiological information on gymnosperm phloem is available, and speculation about translocation in these species is based almost entirely on interpretations of electron micrographs. As discussed previously, the sieve cells of gymnosperms are similar in many respects to sieve tube elements of angiosperms, but the sieve areas of sieve cells are relatively unspecialized and do not appear to consist of open pores (see Figure 10.6).

The pores in gymnosperms are filled with numerous membranes that are continuous with the smooth endoplasmic reticulum adjacent to the sieve areas. Such pores are clearly inconsistent with the requirements of the pressure-flow hypothesis. Although these electron micrographs might be artifactual and fail to show conditions in the intact tissue, translocation in gymnosperms might involve a different mechanism—a possibility that requires further investigation.

PHLOEM LOADING: FROM CHLOROPLASTS TO SIEVE ELEMENTS

Several transport steps are involved in the movement of photosynthate from the mesophyll chloroplasts to the sieve elements of mature leaves, which is called **phloem loading** (Oparka and van Bel 1992):

1. Triose phosphate formed by photosynthesis during the day (see Chapter 8) is transported from the chloroplast to the cytosol, where it is converted to sucrose. During the night, carbon from stored starch exits the chloroplast probably in the form of glucose and is converted to sucrose. (Other transport sugars are later synthesized from sucrose in some species.)

2. Sucrose moves from the mesophyll cell to the vicinity of the sieve elements in the smallest veins of the leaf (Figure 10.13). This **short-distance transport** pathway usually covers a distance of only two or three cell diameters.

3. In a process called **sieve element loading**, sugars are transported into the sieve elements and companion cells. In most of the species studied so far, sugars become more concentrated in the sieve elements and

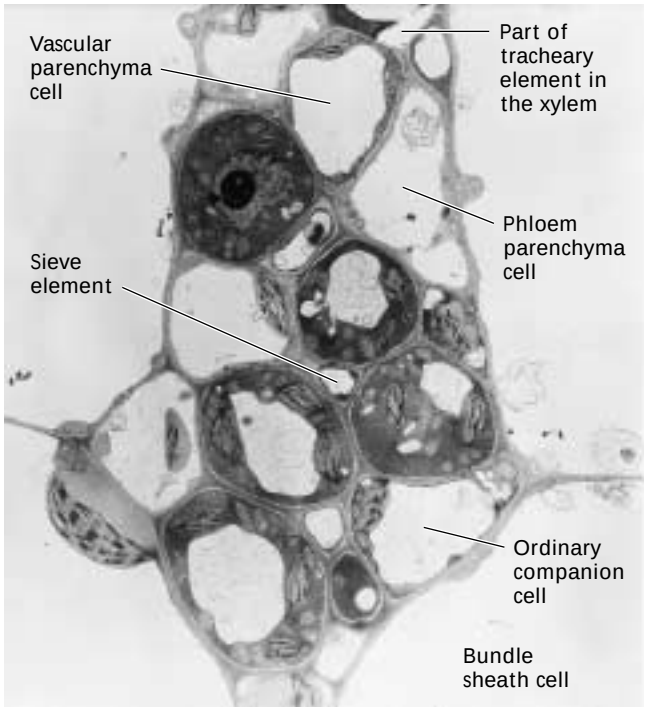


FIGURE 10.13 Electron micrograph showing the relationship between the various cell types of a small vein in a source leaf of sugar beet (*Beta vulgaris*). Photosynthetic cells (mesophyll cells) surround the compactly arranged cells of the bundle sheath layer. Photosynthate from the mesophyll must move a distance equivalent to several cell diameters before being loaded into the sieve elements. (From Evert and Mierzwa 1985, courtesy of R. Evert.)

companion cells than in the mesophyll. Note that with respect to loading, the sieve elements and companion cells are often considered a functional unit, called the *sieve element–companion cell complex*. Once inside the sieve elements, sucrose and other solutes are translocated away from the source, a process known as **export**. Translocation through the vascular system to the sink is referred to as **long-distance transport**.

As discussed earlier, the processes of loading at the source and unloading at the sink provide the driving force that generates the pressure gradient pushing phloem sap in long-distance transport and are thus of considerable basic, as well as agricultural, importance. A thorough understanding of these mechanisms should provide the basis of technology aimed at enhancing crop productivity by increasing the accumulation of photosynthate by edible sink tissues, such as cereal grains.

Photosynthate Can Move from Mesophyll Cells to the Sieve Elements via the Apoplast or the Symplast

We have seen that solutes (mainly sugars) in source leaves must move from the photosynthesizing cells in the mesophyll to the veins. Sugars might move entirely through the symplast (cytoplasm) via the plasmodesmata, or they might enter the apoplast at some point en route to the phloem (Figure 10.14). (See Figure 4.3 for a general description of the symplast and apoplast.) In the latter case, the sugars are actively loaded from the apoplast into the sieve elements and companion cells by an energy-driven, selective transporter located in the plasma membranes of these cells. In fact, the apoplastic and symplastic routes are used in different species.

Early research on phloem loading focused on the apoplastic pathway. Apoplastic phloem loading leads to three basic predictions (Grusak et al. 1996): (1) Transported sugars should be found in the apoplast; (2) in experiments in which sugars are supplied to the apoplast, the exogenously supplied sugars should accumulate in sieve elements and companion cells; and (3) inhibition of sugar uptake from the apoplast should result in inhibition of export from the leaf. Many studies devoted to testing these predictions have provided solid evidence for apoplastic loading in several species (see **Web Topic 10.5**).

Sucrose Uptake in the Apoplastic Pathway Requires Metabolic Energy

In source leaves, sugars become more concentrated in the sieve elements and companion cells than in the mesophyll. This difference in solute concentration, found in most of the species studied, can be demonstrated through measurement of the osmotic potential (Ψ_s) of the various cell types in the leaf (see Chapter 3).

In sugar beet, the osmotic potential of the mesophyll is approximately –1.3 MPa, and the osmotic potential of the sieve elements and companion cells is about –3.0 MPa (Geiger et al. 1973). Most of this difference in osmotic potential is thought to result from accumulated sugar, specifically sucrose because sucrose is the major transport sugar in this species. Experimental studies have also demonstrated that both externally supplied sucrose and sucrose made from photosynthetic products accumulate in the sieve elements and companion cells of the minor veins of sugar beet source leaves (Figure 10.15).

The fact that sucrose is at a higher concentration in the sieve element–companion cell complex than in surrounding cells indicates that sucrose is actively transported against its chemical-potential gradient. The dependence of sucrose accumulation on active transport is supported by the fact that treating source tissue with respiratory inhibitors both decreases ATP concentration and inhibits loading of exogenous sugar. On the other hand, other metabolites, such as organic acids and hormones, may enter sieve elements passively (see **Web Topic 10.6**).

In the Apoplastic Pathway, Sieve Element Loading Involves a Sucrose–H^+ Symporter

A sucrose–H^+ symporter is thought to mediate the transport of sucrose from the apoplast into the sieve element–companion cell complex. Recall from Chapter 6

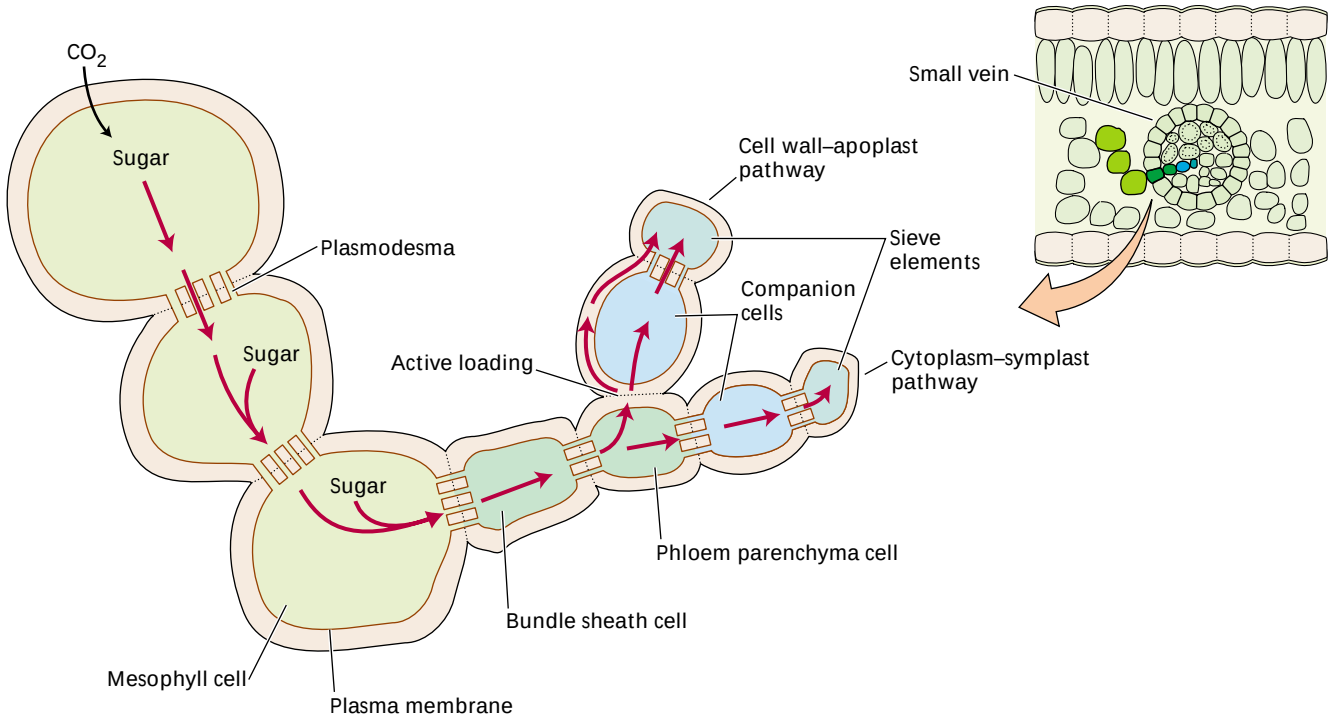


FIGURE 10.14 Schematic diagram of pathways of phloem loading in source leaves. In the totally symplastic pathway, sugars move from one cell to another in the plasmodesmata, all the way from the mesophyll to the sieve elements. In the partly apoplastic pathway, sugars enter the apoplast at some point. For simplicity, sugars are shown here entering the apoplast near the sieve element–companion cell complex, but they could also enter the apoplast earlier in the path and then move to the small veins. In any case, the sugars are actively loaded into the companion cells and sieve elements from the apoplast. Sugars loaded into the companion cells are thought to move through plasmodesmata into the sieve elements.

