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Minor vein structure and sugar transport in *Arabidopsis thaliana*

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Abstract. Leaf and minor vein structure were studied in *Arabidopsis thaliana* (L.) Heynh. to gain insight into the mechanism(s) of phloem loading. Vein density (length of veins per unit leaf area) is extremely low. Almost all veins are intimately associated with the mesophyll and are probably involved in loading. In transverse sections of veins there are, on average, two companion cells for each sieve element. Phloem parenchyma cells appear to be specialized for delivery of photoassimilate from the bundle sheath to sieve element-companion cell complexes: they make numerous contacts with the bundle sheath and with companion cells and they have transfer cell wall ingrowths where they are in contact with sieve elements. Plasmodesmatal frequencies are high at interfaces involving phloem parenchyma cells. The plasmodesmata between phloem parenchyma cells and companion cells are structurally distinct in that there are several branches on the phloem parenchyma cell side of the wall and only one branch on the companion cell side. Most of the translocated sugar in *A. thaliana* is sucrose, but raffinose is also transported. Based on structural evidence, the most likely route of sucrose transport is from bundle sheath to phloem parenchyma cells through plasmodesmata, followed by efflux into the apoplasm across wall ingrowths and carrier-mediated uptake into the sieve element-companion cell complex.

Key words: *Arabidopsis* (sucrose transport) – Minor vein – Phloem loading – Plasmodesmata – Raffinose – Sucrose transport

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Abbreviations: CC = companion cell; SE = sieve element; SE-CCC = sieve element-companion cell complex

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Introduction

Phloem loading is the active accumulation of solute, against a concentration gradient, into the sieve element-companion cell complex (SE-CCC). As a consequence of loading, solute concentration and pressure are elevated at the source end of the phloem.

Phloem loading is not driven by a single ubiquitous mechanism in all plants (Bush 1993; van Bel 1993; Grusak et al. 1996; Komor et al. 1996; Rentsch and Frommer 1996; Turgeon 1996). Two large data sets, derived from the analysis of hundreds of species, have been especially useful in understanding diversity in phloem loading. Gamalei (1989, 1991) analyzed plasmodesmatal frequencies at the interface between the SE-CCC and surrounding cells in leaf minor veins, while Zimmermann and Ziegler (1975) analyzed the sugar composition of phloem exudate.

The apoplasmic loading mechanism has been studied most extensively. In plants that load exclusively via the apoplasm, there are relatively few plasmodesmata connecting the SE-CCC to surrounding cells, i.e. the complex is somewhat isolated symplasmically (Gamalei's Type 2 species). Also, sucrose is the only transport sugar. In these plants, sucrose from mesophyll cells is delivered to the apoplasm in the vicinity of the SE-CCC. From the apoplasm, the sucrose is taken up into the SE-CCC by co-transport with protons (Bush 1993; Sauer et al. 1994). Apoplasmic loaders include many important crop plants such as sugar beet (Geiger 1976), corn (Fritz et al. 1983), and tobacco (von Schaewen et al. 1990).

In other species (Gamalei's Type 1), numerous plasmodesmata link minor-vein companion cells (CCs) to surrounding cell types. It is often assumed that these plants load symplasmically. However, it is clear that plants cannot be neatly divided into apoplasmic and symplasmic loaders based simply on plasmodesmatal frequencies. Type 1 plants fall into at least two categories and evidence for symplasmic loading has only been obtained for one of them. These are species in which the minor-vein CCs are specialized as "intermediary cells."

Intermediary cells have a distinct appearance and are connected to the bundle sheath, not to phloem parenchyma cells, by dense fields of branched plasmodesmata (Turgeon et al. 1993). The presence of intermediary cells is always correlated with the translocation of considerable raffinose and stachyose, especially the latter, in addition to sucrose. Species with intermediary cells include the cucurbits (Turgeon et al. 1975; Schmitz et al. 1987) and members of the Lamiaceae (Fisher 1986; Gamalei 1991), Verbenaceae (Gamalei 1991), Scrophulariaceae (Turgeon et al. 1993), and Oleaceae (Gamalei 1989; Flora and Madore 1993). A "polymer trap" mechanism of phloem loading has been proposed that explains the coincidence of intermediary cell structure and stachyose transport (Turgeon 1991, 1996).

Other Type 1 plants which do not have intermediary cells translocate limited amounts of raffinose-family sugars. This is also true of Gamalei's Type 1-2a species, with plasmodesmatal frequencies between those of Types 1 and 2. While such plants have extensive symplasmic continuity between minor-vein phloem and surrounding cells, they apparently do not phloem load by the same mechanism as those with intermediary cells.

Clearly, there is much to be learned by the comparative approach, especially by focusing on plants that do not have the typical vein structures and sugar chemistry most often associated with apoplasmic or symplasmic loading. We report here on *Arabidopsis thaliana*. Analysis of minor-vein ultrastructure and plasmodesmatal frequencies indicates that *Arabidopsis* is a Type 1-2a species by Gamalei's definition. The predominant transport sugar is sucrose, although some raffinose is also translocated.

Materials and methods

Plant material. Plants of *Arabidopsis thaliana* (L.) Heynh.(Columbia ecotype) were grown in Cornell Mix (Boodley and Sheldrake 1977) without perlite. Plants were grown in a controlled-environment growth room with a 12 h/12 h light/dark cycle at 20 °C in the light and 18 °C in the dark.

Microscopy. Whole leaves were cleared in 95% ethanol, washed in water, and stained for 1 or more days in 0.01% (w/v) saffranin O in water. Leaves were examined and photographed in the same dilute stain solution, without destaining the tissue. For light and electron microscopy, pieces of leaf tissue approximately 2 mm^2 were removed from the leaf and fixed in 2% glutaraldehyde, 2% paraformaldehyde, 0.1% (w/v) tannic acid, and 150 mM sucrose in 70 mM sodium cacodylate buffer (pH 6.8) for 4 h at room temperature. Fixed tissue was washed in buffer and post-fixed for 1 h in 1% OsO_4 in the same buffer. Tissue was then washed in buffer, stained for 30 min with 2% (w/v) uranyl acetate, dehydrated in a graded ethanol series at 4 °C, and embedded in Spurr resin (Electron Microscopy Sciences, Ft. Washington, Pa., USA). Semithin sections for light microscopy were stained with toluidine blue. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate and photographed at 60 kV with a Philips (Eindhoven, The Netherlands) EM-300 transmission electron microscope. For morphometric analysis, images were captured digitally and viewed in either Adobe Photoshop 4.0 or NIH Image (http://rsb.info.nih.gov/nih-image/).

Plasmodesmata were often branched; we refer to each branch as a plasmodesmatal channel. A functional measure of the flow pathway for solutes requires knowledge of the number of channels, not necessarily the number of plasmodesmata. Electron micrographs were used to calculate frequency of plasmodesmatal channels per unit length of vein on each side of the common wall between cell types. Since a plasmodesmatal channel might not cross the entire half of the wall in a given section, it was counted only if it traversed at least half this distance. The frequency of plasmodesmatal channels per μm^2 of vein surface was then determined by the method of Gunning (1978), using a section thickness of 70 nm and a channel radius of 22.5 nm.

Carbohydrate synthesis and transport. Plants were illuminated by an incandescent 1000-W metal-halide lamp at least 1 h before labeling. Photon flux density at leaf level was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Just prior to labeling, each plant was covered with aluminum foil, leaving only a mature leaf exposed to prevent inadvertent fixation of leaked $^{14}CO_2$ by sink tissues. A clear plastic bag was cut to fit the mature leaf and sealed with tape. Radiolabeled CO_2 (1 MBq) was generated in a syringe by adding an excess of 80% lactic acid to $Na_2{}^{14}CO_3$ (6.6×10^5 $MBq \cdot mmol^{-1}$) and was then injected into the bag. Five minutes later, the bag was cut open to release the CO_2. Label was monitored with a Geiger-Müller tube; when label was detected in sink tissues (1–2 h after labeling), sink and source samples were removed and frozen in liquid N_2.

Source samples consisted of the leaf that was enclosed in the plastic bag. For *Arabidopsis* sink samples, plants were removed from soil and the roots were quickly rinsed in distilled water. Mature leaves, and all but the smallest sink leaves, were removed; all the remaining tissue constituted the sample. For *Coleus blumei*, an inflorescence was used as a sink sample.

Tissue was ground in liquid N_2 and soluble carbohydrates were extracted in a mixture of methanol, chloroform, and water (MCW) (12:5:3; by vol.). Aqueous and non-aqueous phases were separated by addition of water, as described previously (Beebe and Turgeon 1992). The aqueous phase was passed through a plastic BioSpin column (BioRad, Richmond, Calif., USA) containing a layer of cation-exchange resin (AG1-X4, purchased in hydroxide form from BioRad and converted to carbonate form by incubation with excess 1 M Na_2CO_3), a layer of polyvinylpolypyrrolidone, and a layer of anion-exchange resin (AG50-X4; BioRad). The neutral fraction was taken to dryness in a heating block at 70 °C under a stream of N_2. Samples were rehydrated in water and spotted on silica plates; radioactive compounds were identified by two-dimensional thin-layer chromatography as previously described (Turgeon and Gowan 1992).

Results

Leaf structure. Unless otherwise indicated, the following descriptions pertain to rosette leaves of plants grown at 200 μmol photons $\cdot m^{-2} \cdot s^{-1}$. Leaves are 1 to 5 cm in length, oblong or narrowly obovate, with sinuous margins and more pronounced teeth toward the base, which is narrowly triangular. The margin is cupped under, especially at the rounded tip (Fig. 1). Only three to four orders of branching from the midrib can be recognized, in comparison to the six or seven orders common in leaves of other dicotyledenous species (Hickey 1973). Approximately five secondaries branch from each side of the midrib at an acute angle, and intersecondaries of smaller dimension also branch from the midrib. The midrib and secondaries reduce considerably in size as they approach the margin. Hydathodes are present at the tips of the teeth, where the veins end. The smallest veins end blindly in most, but not all, areoles.