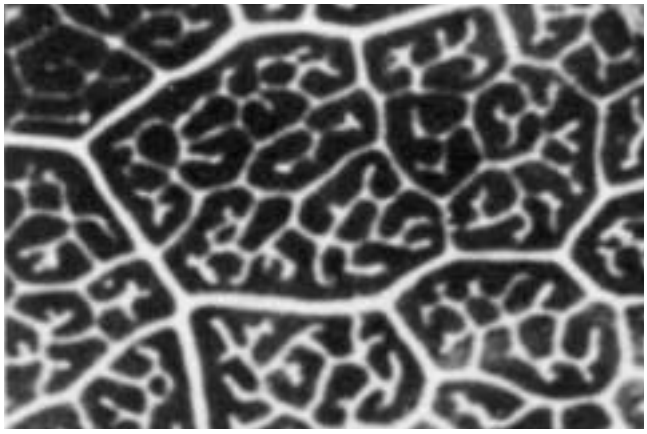


FIGURE 10.15 This autoradiograph shows that labeled sugar moves from the apoplast into sieve elements and companion cells against its concentration gradient. A solution of ^{14}C-labeled sucrose was applied for 30 minutes to the upper surface of a sugar beet (*Beta vulgaris*) leaf that had previously been kept in darkness for 3 hours. The leaf cuticle was removed to allow penetration of the solution to the interior of the leaf. Label accumulates in the small veins, sieve elements, and companion cells of the source leaf, indicating the ability of these cells to transport sucrose against its concentration gradient. (From Fondy 1975, courtesy of D. Geiger.)

that symport is a secondary transport process that uses the energy generated by the proton pump (see Figure 6.10A). The energy dissipated by protons moving back into the cell is coupled to the uptake of a substrate, in this case sucrose (Figure 10.16).

High pH (low H^+ concentration) in the apoplast reduces the uptake of exogenous sucrose into the sieve elements and companion cells of broad bean. This effect occurs because a low proton concentration in the apoplast reduces the driving force for proton diffusion into the symplast and for the sucrose–H^+ symporter.

Data from molecular studies support the operation of a sucrose–H^+ symporter in sieve element loading. Proton-pumping ATPases, localized by immunological techniques, have been found in the plasma membranes of companion cells of *Arabidopsis* and in transfer cells of broad bean. In transfer cells, the H^+-ATPase molecules are most concentrated in the plasma membrane infoldings that face the bundle sheath and phloem parenchyma cells (for details, see **Web Topic 10.7**).

Such localization suggests that the function of these H^+-ATPases is to energize the transport of photosynthate from

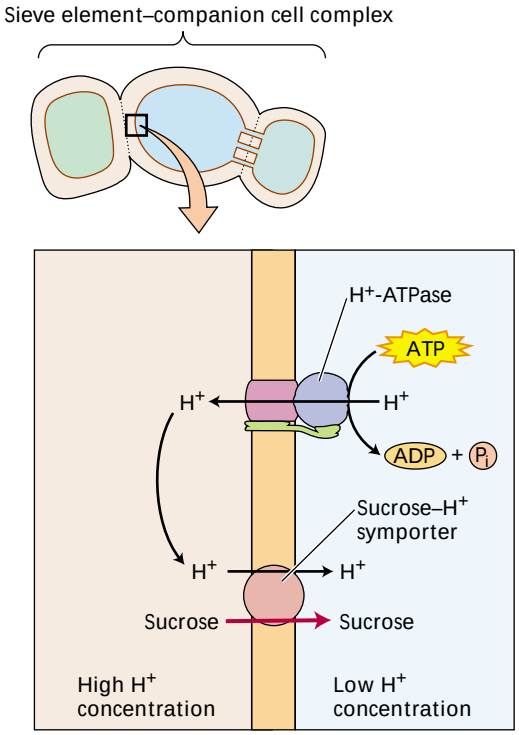


FIGURE 10.16 ATP-dependent sucrose transport in sieve element loading. In the cotransport model of sucrose loading into the symplast of the sieve element–companion cell complex, the plasma membrane ATPase pumps protons out of the cell into the apoplast, establishing a high proton concentration there. The energy in this proton gradient is then used to drive the transport of sucrose into the symplast of the sieve element–companion cell complex through a sucrose–H^+ symporter.

the apoplast to the sieve elements (Bouche-Pillon et al. 1994). Furthermore, the distribution of the H^+-ATPases in companion cells of *Arabidopsis* appears to be correlated with the distribution of a sucrose–H^+ symporter called *SUC2* (DeWitt and Sussman 1995; Truernit and Sauer 1995). The SUC2 transporter has also been localized in companion cells of broad-leaved plantain, *Plantago major* (see **Web Topic 10.7**). H^+-ATPases and sucrose–H^+ symporters are sometimes co-localized in the plasma membranes of sieve elements (Langhans et al. 2001) rather than companion cells.

SUC2 is one of several sucrose–H^+ symporters that have been cloned and localized in the phloem (Table 10.3). The carriers are found in plasma membranes of either sieve elements (SUT1, SUT2, and SUT4) or companion cells (SUC2). Work with SUT1 has shown that the messenger RNAs for symporters found in the sieve element membrane are synthesized in the companion cells (Kuhn et al. 1997). This finding agrees with the fact that sieve elements lack nuclei. The symporter protein is probably also synthesized in the companion cells, since ribosomes do not appear to persist in mature sieve elements.

The roles played by the various carriers listed in Table 10.3 are still being elucidated. Most of the transporters are found in source, path, and sink tissues. SUT1, characterized as a high-affinity / low-capacity transporter found in the minor veins of source tissues, appears to be important in phloem loading. Potato plants transformed with antisense DNA to SUT1 showed reduced transporter activity, a reduction in root and tuber growth, and accumulation of starch and lipids in source leaves (Schulz et al. 1998).

SUT1 is also thought to play a role in the retrieval of sucrose lost in transit. The important role of SUT1 in phloem loading appears to be complemented by SUT4, a low-affinity / high-capacity carrier (Weise et al. 2000). SUT2, on the other hand, appears to function as a sucrose sensor. This is indicated by findings showing that SUT2 is more highly expressed in sink and path tissues than in source leaves, and by the similarity between many structural features of SUT2 and yeast sugar sensors (Lalonde et al. 1999; Barker et al. 2000). Finally, uptake into companion cells appears to be the function of SUC2.

Regulating sucrose loading. The mechanisms that regulate the loading of sucrose from the apoplast to the sieve elements by the sucrose–H^+ symporter await characterization. Possible regulatory factors include the following:

- *The solute potential or, more likely, the turgor pressure of the sieve elements.* A decrease in sieve element turgor below a certain threshold would lead to a compensatory increase in loading.

TABLE 10.3
Sucrose–H^+ symporters in the phloem

Carrier	Location	Species	Affinity	Source
SUT1	Sieve elements	Tobacco, tomato, potato	High	Kuhn et al. 1997
SUT2	Sieve elements	Tomato	Sensor	Barker et al. 2000
SUT4	Sieve elements	*Arabidopsis*, tomato, potato	Low	Weise et al. 2000
SUC2	Companion cells	*Arabidopsis*, plantain	—	Truernit and Sauer, 1995; Stadler et al. 1995

- *Sucrose concentration in the apoplast*. High sucrose concentrations in the apoplast would increase phloem loading.
- *The available number of symporter protein molecules*. The levels of SUT1 transporter mRNA and protein have been shown to be lower after 15 hours of darkness than after a light treatment. These data suggest that the concentration of SUT1 transporter molecules could regulate loading.

Other studies have shown that sucrose efflux into the apoplast is enhanced by potassium availability in the apoplast, suggesting that a better nutrient supply increases translocation to sinks and enhances sink growth.

Phloem Loading Appears to Be Symplastic in Plants with Intermediary Cells

As discussed earlier, many results point to apoplastic phloem loading in species that have ordinary companion cells or transfer cells in the minor veins, and that transport only sucrose. On the other hand, a symplastic pathway has become evident in species that transport raffinose and stachyose in the phloem, in addition to sucrose, and that have intermediary cells in the minor veins. Some examples of such species are common coleus (*Coleus blumei*), squash (*Cucurbita pepo*), and melon (*Cucumis melo*) (see **Web Topic 10.8**).

The operation of a symplastic pathway requires the presence of open plasmodesmata between the different cells in the pathway. Many species have numerous plasmodesmata at the interface between the sieve element–companion cell complex and the surrounding cells (see Figure 10.7C), and experimental studies have demonstrated symplastic continuity in source leaves of some species (see **Web Topic 10.8**).

The Polymer-Trapping Model Explains Symplastic Loading in Source Leaves

The composition of sieve element sap is generally different from the solute composition in tissues surrounding the phloem. This difference indicates that certain sugars are specifically selected for transport in the source leaf. The involvement of symporters in apoplastic phloem loading provides a clear mechanism for selectivity because symporters are specific for certain sugar molecules. Symplastic loading, on the other hand, depends on the diffusion of sugars from the mesophyll to the sieve elements via the plasmodesmata. It is more difficult to envision how diffusion through plasmodesmata during symplastic loading could be selective for certain sugars.