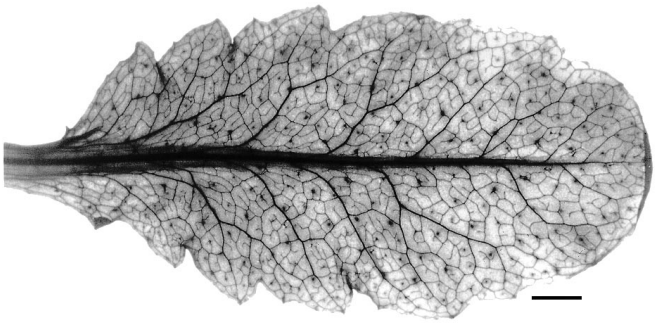

Fig. 1. An *Arabidopsis thaliana* leaf cleared and stained to highlight the veins. Note the wide interveinal spacing and the subdivision of the veins into only three or four branching orders. Bar = 2 mm; ×3.3

Vein density increases with increasing photon flux density. The intervascular interval (Wylie 1939), measured from center to center of veins, is 674 ± 15.8 μm (SE; $n = 60$) and 399 ± 17.0 μm (SE; $n = 85$) when plants are grown at 250 and 600 μmol photons $\cdot m^{-2} \cdot s^{-1}$, respectively. The maximum distance from a mesophyll cell to a vein is half this distance.

Under the low-light conditions at which *Arabidopsis* is commonly raised in growth chambers and growth rooms, leaf blades exhibit well-known characteristics of shade leaves (Fig. 2). Palisade mesophyll cells are short, as is generally the case in leaves with widely spaced veins (Wylie 1939). Palisade cells are also broad: at point of contact with each other they are 52 ± 0.02 μm (SE; $n = 35$) in diameter. Therefore, even though the intervascular interval is very large, photoassimilate does not have to move through more than six or seven cells to reach a vein, as is common in other species. Considering all vein classes, there are 2.45 ± 0.26 mm (SE; $n = 5$) veins $\cdot mm^{-2}$ leaf area.

The most adaxial layer of spongy mesophyll is oriented predominantly in the transverse direction at the level of the phloem, thus providing a conduit for the efficient transport of photoassimilate from the mesophyll tissue to the veins (Fig. 2). In its orientation, this layer of cells resembles the paraveinal mesophyll found in leguminous species, but it lacks certain specialized features of paraveinal mesophyll, such as the absence of chloroplasts and conspicuous protein storage in the vacuoles (Franceschi and Giaquinta 1983).

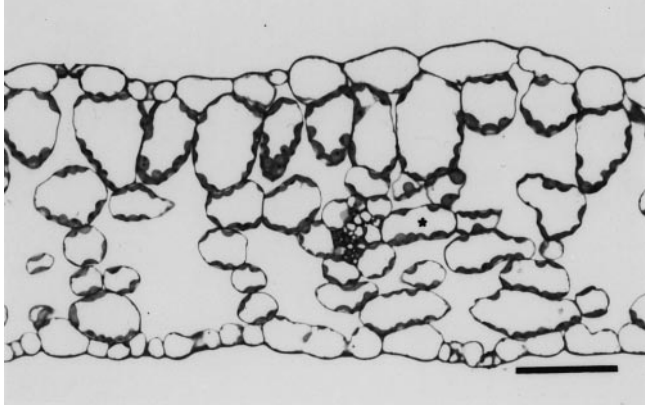

Fig. 2. Transverse section of an *Arabidopsis thaliana* leaf. Palisade mesophyll cells are short, as is common in shade leaves. These cells are also very broad. A transversely oriented mesophyll cell at the level of the minor venation is indicated by an *asterisk*. Bar = 50 μm; ×263

Vein structure. The arrangement of cells in veins can provide clues to developmental patterns and functional relationships between different cell types. Veins of different sizes in *Arabidopsis* are similar in overall organization: although larger veins have more cells, the proportionate numbers of cells of different types is similar. In other words, there is no clear structural distinction between veins of different order, except on the basis of size. Larger veins are found in enlarged regions of the lamina, with a recognizable rib on the abaxial surface. In classical terms, veins with ribs are not "minor" (Esau 1965). However, all veins, with the exception of the larger regions of the midrib and secondaries, are in intimate contact with the mesophyll; they do not have intervening layers of parenchyma between the mesophyll and the phloem. Therefore, in physiological terms, almost all veins in the *Arabidopsis* leaf are "minor" since they appear to participate in phloem loading.

Bundle sheath cells surround the veins tightly and intercellular spaces within the veins are rare (Fig. 3). Cells of the bundle sheath are similar in internal structure to associated mesophyll cells. The xylem is represented by one to several tracheary elements, with associated parenchyma on the adaxial and lateral sides (Fig. 3). These parenchyma cells are variable in size and number. In some cases they are approximately the dimensions of tracheary elements, with small chloroplasts, whereas in others they are almost indistinguishable from mesophyll cells.

Below the xylem, cells are arranged in a reasonably distinct layered pattern, especially in the adaxial portion (Fig. 3). Below the xylem, there are commonly one or two layers of vascular parenchyma, then a layer of cells composed primarily of SE-CCCs, and a layer of phloem parenchyma. The composition of these layers varies somewhat and the layered appearance is replaced by a more clustered cell arrangement in the abaxial region of the phloem.

The number of sieve elements (SEs) in a transverse section of a vein ranges from 4 to more than 20. Most commonly, there are 5 SEs in veins delimiting the areoles, and in the blind endings of areoles, as in Fig. 3. Considering only veins with 10 or fewer SEs, there are 1.94 CCs and 1.34 phloem parenchyma cells per SE in a transverse section ($n = 13$ veins).

In transverse section, CCs are rounded to oval in outline and densely cytoplasmic, with small vacuoles and many mitochondria (Fig. 3). In some electron micrographs, CCs are so dense that their internal structure is obscured. Chloroplasts are small and rarely have starch grains. They are joined to SEs by the specialized branched plasmodesmata noted at this interface in other species (Evert 1990).

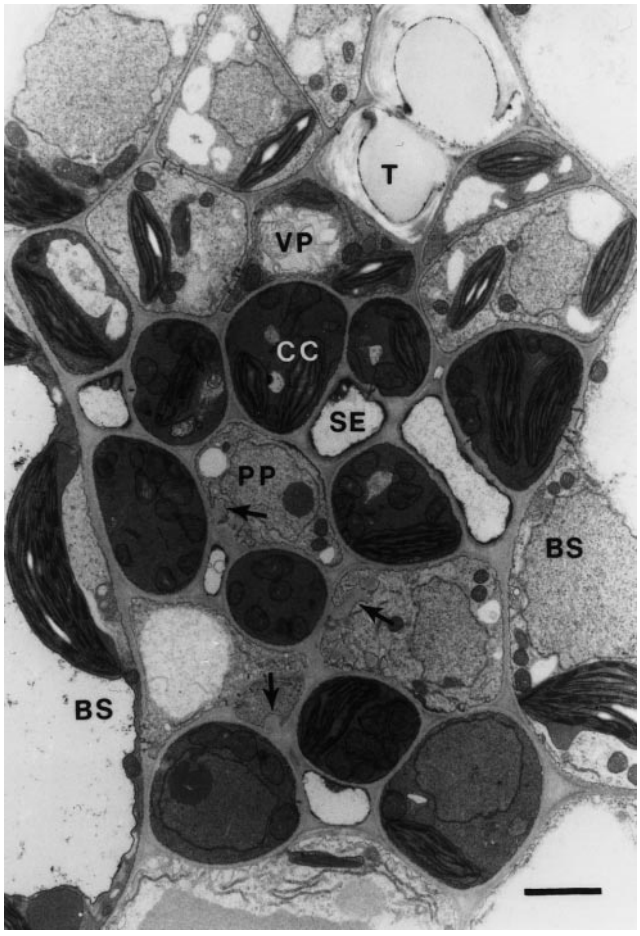

Fig. 3. Transverse section of a typical *Arabidopsis thaliana* minor vein, with five sieve elements. Transfer cell ingrowths are marked by arrows in phloem parenchyma cells. *BS*, bundle sheath cell; *CC*, companion cell; *PP*, phloem parenchyma cell; *SE*, sieve element; *T*, tracheary element; *VP*, vascular parenchyma cell. Bar = 2 μm; ×4990

Sieve elements are smaller than CCs (Fig. 3), but the size discrepancy is not as great as it is in plants, such as the cucurbits, in which the minor-vein CCs are specialized as intermediary cells. Sieve elements have the reduced internal structure described in other plants (Evert 1990) and are connected end-to-end by sieve plates (not shown). Sieve elements are flanked by two or more CCs, and in most cases they are in close association with one or more phloem parenchyma cells.

Phloem parenchyma cells appear to be positioned for the efficient transfer of materials into veins. They abut the bundle sheath with the same frequency as CCs, although there are fewer of them in a vein. Every phloem parenchyma cell comes in contact with one or more CCs, and vice versa. The number of contacts between phloem parenchyma cells and CCs is far higher than those between adjacent CCs, or between adjacent phloem parenchyma cells (Table 1; Fig. 3). Phloem parenchyma cells are larger than CCs, more irregular in outline, and their cytoplasm is much less dense.