Furthermore, data from several species showing symplastic loading indicate that sieve elements and companion cells have a higher osmotic content than the mesophyll. How could diffusion-dependent symplastic loading account for the observed selectivity for transported molecules and the accumulation of sugars against a concentration gradient?

The **polymer-trapping model** (Figure 10.17) has been developed to address these questions (Turgeon and Gowan

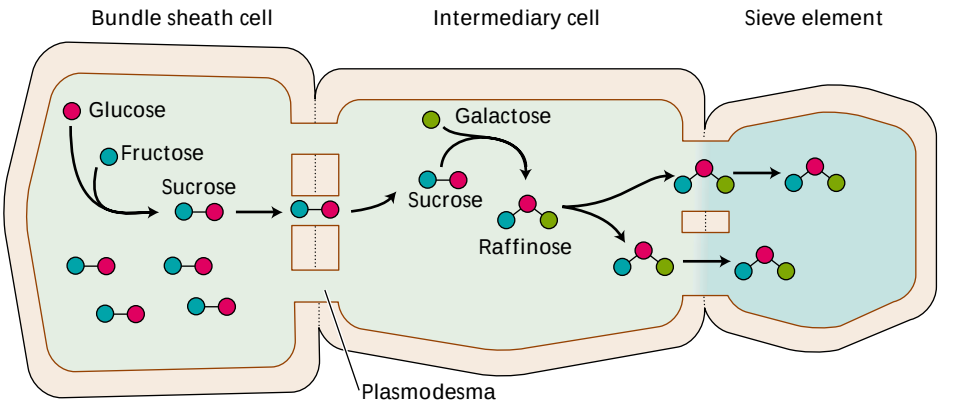


FIGURE 10.17 Polymer-trapping model of phloem loading. For simplicity, the trisaccharide stachyose is omitted. (After van Bel 1992.)

1990). This model states that the sucrose synthesized in the mesophyll diffuses from the bundle sheath cells into the intermediary cells through the abundant plasmodesmata that connect the two cell types. In the intermediary cells, raffinose and stachyose (polymers made of three and four hexose sugars, respectively; see Figure 10.9B) are synthesized from the transported sucrose and from galactose. Because of the anatomy of the tissue and the relatively large size of raffinose and stachyose, the polymers cannot diffuse back into the bundle sheath cells, but they can diffuse into the sieve element. Sucrose can continue to diffuse into the intermediary cells because its synthesis in the mesophyll and its utilization in the intermediary cells maintain the concentration gradient (see Figure 10.17).

The polymer-trapping model makes three predictions:

1. Sucrose should be more concentrated in the mesophyll than in the intermediary cells.
2. The enzymes for raffinose and stachyose synthesis should be preferentially located in the intermediary cells.
3. The plasmodesmata linking the bundle sheath cells and the intermediary cells should exclude molecules larger than sucrose.

Many studies support the polymer-trapping model. For instance, all of the enzymes required to synthesize stachyose from sucrose have been found in intermediary cells. In melon, raffinose and stachyose are present in high concentrations in intermediary cells, but not in mesophyll cells.

The Type of Phloem Loading Is Correlated with Plant Family and with Climate

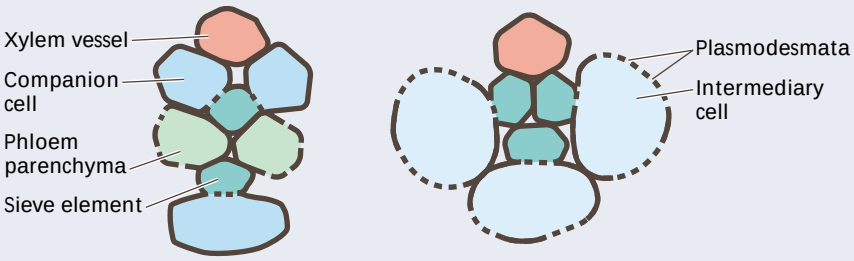
As discussed earlier, the operation of apoplastic and symplastic phloem-loading pathways is correlated with the transport sugar, the type of companion cell in the minor veins, and the number of plasmodesmata connecting the sieve elements and companion cells to the surrounding photosynthetic cells (Table 10.4) (van Bel et al. 1992):

- Species showing apoplastic phloem loading translocate sucrose almost exclusively, have either ordinary companion cells or transfer cells in the minor veins, and possess few connections between the sieve element–companion cell complex and the surrounding cells.
- Species having symplastic phloem loading translocate oligosaccharides such as raffinose in addition to sucrose, have intermediary-type companion cells in the minor veins, and possess abundant connections between the sieve element–companion cell complex and the surrounding cells.

Plants that have abundant plasmodesmata between the phloem and surrounding cells are often trees, shrubs, or vines. Plants with few plasmodesmata at this interface are more typically herbaceous plants. In general, plants with abundant plasmodesmata between the phloem and surrounding cells tend to be found in tropical and subtropical regions, and plants with few plasmodesmata at this interface tend to be found in temperate and arid climates.

TABLE 10.4
Patterns in apoplastic and symplastic loading

	Apoplastic loading	Symplastic loading
Transport sugar	Sucrose	Oligosaccharides in addition to sucrose
Type of companion cell in the minor veins	Ordinary companion cells or transfer cells	Intermediary cells
Number of plasmodesmata connecting the sieve elements and companion cells to surrounding cells	Few	Abundant



Source: Drawings after van Bel et al. 1992.

Note: Some species may load both apoplastically and symplastically, since different types of companion cells can be found within the veins of a single species.

There are, of course, intermediate cases and exceptions to these generalizations. Some species with apoplastic loading have more plasmodesmata linking their companion cells to surrounding cells than might be predicted from known apoplastically loading species (Goggin et al. 2001). A number of species have more than one type of companion cell in their minor veins. For example, coleus has both intermediary cells and ordinary companion cells. It has been suggested that the symplastic and apoplastic pathways may coexist in some species, simultaneously or at different times, in different sieve elements in the same vein or in sieve elements in veins of different sizes (Turgeon et al. 2001).

Future research may reveal new loading pathways or combinations of pathways (Flora and Madore 1996). Certainly, the evolution of different loading types and how these types adapt species to their environment will be important research areas in the future, as loading pathways are clarified in more species.

PHLOEM UNLOADING AND SINK-TO-SOURCE TRANSITION

Now that we have learned about the events leading up to the export of sugars from sources, let's take a look at phloem unloading. In many ways the events in sink tissues are simply the reverse of the events in sources. Transport into sink organs, such as developing roots, tubers, and reproductive structures, is termed **import**. The following steps are involved in the import of sugars into sink cells.

1. *Sieve element unloading*. This is the process by which imported sugars leave the sieve elements of sink tissues.
2. *Short-distance transport*. After sieve element unloading, the sugars are transported to cells in the sink by means of a short-distance transport pathway. This pathway has also been called *post–sieve element transport*.
3. *Storage and metabolism*. In the final step, sugars are stored or metabolized in sink cells.

These three transport steps together constitute **phloem unloading**, the movement of photosynthates from the sieve elements and their distribution to the sink cells that store or metabolize them (Oparka and van Bel 1992).

In this section we will discuss the following questions: Is phloem unloading symplastic or apoplastic? Is sucrose hydrolyzed during the process? Does phloem unloading require energy? Finally, we will examine the transition process by which a young, importing leaf becomes an exporting source leaf.

Phloem Unloading Can Occur via Symplastic or Apoplastic Pathways

In sink organs, sugars move from the sieve elements to the cells that store or metabolize them. Sinks vary widely from growing vegetative organs (root tips and young leaves) to storage tissues (roots and stems) to organs of reproduction and dispersal (fruits and seeds). Because sinks vary so greatly in structure and function, there is no single scheme of phloem unloading. As in sources, the sugars may move entirely through the symplast via the plasmodesmata, or they may enter the apoplast at some point.

Figure 10.18 diagrams several possible phloem-unloading pathways. The unloading pathway appears to be completely symplastic in some young dicot leaves, such as sugar beet and tobacco (Figure 10.18A). Evidence for the symplastic pathway of unloading includes insensitivity to PCMBS (*p*-chloromercuribenzenesulfonic acid), a reagent that inhibits the transport of sucrose across plasma membranes but does not permeate the symplastic pathway. Meristematic and elongating regions of primary root tips also appear to unload symplastically. Sufficient plasmodesmata exist in these pathways to support symplastic unloading.

In some sink organs, part of the phloem-unloading pathway is apoplastic (Figure 10.18B). In principle, the apoplastic step could be located at the site of the sieve element–companion cell complex (type 1 in Figure 10.18B), although this pattern has yet to receive experimental support. The apoplastic step could also be farther removed from the sieve elements (type 2). This arrangement, typical of developing seeds, appears to be the most common in apoplastic phloem unloading.

An apoplastic step is required in developing seeds because there are no symplastic connections between the maternal tissues and the tissues of the embryo. Sugars exit the sieve elements (sieve element unloading) via a symplastic pathway and are transferred from the symplast to the apoplast at some point removed from the sieve element–companion cell complex (type 2 in Figure 10.18B). The apoplastic step permits membrane control over the substances that enter the embryo because two membranes must be crossed in the process.

When phloem unloading is apoplastic, the transport sugar can be partly metabolized in the apoplast, or it can cross the apoplast unchanged (see **Web Topic 10.9**). For example, sucrose can be hydrolyzed into glucose and fructose in the apoplast by invertase, a sucrose-splitting enzyme, and glucose and/or fructose would then enter the sink cells. As we will discuss later, such sucrose-cleaving enzymes play a role in the control of phloem transport by sink tissues.

Transport into Sink Tissues Requires Metabolic Energy

Inhibitor studies have shown that import into sink tissues is energy dependent. Growing leaves, roots, and storage sinks in which carbon is stored in starch or protein utilize symplastic phloem unloading. Transport sugars are used as substrate for respiration and are metabolized into storage polymers and into compounds needed for growth. Sucrose

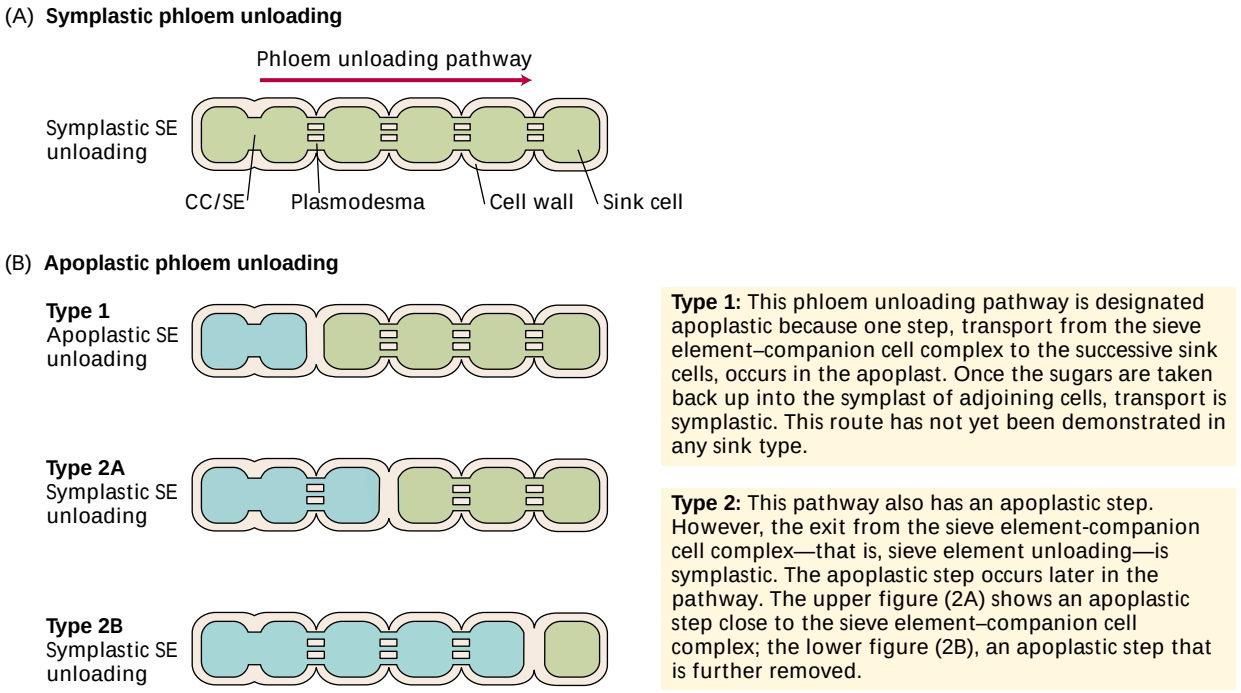


FIGURE 10.18 Pathways for phloem unloading. The sieve element–companion cell complex (CC / SE) is considered a single functional unit. The presence of plasmodesmata is assumed to provide functional symplastic continuity. An absence of plasmodesmata between cells indicates an apoplastic transport step. (A) Symplastic phloem unloading. (B) Three types of apoplastic phloem unloading. (After Oparka and van Bel 1992.)

metabolism results in a low sucrose concentration in the sink cells, thus maintaining a concentration gradient for sugar uptake. No membranes are crossed during sugar uptake into the sink cells, and unloading through the plasmodesmata is passive because transport sugars move from a high concentration in the sieve elements to a low concentration in the sink cells. Metabolic energy is thus required in these sink organs for respiration and for biosynthesis reactions.

In apoplastic phloem unloading, sugars must cross at least two membranes: the plasma membrane of the cell that is exporting the sugar, and the plasma membrane of the sink cell. When sugars are transported into the vacuole of the sink cell, they must also traverse the tonoplast.

As discussed earlier, transport across membranes in an apoplastic pathway may be energy dependent. Developing seeds are valuable experimental systems for studying unloading processes. In legumes such as soybean, the embryo can be removed from the seed, and unloading from the seed coat into the apoplast can be studied without the influence of the embryo. Uptake into the embryo can also be investigated separately. Such studies have shown that energy-requiring transporters mediate both unloading of sucrose into the apoplast and uptake of sucrose into the embryo in soybean (see **Web Topic 10.10**).

The Transition of a Leaf from Sink to Source Is Gradual

Leaves of dicots such as tomato or bean begin their development as sink organs. A transition from sink to source status occurs later in development, when the leaf is approximately 25% expanded, and it is usually complete when the leaf is 40 to 50% expanded.

Export from the leaf begins at the tip or apex of the blade and progresses toward the base until the whole leaf becomes a sugar exporter. During the transition period, the tip exports sugar while the base imports it from the other source leaves (Figure 10.19).

The maturation of leaves is accompanied by a large number of functional and anatomic changes, many of which are needed for the export of photosynthate. The sink-to-source transition is quite different in species with apoplastic versus symplastic loading. In leaves with apoplastic phloem loading, a drastic switch from a symplastic unloading pathway to an apoplastic loading pathway must be made.

In the development of a leaf that will load apoplastically, the cessation of import and the initiation of export are independent events (Turgeon 1984). In albino leaves of tobacco, which have no chlorophyll and therefore are incapable of

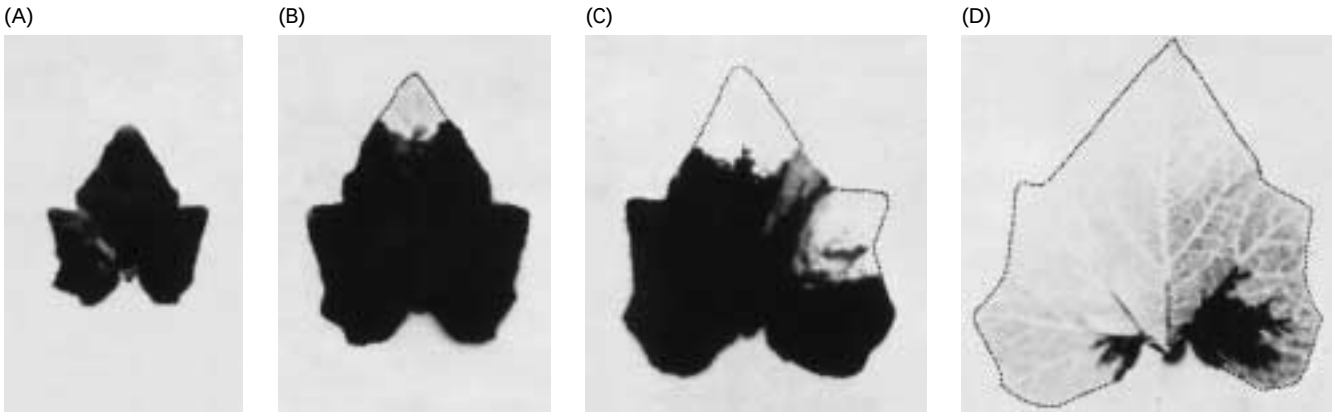


FIGURE 10.19 Autoradiographs of a leaf of summer squash (*Cucurbita pepo*), showing the transition of the leaf from sink to source status. In each case, the leaf imported ^{14}C from the source leaf on the plant for 2 hours. Label is visible as black accumulations. (A) The entire leaf is a sink, importing sugar from the source leaf. (B–D) The base is still a sink. As the tip of the leaf loses the ability to unload and stops importing sugar (as shown by the loss of black accumulations), it gains the ability to load and to export sugar. (From Turgeon and Webb 1973.)

photosynthesis, import stops at the same developmental stage as in green leaves, even though export is not possible. Therefore some other change must occur in developing leaves of tobacco that causes them to cease importing sugars.

Such a change could involve blockage of the unloading pathway at some point in the development of mature leaves. In dicot sink leaves with symplastic unloading, factors that could account for the cessation of unloading include plasmodesmatal closure, a decrease in plasmodesmatal frequency, or another change in symplastic continuity. Experimental data have shown that the unloading pathway is blocked in mature leaves of apoplastic loaders.

Export of sugars begins when phloem loading has accumulated sufficient photosynthate in the sieve elements to drive translocation out of the leaf. In normal leaves with apoplastic loading, export is initiated when

- The symplastic unloading pathway is closed.
- The leaf is synthesizing photosynthate in sufficient quantity that some is available for export.
- The sucrose-synthesizing genes are being expressed.
- The sucrose–H^+ symporter is in place in the plasmalemma of the sieve element–companion cell complex.

In leaves of plants like sugar beet and tobacco, the ability to accumulate exogenous [^{14}C]sucrose in the sieve element–companion cell complex is acquired as the leaves undergo the sink-to-source transition, suggesting that the symporter required for loading has become functional. In developing leaves of *Arabidopsis*, expression of the symporter that is thought to transport sugars during loading begins in the tip and proceeds to the base during a sink-to-source transition. The same basipetal pattern is seen in the development of export capacity.

In tobacco and other *Nicotiana* species, the minor veins that are eventually responsible for most of the loading do not mature until about the time import ceases. Thus, sugars are unloaded and loaded almost entirely via different veins (Roberts et al. 1997).

In leaves in which the symplastic route for unloading is maintained for loading, the transition from import to export is to some extent reversible. In variegated leaves of coleus that have both green and albino regions, the albino portions of mature leaves retain many sinklike characteristics. The green regions of the leaves can export photosynthate to the albino regions; if the green regions are removed, the albino regions can import and unload sugars from other mature leaves.