Phloem parenchyma cells sometimes, but not always, have transfer-cell wall ingrowths where they are in close contact with SEs (Figs. 3, 4). These ingrowths, which are variable in number, may extend over part of the interface with the adjacent CC. Wall ingrowths apparently make up for the limited cell contact between phloem parenchyma cells and SEs. Note in Table 1 that

Table 1. Interface lengths and frequencies of plasmodesmatal channels (PC) in *Arabidopsis thaliana* leaves. Data are normalized to represent frequencies in a vein with five sieve elements, the most common vein size in the leaf. The PC frequencies were obtained from electron micrographs of 28 veins. For interfaces between two different cell types, frequencies are given for each side of the interface. The PC frequencies are also given for interfaces between mesophyll cells ($n = 56$) for comparison. BS, bundle-sheath cell; CC, companion cell; PP, phloem parenchyma cell; SE, sieve element; Mes, mesophyll cell; NA, not applicable

Interface	Number of interfaces[a]	Total interface length (μm)[a]	PC · μm^{-2}
PP/CC	19.0 ± 2.0	33.2 ± 5.4	1.3/0.5
BS/PP	7.0 ± 0.7	19.9 ± 2.4	2.1/2.3
BS/CC	6.8 ± 1.3	19.1 ± 4.0	0.5/0.4
CC/SE	13.0 ± 0.6	18.8 ± 1.8	1.8 /0.1
PP/SE	8.4 ± 0.5	6.9 ± 0.8	0.4/0.4
CC/CC	4.2 ± 0.7	5.8 ± 1.5	1.0
PP/PP	4.2 ± 0.9	5.3 ± 1.3	8.5
BS/SE	0.6 ± 0.3	0.6 ± 0.3	Rare
SE/SE	0.2 ± 0.2	0.2 ± 0.2	Rare
Mes/Mes	NA	NA	0.66

[a]Mean ± SE

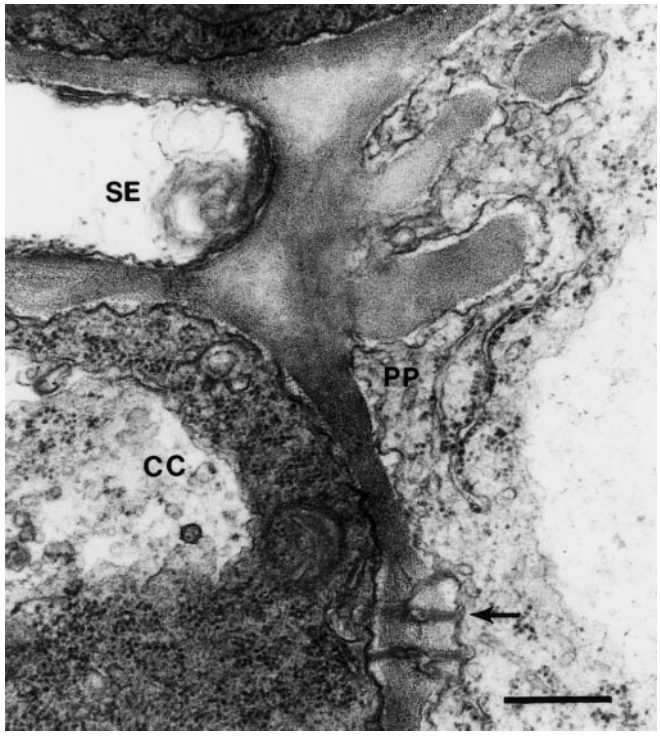

Fig. 4. Junction of a companion cell (*CC*), phloem parenchyma (*PP*) cell, and a sieve element (*SE*) in a transverse section of an *Arabidopsis thaliana* minor vein. The PP cell wall is elaborated by ingrowths where it abuts the SE and the common wall with the CC is traversed by plasmodesmata that are branched only on the PP cell side (*arrow*). Bar = 0.4 μm; ×36700

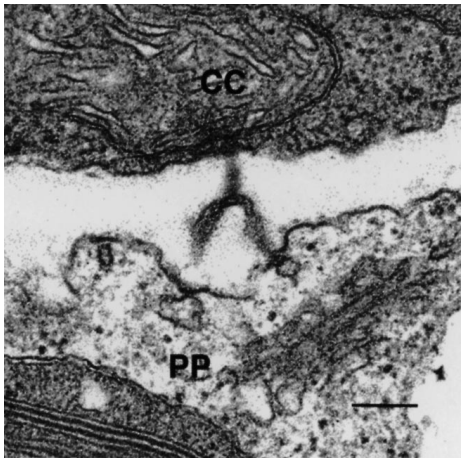

Fig. 5. Plasmodesmata between phloem parenchyma (*PP*) cells and companion cells (*CC*) in *Arabidopsis thaliana* minor veins are branched on the PP side, but not on the CC side. Bar = 0.3 μm, ×29300

the total length of interface between these two cell types, ignoring wall ingrowths, is approximately one-third that of other interfaces with the same number of contacts. Transfer-cell ingrowths, where they occur, effectively increase the contact areas of plasma membrane between phloem parenchyma cells and SEs by a factor of less than 1 to more than 7.