PHOTOSYNTHATE ALLOCATION AND PARTITIONING

The photosynthetic rate determines the total amount of fixed carbon available to the leaf. However, the amount of fixed carbon available for translocation depends on subsequent metabolic events. The regulation of the diversion of fixed carbon into the various metabolic pathways is termed **allocation**.

The vascular bundles in a plant form a system of pipes that can direct the flow of photosynthates to various sinks: young leaves, stems, roots, fruits, or seeds. However, the vascular system is often highly interconnected, forming an open network that allows source leaves to communicate with multiple sinks. Under these conditions, what determines the volume of flow to any given sink? The differential distribution of photosynthates within the plant is termed **partitioning**.

After giving an overview of allocation and partitioning, we will examine the coordination of starch and sucrose

synthesis. We will conclude by discussing how sinks compete, how sink demand might regulate photosynthetic rate in the source leaf, and how sources and sinks communicate with each other.

Allocation Includes the Storage, Utilization, and Transport of Fixed Carbon

The carbon fixed in a source cell can be used for storage, metabolism, and transport:

- *Synthesis of storage compounds.* Starch is synthesized and stored within chloroplasts and, in most species, is the primary storage form that is mobilized for translocation during the night. Plants that store carbon primarily as starch are called *starch storers.*
- *Metabolic utilization*. Fixed carbon can be utilized within various compartments of the photosynthesizing cell to meet the energy needs of the cell or to provide carbon skeletons for the synthesis of other compounds required by the cell.
- *Synthesis of transport compounds.* Fixed carbon can be incorporated into transport sugars for export to various sink tissues. A portion of the transport sugar can also be stored temporarily in the vacuole (see **Web Topic 10.9**).

Allocation is also a key process in sink tissues. Once the transport sugars have been unloaded and enter the sink cells, they can remain as such or can be transformed into various other compounds. In storage sinks, fixed carbon can be accumulated as sucrose or hexose in vacuoles or as starch in amyloplasts. In growing sinks, sugars can be utilized for respiration and for the synthesis of other molecules required for growth.

Transport Sugars Are Partitioned among the Various Sink Tissues

The greater the ability of a sink to store or metabolize imported sugars (the process of allocation), the greater its ability to compete for photosynthate being exported by the sources. Such competition determines the distribution of transport sugars among the various sink tissues of the plant (photosynthate partitioning), at least in the short term.

Of course, events in sources and sinks must be synchronized. Partitioning determines the patterns of growth, and such growth must be balanced between shoot growth (photosynthetic productivity) and root growth (water and mineral uptake). So an additional level of control lies in the interaction between areas of supply and demand.

Turgor pressure in the sieve elements could be an important means of communication between sources and sinks, acting to coordinate rates of loading and unloading. Chemical messengers are also important in signaling to one organ the status of the other. Such chemical messengers include plant hormones and nutrients, such as potassium and phosphate and even the transport sugars themselves.

Attainment of higher yields of crop plants is one goal of research on photosynthate allocation and partitioning. Whereas grains and fruits are examples of edible yields, total yield includes inedible portions of the shoot. An understanding of partitioning enables plant breeders to select and develop varieties that have improved transport to edible portions of the plant. Significant improvements have been made in the ratio of commercial or edible yield to total shoot yield.

Allocation and partitioning in the whole plant must be coordinated such that increased transport to edible tissues does not occur at the expense of other essential processes and structures. Crop yield will also be improved if photosynthates that are normally "lost" by the plant are retained. For example, losses due to nonessential respiration or exudation from roots could be reduced. In the latter case, care must be taken not to disrupt essential processes outside the plant, such as growth of beneficial microbial species in the vicinity of the root that obtain nutrients from the root exudate.

Allocation in Source Leaves Is Regulated

Increases in the rate of photosynthesis in a source leaf generally result in an increase in the rate of translocation from the source. Control points for the allocation of photosynthate (Figure 10.20) include the allocation of triose phosphates to the following processes:

- Regeneration of intermediates in the C_3 photosynthetic carbon reduction cycle (the Calvin cycle; see Chapter 8)
- Starch synthesis
- Sucrose synthesis, as well as distribution of sucrose between transport and temporary storage pools

Various enzymes operate in the pathways that process the photosynthate, and the control of these steps is complex (Geiger and Servaites 1994.)

During the day the rate of starch synthesis in the chloroplast must be coordinated with sucrose synthesis in the cytosol. Triose phosphates (glyceraldehyde-3-phosphate and dihydroxyacetone phosphate) produced in the chloroplast by the C_3 Calvin cycle (see Chapter 8) can be used for either starch or sucrose synthesis. Sucrose synthesis in the cytoplasm diverts triose phosphate away from starch synthesis and storage. For example, it has been shown that when the demand for sucrose by other parts of a soybean plant is high, less carbon is stored as starch by the source leaves. The key enzymes involved in the regulation of sucrose synthesis in the cytoplasm and of starch synthesis in the chloroplast are sucrose phosphate synthase and fructose-1,6-bisphosphatase in the cytoplasm and ADP-glucose pyrophosphorylase in the chloroplast (see Chapter 8, Figure 10.20, and **Web Topic 10.9**).

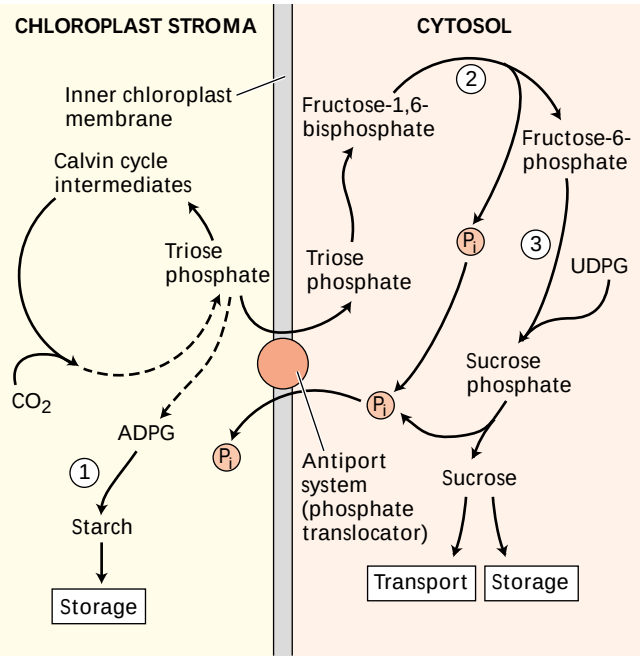


FIGURE 10.20 A simplified scheme for starch and sucrose synthesis during the day. Triose phosphate, formed in the Calvin cycle, can either be utilized in starch formation in the chloroplast or transported into the cytosol in exchange for inorganic phosphate (P_i) via the phosphate translocator in the inner chloroplast membrane. The outer chloroplast membrane is porous to small molecules and is omitted here for clarity. In the cytosol, triose phosphate can be converted to sucrose for either storage in the vacuole or transport. Key enzymes involved are starch synthetase (1), fructose-1,6-bisphosphatase (2), and sucrose phosphate synthase (3). The second and third enzymes, along with ADP-glucose pyrophosphorylase, which forms adenosine diphosphate glucose (ADPG), are regulated enzymes in sucrose and starch synthesis (see Chapter 8). UDPG, uridine diphosphate glucose. (After Preiss 1982.)

However, there is a limit to the amount of carbon that normally can be diverted from starch synthesis in species that store carbon primarily as starch. Studies of allocation between starch and sucrose under different conditions suggest that a fairly steady rate of translocation throughout the 24-hour period is a priority for most plants.

The use of mutants and transgenic plants enables us to ask a new set of questions about allocation. For example, what happens when one of the competing processes, such as starch synthesis, is inhibited or even eliminated? The results have revealed the amazing flexibility of plants. For example, starch-deficient tobacco mutants synthesize only trace amounts of starch but are able to compensate for a lack of stored carbon by doubling the rate of sucrose synthesis and export during the day and by switching most of their growth to the day (Geiger et al. 1995). On the other hand, plants with enhanced starch synthesis during the day often export more of their fixed carbon during the night.

Sink Tissues Compete for Available Translocated Photosynthate

As discussed earlier, translocation to sink tissues depends on the position of the sink in relation to the source and on the vascular connections between source and sink. Another factor determining the pattern of transport is competition between sinks. For example, reproductive tissues (seeds) might compete with growing vegetative tissues (young leaves and roots) for photosynthates in the translocation stream. Competition has been shown by numerous experiments in which removal of a sink tissue from a plant generally results in increased translocation to alternative, and hence competing, sinks.

In the reverse type of experiment, the source supply can be altered while the sink tissues are left intact. When the supply of photosynthates from sources to competing sinks is suddenly and drastically reduced by shading of all the source leaves but one, the sink tissues become dependent on a single source. In sugar beet and bean plants, the rates of photosynthesis and export from the single remaining source leaf usually do not change over the short term (approximately 8 hours; Fondy and Geiger 1980). However, the roots receive less sugar from the single source, while the young leaves receive relatively more. Thus the young leaves are stronger sinks than the roots in these conditions. A stronger sink can deplete the sugar content of the sieve elements more readily and thus increase the pressure gradient and the rate of translocation toward itself.

An effect on the pressure gradient is also indicated indirectly by experiments in which investigators enhance transport to a sink by making the sink water potential more negative. Treatment of the root tips of pea seedlings with 350 m*M* mannitol solutions increased the import of [^{14}C]sucrose by more than 300%, presumably because of a turgor decrease in the sink cells (Schulz 1994).

Sink Strength Is a Function of Sink Size and Sink Activity

Various experiments indicate that the ability of a sink to mobilize photosynthate toward itself, the **sink strength**, depends on two factors—sink size and sink activity—as follows:

$$\text{Sink strength} = \text{sink size} \times \text{sink activity}$$

Sink size is the total weight of the sink tissue, and **sink activity** is the rate of uptake of photosynthates per unit weight of sink tissue. Altering either the size or the activity of the sink results in changes in translocation patterns. For example, the ability of a pea pod to import carbon depends on the dry weight of that pod as a proportion of the total number of pods (Jeuffroy and Warembourg 1991).

Changes in sink activity can be complex because various activities in sink tissues can potentially limit the rate of uptake by the sink. These activities include unloading from the sieve elements, metabolism in the cell wall, uptake from

the apoplast, and metabolic processes that use the photosynthate in either growth or storage.

Cooling a sink tissue inhibits activities that require metabolic energy and results in a decrease in the speed of transport toward the sink. In corn, a mutant that has a defective enzyme for starch synthesis in the kernels transports less material to the kernels than does its normal counterpart (Koch et al. 1982). In this mutant, a deficiency in photosynthate storage leads to an inhibition of transport.

Sink activity and thus sink strength are also thought to be related to the presence and activity of the sucrose-splitting enzymes acid invertase and sucrose synthase because they catalyze the first step in sucrose utilization. Whether these enzymes control sink strength or are simply correlated with sink metabolism and growth is currently an active topic of research. Interestingly, the genes for sucrose synthase and invertase are among those regulated by carbohydrate supply. In general, carbohydrate depletion enhances the expression of genes for photosynthesis, reserve mobilization, and export processes, while abundant carbon resources favor genes for storage and utilization (Koch 1996).

However, the finding that different isoforms of sucrose synthase, encoded by different genes, respond in opposite ways to carbohydrate supply, indicates that the overall picture is complex. For example, the mRNA for one gene for sucrose synthase in corn roots is widely distributed in root tissues and is maximally expressed when sugars are abundant. The mRNA of a second sucrose synthase gene is most abundant in the epidermis and outer tissues of the root and is maximally expressed under conditions of sugar depletion. Thus, utilization of imported sugars is broadly maximized when sugars are abundant, but when sugar supply is low, utilization is increasingly restricted to sites that are crucial for uptake of water and minerals (Koch et al. 1996).

In addition, genes for invertase and sucrose synthase are often expressed at different times during sink development. In bean pods and corn kernels, changes in invertase activity are found to precede changes in photosynthate import. These results point to a key role of invertase and sucrose synthase in controlling import patterns, both during the genetic program of sink development and during responses to environmental stresses (see **Web Topic 10.9**).

Changes in the Source-to-Sink Ratio Cause Long-Term Alterations in the Source

If all but one of the source leaves of a soybean plant are shaded for an extended period (e.g., 8 days), many changes occur in the single remaining source leaf. These changes include a decrease in starch concentration and increases in photosynthetic rate, rubisco activity, sucrose concentration, transport from the source, and orthophosphate concentration (Thorne and Koller 1974). These data indicate that, besides the observed short-term changes in the distribution of photosynthate among different sinks, the metabolism of the source adjusts to the altered conditions in long-term experiments.

Photosynthetic rate (the net amount of carbon fixed per unit leaf area per unit time) often increases over several days when sink demand increases, and it decreases when sink demand decreases. Photosynthesis is most strongly inhibited under conditions of reduced sink demand in plants that normally store starch, rather than sucrose, during the day. Perhaps an accumulation of photosynthate (starch, sucrose, or hexoses) in the source leaf could account for the linkage between sink demand and photosynthetic rate in starch-storing plants (see **Web Topic 10.11**).

Long-Distance Signals May Coordinate the Activities of Sources and Sinks

Besides having a major function in the long-distance transport of photosynthate, the phloem is a conduit for the transport of signal molecules from one part of the organism to another. Signals between sources and sinks might be physical (such as turgor pressure) or chemical (such as plant hormones and carbohydrates). Signals indicating turgor change could be transmitted rapidly via the interconnecting system of sieve elements.

For example, if phloem unloading were rapid under conditions of rapid sugar utilization at the sink tissue, turgor pressures in the sieve elements of sinks would be reduced, and this reduction would be transmitted to the sources. If loading were controlled in part by turgor in the sieve elements of the source, it would increase in response to this signal from the sinks. The opposite response would be seen when unloading was slow in the sinks. Some data suggest that cell turgor can modify the activity of the proton-pumping ATPase at the plasma membrane and therefore alter transport rates.

Shoots produce growth regulators such as auxin (see Chapter 19), which can be rapidly transported to the roots via the phloem; and roots produce cytokinins (see Chapter 21), which move to the shoots through the xylem. Gibberellins (GA) and abscisic acid (ABA) (see Chapters 20 and 23) are also transported throughout the plant in the vascular system. Plant hormones play a role in regulating source–sink relationships. They affect photosynthate partitioning by controlling sink growth, leaf senescence, and other developmental processes.

Loading of sucrose in castor bean is stimulated by exogenous auxin but inhibited by ABA, while exogenous ABA enhances, and auxin inhibits, sucrose uptake by sugar beet taproot tissue. Active transporters in plasma membranes are obvious targets for regulation of apoplastic loading and unloading by hormones. Other potential sites of hormone regulation of unloading include tonoplast transporters, enzymes for metabolism of incoming sucrose, wall extensibility, and plasmodesmatal permeability in the case of symplastic unloading (see the next section).

As indicated earlier, carbohydrate levels can influence the expression of encoding photosynthesis component genes, as well as genes involved in sucrose hydrolysis. Many genes have been shown to be responsive to sugar depletion and abundance (Koch 1996). Thus, not only is sucrose transported in the phloem, but sucrose or its metabolites can act as signals that modify the activities of sources and sinks. In sugar beet, for example, proton–sucrose symporter activity declines in plasma membrane vesicles isolated from source leaves fed exogenous sucrose through the xylem.

The loss of symporter activity is accompanied by a decline in symporter mRNA, suggesting an effect on transcription or mRNA stability. A working model includes the following steps: (1) Decreased sink demand leads to high sucrose levels in the vascular tissue; (2) high sucrose levels lead to down-regulation of the symporter in the source; (3) decreased loading results in increased sucrose concentrations in the source (Chiou and Bush 1998). Increased sucrose concentrations in the source can result in a lower photosynthetic rate (see **Web Topic 10.11**). An increase of starch accumulation in source leaves of plants transformed with antisense DNA to the sucrose symporter SUT1 also supports this model (Schulz et al. 1998).

In some source–sink systems, sugars and other metabolites have been shown to interact with hormonal signals to control gene expression (Thomas and Rodriguez 1994).

Long-Distance Signals May Also Regulate Plant Growth and Development

It has long been known that viruses can move in the phloem, traveling as complexes of proteins and nucleic acids or as intact virus particles. More recently, endogenous mRNA molecules and proteins have been found in phloem sap, and at least some of these are thought to be signal molecules.

The following pathway appears to be open to the movement of macromolecules over long distances: from companion cells of sources to source sieve elements, through the path to sink sieve elements, to companion cells of the sink, and finally to cells of the sink itself.

Proteins synthesized in companion cells can clearly enter the sieve elements through the plasmodesmata that connect the two cell types. As noted earlier, both the SUT1 transporter in the plasma membrane of the sieve element and P-proteins in cucurbit sap (PP1 and PP2) appear to be synthesized in companion cells. The plasmodesmata connecting the companion cells and sieve elements must thus allow these macromolecules to move across them. Viral particles have been observed in the plasmodesmata.

Some of the proteins that enter sieve elements may simply diffuse through the plasmodesmata into the sieve elements, others may mediate their own cell-to-cell transport, and yet others may be aided by specific control proteins (Mezitt and Lucas 1996). Passive movement of proteins from companion cells to sieve elements has been demonstrated in *Arabidopsis* and tobacco plants, transformed with the gene for a green fluorescent protein (GFP) from jellyfish, under the control of the SUC2 promoter from *Arabidopsis*.

The SUC2 sucrose–H^+ symporter is synthesized within the companion cells, so proteins expressed under the control of its promoter are also synthesized in the companion cells. GFP, which is localized by its fluorescence after excitation with blue light, moves through plasmodesmata from companion cells into sieve elements and migrates within the phloem to sink tissues. Because jellyfish GFP is unlikely to possess specific sequences for interaction with plasmodesmatal structures, its movement into sieve elements is likely to occur by passive diffusion (Imlau et al. 1999).

Once in the sieve elements, some proteins (e.g., SUT1) are targeted to the plasma membrane or other cellular locations, while other proteins move with the translocation stream to sink tissues. Proteins moving to sinks in the phloem include the P-proteins PP1 and PP2. Subunits of P-proteins from cucumber (*Cucumis sativus*) can move across graft unions from the cucumber stock (basal graft partner) to a pumpkin (*Cucurbita maxima*) scion (upper graft partner). One experiment showed that the smaller PP2 protein is able to move from the sieve elements to companion cells of the scion stem; the larger PP1 was not detected in the companion cells. Neither protein was able to move beyond the sieve element–companion cell complex (Golecki et al. 1999). These proteins may be too large to pass through the plasmodesmata that surround the sieve element–companion cell complex, or they may lack recognition factors allowing interaction with the plasmodesmata (Oparka and Santa Cruz 2000). In contrast, the jellyfish green fluorescent protein is unloaded symplastically through the plasmodesmata into sink tissues, such as seed coats, anthers, root tips, and mesophyll cells in importing leaves (Imlau et al. 1999).