Plasmodesmatal frequencies. The number of plasmodesmata between SE-CCCs and contiguous cells places *Arabidopsis* in Gamalei's Type 1-2a category, i.e. between those with the most abundant (Type 1) and least abundant (Type 2) plasmodesmata at this interface (Table 1; see *Discussion*). As usual, few plasmodesmata are found between SEs and any other cell type but the CC.

Symplasmic continuity is highest at interfaces that involve phloem parenchyma cells. For example, total interface length between bundle sheath cells and either CCs or phloem parenchyma cells is similar, but plasmodesmata are four times more frequent at the latter border. Plasmodesmata between adjacent phloem parenchyma cells are eight times more frequent than those between CCs. Plasmodesmata joining phloem parenchyma cells and CCs are branched on the phloem parenchyma cell side only (Fig. 5).

Photoassimilate translocation. Following exposure of mature *Arabidopsis* leaves to $^{14}CO_2$, radiolabel was found in sucrose, raffinose, and galactinol (Table 2). The experiment was conducted four times, with virtually identical results. All three compounds were translocated to sink leaves, although proportionately little galactinol was transported (Table 2). For comparison, the same studies were conducted with *Coleus blumei* plants (Table 2). As noted in studies on other species with intermediary cells (Mitchell et al. 1992; Turgeon et al. 1993), *Coleus* synthesizes and translocates a considerable amount of raffinose and stachyose, but little galactinol.

Table 2. Synthesis of radiolabeled neutral compounds in mature leaves of *Arabidopsis thaliana* and *Coleus blumei* following exposure to $^{14}CO_2$, and translocation of radiolabel to sink leaves. Data are expressed as the percentage of activity in sucrose. Results from a representative experiment

	Arabidopsis		*Coleus*	
	Source	Sink	Source	Sink
Sucrose	100	100	100	100
Stachyose	0	0	118	76
Raffinose	22	47	37	61
Galactinol	3.0	0.7	40	1.3

Discussion

By definition (Gamalei 1991), *Arabidopsis thaliana* is a Type 1-2a species since it has 1–10 plasmodesmata · μm^{-2} interface leading into minor vein CCs. Our absolute counts of plasmodesmatal frequencies in *Arabidopsis* are lower than this value (0.5 and 1.3 plasmodesmatal channels · μm^{-2} leading from bundle sheath and phloem parenchyma cells into CCs, respectively; see Table 1). However, unlike Gamalei we use a correction factor for section thickness (Gunning 1978), and we count only those channels that can be seen to cross at least half the cell wall (Gunning 1978), conventions which reduce our counts by a factor of approximately 3.4 (compare the data for cucurbits in Volk et al. 1996 and Gamalei 1991). If our data on *Arabidopsis* are multiplied by 3.4, they fall within the range that defines Gamalei's Type 1-2a species.

As is common in plants of the 1-2a type, *Arabidopsis* synthesizes and translocates raffinose. Galactinol is also present, as expected, since galactinol serves as the galactose donor in raffinose oligosaccharide synthesis (Keller and Pharr 1996). In species with intermediary cells, very little galactinol is translocated (Mitchell et al. 1992; Turgeon et al. 1993). This is also the case in *Arabidopsis*, although the proportion of labeled galactinol to labeled raffinose is higher in the transport stream than it is in *Coleus blumei*.

Gamalei (1989) did not include *Arabidopsis* in his survey of the Brassicaceae, but the structure of *Moricandia arvensis* (Brassicaceae) leaves and minor veins has been thoroughly documented (Beebe and Evert 1990, 1992). The veins of *Arabidopsis* and *Moricandia* are similar in overall appearance, though the latter have fewer SE-CC complexes. Perhaps this is a reflection of the greater density of veins in *Moricandia* (Beebe and Evert 1990), which presumably require fewer SE-CC complexes per vein to export the same amount of photoassimilate. Also, in *Moricandia* there appear to be fewer phloem parenchyma cells in intimate contact with SE-CC complexes (Beebe and Evert 1992), and *Moricandia* phloem parenchyma cells do not have transfer-cell wall ingrowths. There is a close correspondence between plasmodesmatal frequencies in *Moricandia* (Beebe and Evert 1992) and *Arabidopsis* at the various interfaces leading into and within the veins. In both species, the highest frequency, per μm^2 of interface, is

between phloem parenchyma cells, a feature that is even more pronounced in *Arabidopsis* than in *Moricandia*.

Phloem parenchyma cells in the *Arabidopsis* minor vein are Type B, as defined by Gunning and Pate (1969), with ingrowths restricted to the region of the wall closest to the SE and CC. In the minor veins of pea leaves, wall ingrowths of transfer cells are more extensive when plants are grown in high light (Wimmers and Turgeon 1991), and this might also be the case in *Arabidopsis*, although not tested in these studies.

What role do transfer cell ingrowths play in *Arabidopsis* minor veins? In the figures in Gunning and Pate (1969), Type B cells in *Impatiens balsamina* (Balsaminaceae) and *Ammobium alatum* (Compositae) appear to be phloem parenchyma, as in *Arabidopsis*, and Type A transfer cells (ingrowths predominantly on walls not adjoining the SEs) are CCs. While Type A cells are known to facilitate uptake of photoassimilate from the apoplasm (Wimmers and Turgeon 1991), the function of Type B cells has not been as obvious (Gunning and Pate 1969). Since Type B cells in minor veins appear to be phloem parenchyma, and they have similar structure in the various families in which they have been examined (Gunning and Pate 1969; Fisher 1991), it is likely that they fulfill the same function in different plants. The most likely function, considering the spatial arrangement of cells in the veins, frequency of cell contacts, and frequency of plasmodesmata, is that they serve as conduits, accepting photoassimilate symplasmically from bundle sheath cells and other phloem parenchyma cells, and releasing it to the apoplasm along the common walls with SE-CC complexes. Wall ingrowths would facilitate this release. It is also possible that sucrose is released from bundle sheath cells.

At present, the mechanism(s) of phloem loading in *Arabidopsis* are largely conjectural. The most likely mechanism is carrier-mediated retrieval from the apoplasm by co-transport with protons. The sucrose-proton symporter *AtSUC1* is expressed in companion cells of *Arabidopsis* minor veins (Stadler and Sauer 1996). Presumably, raffinose is synthesized in CCs and is transported along with sucrose.

What prevents sucrose from diffusing through plasmodesmata from CCs into phloem parenchyma and bundle sheath cells, thus lowering the efficiency of phloem loading? It is notable that the plasmodesmata of minor-vein CCs in *Arabidopsis* and *Moricandia* are structurally distinct from those of other species that have been examined. They have only a single branch on the CC side, whereas in many other species the branches on the CC side are especially numerous (Turgeon 1996).

In *Moricandia*, the CC channels are apparently occluded with electron-dense material. Beebe and his co-workers (Beebe and Evert 1992; Gagnon and Beebe 1996) suggested that diffusion out of CCs in *Moricandia* is prevented by structural modification of the CC channels. Whether the plasmodesmata at this interface in *Arabidopsis* are also structurally modified is not known; this question will require further microscopic and functional analyses.

Recent evidence indicates that plasmodesmata may be involved in loading in more ways than previously thought. In *Ricinus communis*, a Type 1-2a species that translocates sucrose only, loading occurs primarily from the apoplasm, but there also appears to be a symplasmic component (Orlich et al. 1998). In willow (*Salix babylonica* L.), a Type 1 species, there is apparently no phloem loading at all; sucrose enters the SE-CCC from the mesophyll by diffusion, without being elevated in concentration (Turgeon and Medville 1998).

These data indicate that more attention should be devoted to understanding the role of plasmodesmata in transferring material to the SE-CC complexes in the minor vein of all species, not only those with intermediary cells. The plasmodesmata at this interface are particularly important in controlling assimilate flux and the spread of systemic virus (Oparka and Turgeon 1999); their functional characteristics need to be understood in much more detail.

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References

Beebe DU, Evert RF (1990) The morphology and anatomy of the leaf of *Moricandia arvensis* (L.) DC. (Brassicaceae). Bot Gaz 151: 184–203

Beebe DU, Evert RF (1992) Photoassimilate pathway(s) and phloem loading in the leaf of *Moricandia arvensis* (L.) DC. (Brassicaceae). Int J Plant Sci 153: 61–72

Beebe DU, Turgeon R (1992) Localization of galactinol, raffinose, and stachyose synthesis in *Cucurbita pepo* leaves. Planta 188: 354–361

Boodley JW, Sheldrake R, Jr. (1977) Cornell peat-lite mixes for commercial plant growing. NY State Coll Agric Life Sci, Info. Bull. 43

Bush DR (1993) Proton-coupled sugar and amino acid transporters in plants. Annu Rev Plant Physiol Plant Mol Biol 44: 513–542

Esau K (1965) Plant Anatomy. Wiley, New York

Evert RF (1990) Dicotyledons. In: Behnke H-D, Sjolund RD (eds) Sieve elements: comparative structure, induction and development. Springer, Berlin, pp 103–137

Fisher DG (1986) Ultrastructure, plasmodesmatal frequency, and solute concentration in green areas of variegated *Coleus blumei* Benth. leaves. Planta 169: 141–152

Fisher DG (1991) Plasmodesmatal frequency and other structural aspects of assimilate collection and phloem loading in leaves of *Sonchus oleraceus* (Asteraceae), a species with minor vein transfer cells. Amer J Bot 78: 1549–1559