Clearly, proteins can be transported from the companion cells in the source through the intervening sieve elements to sink companion cells. However, little evidence exists for a similar movement of proteins synthesized outside the companion cells. Other signals from outside the sieve element–companion cell complex may give rise to the production of mobile proteins in the companion cells. Evidence also exists for the translocation via the phloem of mRNA molecules that are involved in sink tissue development (Oparka and Santa Cruz 2000). To be assigned a signaling role in plants, a macromolecule must be able to leave the sieve element–companion cell complex in sink tissues, and perhaps most importantly, it must be able to modify the functions of specific cells in the sink (Oparka and Santa Cruz 2000). Such demonstrations await the results of future experimentation.

Plasmodesmata can exercise dynamic control of the intercellular diffusion of small molecules (Lucas et al. 1993; Baluska et al. 2001). RNA and protein also move from cell to cell in plants via plasmodesmata. Virally encoded

"movement proteins" interact directly with plasmodesmata to allow the passage of viral nucleic acids. Potato plants transformed with the tobacco mosaic virus movement protein show altered allocation patterns in source leaves (Olesinski et al. 1996) and modified whole-plant partitioning patterns (Almon et al. 1997). The modification of source leaf allocation depends on whether the movement protein is expressed in mesophyll and bundle sheath cells or in phloem parenchyma and companion cells.

Plasmodesmata have been implicated in nearly every aspect of phloem translocation, from loading to long-distance transport (remember that pores in sieve areas and sieve plates are modified plasmodesmata) to allocation and partitioning. Future research on phloem translocation and on the roles of plasmodesmata in plant growth and development will surely go hand in hand.

SUMMARY

Translocation in the phloem is the movement of the products of photosynthesis from mature leaves to areas of growth and storage. The phloem also redistributes water and various compounds throughout the plant body.

Some aspects of phloem translocation have been well established by extensive research over many years. These include the following:

- *The pathway of translocation*. Sugars and other organic materials are conducted throughout the plant in the phloem, specifically in cells called sieve elements. Sieve elements display a variety of structural adaptations that make them well suited for transport.
- *Patterns of translocation*. Materials are translocated in the phloem from sources (areas of photosynthate supply) to sinks (areas of metabolism or storage of photosynthate). Sources are usually mature leaves. Sinks include organs such as roots and immature leaves and fruits.
- *Materials translocated in the phloem*. The translocated solutes are mainly carbohydrates, and sucrose is the most commonly translocated sugar. Phloem sap also contains other organic molecules, such as amino acids, proteins, and plant hormones, as well as inorganic ions.
- *Rates of movement*. Rates of movement in the phloem are quite rapid, well in excess of rates of diffusion. Velocities average 1 m h^{-1}, and mass transfer rates range from 1 to 15 g h^{-1} cm^{-2} of sieve elements.

Other aspects of phloem translocation require further investigation, and most of these are being studied intensively at the present time. These aspects include the following:

- *Phloem loading and unloading*. Transport of sugars into and out of the sieve elements is called sieve element loading and unloading, respectively. In some species, sugars must enter the apoplast of the source leaf before loading. In these plants, loading into the sieve elements requires metabolic energy, provided in the form of a proton gradient. In other species, the whole pathway from the photosynthesizing cells to the sieve elements occurs in the symplast of the source leaf. In either case, phloem loading is specific for the transported sugar. Phloem unloading requires metabolic energy, but the transport pathway, the site of metabolism of transport sugars, and the site where energy is expended vary with the organ and species.
- *Mechanism of translocation*. Pressure flow is well accepted as the most probable mechanism of phloem translocation. In this model the bulk flow of phloem sap occurs in response to an osmotically generated pressure gradient. A variety of structural and physiological data indicate that materials are translocated in the phloem of angiosperms by pressure flow. The mechanism of translocation in gymnosperms requires further investigation.
- *Photosynthate allocation and partitioning*. Allocation is the regulation of the quantities of fixed carbon that are channeled into various metabolic pathways. In sources, the regulatory mechanisms of allocation determine the quantities of fixed carbon that will be stored (usually as starch), metabolized within the cells of the source, or immediately transported to sink tissues. In sinks, transport sugars are allocated to growth processes or to storage. Partitioning is the differential distribution of photosynthates within the whole plant. Partitioning mechanisms determine the quantities of fixed carbon delivered to specific sink tissues. Phloem loading and unloading, and photosynthate allocation and partitioning, are of great research interest because of their roles in crop productivity.

Web Material

Web Topics

10.1 Classical Studies on Phloem Transport
Classical experiments illustrate some basic properties of phloem transport.

10.2 Sampling Phloem Sap
Aphid stylets are optimally suited to sample phloem sap.

10.3 Nitrogen Transport in Soybean
Nitrogen compounds synthesized in the roots are transferred from the xylem to the phloem.

10.4 Monitoring Traffic on the Sugar Freeway
Sugar transport rates in the phloem are measured with radioactive tracers.

10.5 Evidence for Apoplastic Loading of Sieve Elements

Transgenic plants have provided experimental support for apoplastic loading.

10.6 Some Substances Enter the Phloem by Diffusion

Substances such as plant hormones might enter the phloem by diffusion.

10.7 Localization of the Sucrose–H^+ Symporter in the Phloem of Apoplastic Loaders

The sucrose H^+ symporter of companion cells has been localized using fluorescent dyes.

10.8 Physiological Evidence for Symplastic Continuity in Source Leaves

Fluorescent dyes have also been used to show symplastic continuity in source leaves.

10.9 Sugars in the Phloem

The transport, allocation, and metabolism of phloem sugars are tightly regulated.

10.10 Energy Requirements for Unloading in Developing Seeds and Storage Organs

Unloading of seed storage sugars into the embryo is mediated by active transporters.

10.11 Possible Mechanisms Linking Sink Demand and Photosynthetic Rate in Starch Storers

Photosynthate accumulation increases sink demand.

Chapter References

Almon, E., Horowit, M., Wang, H.-L., Lucas, W. J., Zamski, E., and Wolf, S. (1997) Phloem-specific expression of the tobacco mosaic virus movement protein alters carbon metabolism and partitioning in transgenic potato plants. *Plant Physiol.* 115: 1599–1607.

Baluska, F., Cvrckova, F., Kendrick-Jones, J., and Volkmann, D. (2001) Sink plasmodesmata as gateways for phloem unloading. Myosin VIII and calreticulin as molecular determinants of sink strength? *Plant Physiol.* 126: 39–46.

Barker, L., Kuehn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., Hellmann, H., Schulze, W., Ward, J. M., and Frommer, W. B. (2000) SUT2, a putative sucrose sensor in sieve elements. *Plant Cell* 12: 1153–1164.

Bostwick, D. E., Dannenhoffer, J. M., Skaggs, M. I., Lister, R. M., Larkins, B. A., and Thompson, G. A. (1992) Pumpkin phloem lectin genes are specifically expressed in companion cells. *Plant Cell* 4: 1539–1548.

Bouche-Pillon, S., Fleurat-Lessard, P., Fromont, J.-C., Serrano, R., and Bonnemain, J.-L. (1994) Immunolocalization of the plasma membrane H^+-ATPase in minor veins of *Vicia faba* in relation to phloem loading. *Plant Physiol.* 105: 691–697.

Brentwood, B., and Cronshaw, J. (1978) Cytochemical localization of adenosine triphosphatase in the phloem of *Pisum sativum* and its relation to the function of transfer cells. *Planta* 140: 111–120.

Chiou, T.-J., and Bush, D. R. (1998) Sucrose is a signal molecule in assimilate partitioning. *Proc. Natl. Acad. Sci. USA* 95: 4784–4788.

Clark, A. M., Jacobsen, K. R., Bostwick, D. E., Dannenhoffer, J. M., Skaggs, M. I., and Thompson, G. A. (1997) Molecular characterization of a phloem-specific gene encoding the filament protein, phloem protein 1 (PP1), from *Cucurbita maxima*. *Plant J.* 12: 49–61.

DeWitt, N. D., and Sussman, M. R. (1995) Immunocytological localization of an epitope-tagged plasma membrane proton pump (H^+-ATPase) in phloem companion cells. *Plant Cell* 7: 2053–2067.

Eschrich, W. (1975) Bidirectional transport. In *Transport in Plants*, 1: *Phloem Transport* (Encyclopedia of Plant Physiology, New Series, Vol. 1), M. H. Zimmermann and J. A. Milburn, eds., Springer, New York, pp. 245–255.

Evert, R. F. (1982) Sieve-tube structure in relation to function. *BioScience* 32: 789–795.

Evert, R. F., and Mierzwa, R. J. (1985) Pathway(s) of assimilate movement from mesophyll cells to sieve tubes in the *Beta vulgaris* leaf. In *Phloem Transport. Proceedings of an International Conference on Phloem Transport, Asilomar, CA*, J. Cronshaw, W. J. Lucas, and R. T. Giaquinta, eds. Liss, New York, pp. 419–432.

Fisher, D. B. (1978) An evaluation of the Munch hypothesis for phloem transport in soybean. *Planta* 139: 25–28.

Flora, L. L., and Madore, M. A. (1996) Significance of minor-vein anatomy to carbohydrate transport. *Planta* 198: 171–178.

Fondy, B. R. (1975) Sugar selectivity of phloem loading in *Beta vulgaris*, *vulgaris* L. and *Fraxinus americanus*, *americana* L. Thesis, University of Dayton, Dayton, OH.

Fondy, B. R., and Geiger, D. R. (1980) Effect of rapid changes in sink–source ratio, on export and distribution of products of photosynthesis in leaves of *Beta vulgaris* L. and *Phaseolus vulgaris* L. *Plant Physiol.* 66: 945–949.

Geiger, D. R., and Servaites, J. C. (1994) Diurnal regulation of photosynthetic carbon metabolism in C_3 plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45: 235–256.

Geiger, D. R., and Sovonick, S. A. (1975) Effects of temperature, anoxia and other metabolic inhibitors on translocation. In *Transport in Plants*, 1: *Phloem Transport* (Encyclopedia of Plant Physiology, New Series, Vol. 1), M. H. Zimmerman and J. A. Milburn, eds., Springer, New York, pp. 256–286.

Geiger, D. R., Giaquinta, R. T., Sovonick, S. A., and Fellows, R. J. (1973) Solute distribution in sugar beet leaves in relation to phloem loading and translocation. *Plant Physiol.* 52: 585–589.

Geiger, D. R., Shieh, W.-J., and Yu, X.-M. (1995) Photosynthetic carbon metabolism and translocation in wild-type and starch-deficient mutant *Nicotiana sylvestris* L. *Plant Physiol.* 107: 507–514.

Giaquinta, R. T., and Geiger, D. R. (1977) Mechanism of cyanide inhibition of phloem translocation. *Plant Physiol.* 59: 178–180.

Goggin, F. L., Medville, R., and Turgeon, R. (2001) Phloem loading in the tulip tree. Mechanisms and evolutionary implications. *Plant Physiol.* 124: 891–899.

Golecki, B., Schulz, A., and Thompson, G. A. (1999) Translocation of structural P proteins in the phloem. *Plant Cell* 11: 127–140.

Grusak, M. A., Beebe, D. U., and Turgeon, R. (1996) Phloem loading. In *Photoassimilate Distribution in Plants and Crops: Source–Sink Relationships*, E. Zamski and A. A. Schaffer, eds., Dekker, New York, pp. 209–227.

Hall, S. M., and Baker, D. A. (1972) The chemical composition of *Ricinus* phloem exudate. *Planta* 106: 131–140.

Imlau, A., Truernit, E., and Sauer, N. (1999) Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. *Plant Cell* 11: 309–322.

Jeuffory, M.-H., and Warembourg, F. R. (1991) Carbon transfer and partitioning between vegetative and reproductive organs in *Pisum sativum* L. *Plant Physiol.* 97: 440–448.

Joy, K. W. (1964) Translocation in sugar beet. I. Assimilation of $^{14}CO_2$ and distribution of materials from leaves. *J. Exp. Bot.* 15: 485–494.

Knoblauch, M., and Van Bel, A. J. E. (1998) Sieve tubes in action. *Plant Cell* 10: 35–50.

Koch, K. E. (1996) Carbohydrate-modulated gene expression in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 509–540.

Koch, K. E., Tsui, C.-L., Schrader, L. E., and Nelson, O. E. (1982) Source–sink relations in maize mutants with starch deficient endosperms. *Plant Physiol.* 70: 322–325.

Koch, K. E., Wu, Y., and Xu, J. (1996) Sugar and metabolic regulation of genes for sucrose metabolism: Potential influence of maize sucrose synthase and soluble invertase responses on carbon partitioning and sugar sensing. *J. Exp. Bot.* 47 (special issue): 1179–1185.

Kuehn, C., Franceschi, V. R., Schulz, A., Lemoine, R., and Frommer, W. B. (1997) Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. *Science* 275: 1298–1300.

Lalonde, S., Boles, E., Hellmann, H., Barker, L., Patrick, J. W., Frommer, W. B., and Ward, J. M. (1999) The dual function of sugar carriers: Transport and sugar sensing. *Plant Cell* 11: 707–726.

Langhans, M., Ratajczak, R., Luetzelschwab, M., Michalke, W., Waechter, R., Fischer-Schliebs, E., and Ullrich, C. I. (2001) Immunolocalization of plasma-membrane H^+-ATPase and tonoplast-type pyrophosphatase in the plasma membrane of the sieve element-companion cell complex in the stem of *Ricinus communis* L. *Planta* 213: 11–19.

Lucas, W. J., Ding, B., and Van der Schoot, C. (1993) Plasmodesmata and the supracellular nature of plants. *New Phytol.* 125: 435–476.

Mezitt, L. A., and Lucas, W. J. (1996) Plasmodesmal cell-to-cell transport of proteins and nucleic acids. *Plant Mol. Biol.* 32: 251–273.

Münch, E. (1930) *Die Stoffbewegungen in der Pflanze*. Gustav Fischer, Jena, Germany.

Nobel, P. S. (1991) *Physicochemical and Environmental Plant Physiology*. Academic Press, San Diego, CA.

Olesinski, A. A., Almon, E., Navot, N., Perl, A., Galun, E., Lucas, W. J., and Wolf, S. (1996) Tissue-specific expression of the tobacco mosaic virus movement protein in transgenic potato plants alters plasmodesmal function and carbohydrate partitioning. *Plant Physiol.* 111: 541–550.

Oparka, K. J., and Santa Cruz, S. (2000) The great escape: Phloem transport and unloading of macromolecules. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51: 323–347.

Oparka, K. J., and van Bel, A. J. E. (1992) Pathways of phloem loading and unloading: a plea for a uniform terminology. In *Carbon Partitioning within and between Organisms*, C. J. Pollock, J. F. Farrar, and A. J. Gordon, eds., BIOS Scientific, Oxford, pp. 249–254.

Preiss, J. (1982) Regulation of the biosynthesis and degradation of starch. *Annu. Rev. Plant Physiol.* 33: 431–454.

Roberts, A. G., Santa Cruz, S., Roberts, I. M., Prior, D. A. M., Turgeon, R., and Oparka, K. J. (1997) Phloem unloading in sink leaves of *Nicotiana benthamiana*: Comparison of a fluorescent solute with a fluorescent virus. *Plant Cell* 9: 1381–1396.

Schobert, C., Grossmann, P., Gottschalk, M., Komor, E., Pecsvaradi, A., and zur Nieden, U. (1995) Sieve-tube exudate from *Ricinus communis* L. seedlings contains ubiquitin and chaperones. *Planta* 196: 205–210.

Schulz, A. (1990) Conifers. In *Sieve Elements: Comparative Structure, Induction and Development*. H.-D. Behnke and R. D. Sjolund, eds. Springer-Verlag, Berlin.

Schulz, A. (1992) Living sieve cells of conifers as visualized by confocal, laser-scanning fluorescence microscopy. *Protoplasma* 166: 153–164.

Schulz, A. (1994) Phloem transport and differential unloading in pea seedlings after source and sink manipulations. *Planta* 192: 239–248.

Schulz, A., Kuhn, C., Riesmeier, J. W., and Frommer, W. B. (1998) Ultrastructural effects in potato leaves due to antisense-inhibition of the sucrose transporter indicate an apoplasmic mode of phloem loading. *Planta* 206: 533–543.

Stadler, R., Brandner, J., Schulz, A., Gahrtz, M., and Sauer, N. (1995) Phloem loading by the PmSUC2 sucrose carrier from *Plantago major* occurs into companion cells. *Plant Cell* 7: 1545–1554.

Thomas, B. R., and Rodriguez, R. L. (1994) Metabolite signals regulate gene expression and source/sink relations in cereal seedlings. *Plant Physiol.* 106: 1235–1239.

Thorne, J. H., and Koller, H. R. (1974) Influence of assimilate demand on photosynthesis, diffusive resistances, translocation, and carbohydrate levels of soybean leaves. *Plant Physiol.* 54: 201–207.

Truernit, E., and Sauer, N. (1995) The promoter of the *Arabidopsis thaliana* SUC2 sucrose-H^+ symporter gene directs expression of β-glucuronidase to the phloem: Evidence for phloem loading and unloading by SUC2. *Planta* 196: 564–570.

Turgeon, R. (1984) Termination of nutrient import and development of vein loading capacity in albino tobacco leaves. *Plant Physiol.* 76: 45–48.

Turgeon, R., and Gowan, E. (1990) Phloem loading in *Coleus blumei* in the absence of carrier-mediated uptake of export sugar from the apoplast. *Plant Physiol.* 94: 1244–1249.

Turgeon, R., and Webb, J. A. (1973) Leaf development and phloem transport in *Cucurbita pepo*: Transition from import to export. *Planta* 113: 179–191.

Turgeon, R., Beebe, D. U., and Gowan, E. (1993) The intermediary cell: Minor-vein anatomy and raffinose oligosaccharide synthesis in the Scrophulariaceae. *Planta* 191: 446–456.

Turgeon, R., Medville, R., and Nixon, K. C. (2001) The evolution of minor vein phloem and phloem loading. *Am. J. Bot.* 88: 1331–1339.

van Bel, A. J. E. (1992) Different phloem-loading machineries correlated with the climate. *Acta Bot. Neerl.* 41: 121–141.

van Bel, A. J. E., Gamalei, Y. V., Ammerlaan, A., and Bik, L. P. M. (1992) Dissimilar phloem loading in leaves with symplasmic or apoplasmic minor-vein configurations. *Planta* 186: 518–525.

Warmbrodt, R. D. (1985) Studies on the root of *Hordeum vulgare* L.—Ultrastructure of the seminal root with special reference to the phloem. *Am. J. Bot.* 72: 414–432.

Weise, A., Barker, L., Kuehn, C., Lalonde, S., Buschmann, H., Frommer, W. B., and Ward, J. M. (2000) A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *Plant Cell* 12: 1345–1355.

Wright, J. P., and Fisher, D. B. (1980) Direct measurement of sieve tube turgor pressure using severed aphid stylets. *Plant Physiol.* 65: 1133–1135.

Yoo, B.-C., Aoki, K., Xiang, Y., Campbell, L. R., Hull, R. J., Xoconostle-Cazares, B., Monzer, J., Lee, J.-Y., Ullman, D. E., and Lucas, W. J. (2000) Characterization of *Cucurbita maxima* phloem serpin-1 (CmPS-1): A developmentally regulated elastase inhibitor. *J. Biol. Chem.* 275: 35122–35128.

Zimmermann, M. H., and Milburn, J. A., eds. (1975) *Transport in Plants*, 1: *Phloem Transport* (Encyclopedia of Plant Physiology, New Series, Vol. 1). Springer, New York.