Flora LL, Madore MA (1993) Stachyose and mannitol transport in olive (*Olea europaea* L.) Planta 189: 484–490

Franceschi VR, Giaquinta RT (1983) Specialized cellular arrangements in legume leaves in relation to assimilate transport and compartmentation; comparison of the paraveinal mesophyll. Planta 159: 415–422

Fritz E, Evert RF, Heyser W (1983) Microautoradiographic studies of phloem loading and transport in the leaf of *Zea mays*. Planta 159: 193–206

Gagnon M-J, Beebe DU (1996) Minor vein differentiation and the development of specialized plasmodesmata between companion cells and contiguous cells in expanding leaves of *Moricandia arvensis* (L.) DC. (Brassicaceae). Int J Plant Sci 157: 685–697

Gamalei YV (1989) Structure and function of leaf minor veins in trees and herbs. A taxonomic review. Trees 3: 96–110

Gamalei YV (1991) Phloem loading and its development related to plant evolution from trees to herbs. Trees 5: 50–64

Geiger DR (1976) Phloem loading in source leaves. In: Wardlaw IF, Passioura JB (eds) Transport and transfer processes in plants: proceedings of a symposium. Academic Press, New York, pp 167–183

Grusak MA, Beebe DU, Turgeon R (1996) Phloem loading. In: Zamski E, Schaffer AA (eds) Photoassimilate distribution in plants and crops. Source-sink relationships. Marcel Dekker, New York, pp 220–227

Gunning BES (1978) Age-related and origin-related control of the numbers of plasmodesmata in cell walls of developing *Azolla* roots. Planta 143: 181–190

Gunning BES, Pate JS (1969) "Transfer cells." Plant cells with wall ingrowths, specialized in relation to short distance transport of solutes – their occurrence, structure, and development. Protoplasma 68: 107–133

Hickey LJ (1973) Classification of the architecture of dicotyledonous leaves. Am J Bot 60: 17–33

Keller F, Pharr DM (1996) Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides. In: Zamski E, Schaffer AA (eds) Photoassimilate distribution in plants and crops. Source-sink relationships. Marcel Dekker, New York, pp 157–183

Komor E, Orlich G, Alfons W, Koeckenberger W (1996) Phloem loading – not metaphysical, only complex: towards a unified model of phloem loading. J Exp Bot 47: 1155–1164

Mitchell DE, Gadus MV, Madore MA (1992) Patterns of assimilate production and translocation in muskmelon (*Cucumis melo* L.) 1. Diurnal patterns. Plant Physiol 99: 959–965

Oparka KJ, Turgeon R (1999) Sieve elements and companion cells – traffic control centers of the phloem. Plant Cell 11: 739–750

Orlich G, Hofbrueckl M, Schulz A (1998) A symplasmic flow of sucrose contributes to phloem loading in *Ricinus* cotyledons. Planta 206: 108–116

Rentsch D, Frommer WB (1996) Molecular approaches towards an understanding of loading and unloading of assimilates in higher plants. J Exp Bot 47: 1199–1204

Sauer N, Baier K, Gahrtz M, Stadler R, Stolz J, Truernit E (1994) Sugar transport across the plasma membrane of higher plants. Plant Mol Biol 26: 1671–1679

Schmitz K, Cuypers B, Moll M (1987) Pathway of assimilate transfer between mesophyll cells and minor veins in leaves of *Cucumis melo* L. Planta 171: 19–29

Stadler R, Sauer N (1996) The *Arabidopsis thaliana AtSUC2* gene is specifically expressed in companion cells. Bot Acta 109: 299–306

Turgeon R (1991) Symplastic phloem loading and the sink-source transition in leaves: a model. In: Bonnemain J-L, Delrot S, Lucas WJ, Dainty J (eds) Recent advances in phloem transport and assimilate compartmentation. Ouest Editions, Paris, pp 18–22

Turgeon R (1996) Phloem loading and plasmodesmata. Trends Plant Sci 1: 418–423

Turgeon R, Gowan E (1992) Sugar synthesis and phloem loading in *Coleus blumei* leaves. Planta 187: 388–394

Turgeon R, Medville R (1998) The absence of phloem loading in willow leaves. Proc Natl Acad Sci 95: 12055–12060

Turgeon R, Webb JA, Evert RF (1975) Ultrastructure of minor veins in *Cucurbita pepo* leaves. Protoplasma 83: 217–232

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

van Bel AJE (1993) Strategies of phloem loading. Annu Rev Plant Physiol Plant Mol Biol 44: 253–281

Volk GM, Turgeon R, Beebe DU (1996) Secondary plasmodesmata formation in the minor-vein phloem of *Cucumis melo* L. and *Cucurbita pepo* L. Planta 199: 425–432

von Schaewen A, Stitt M, Schmidt R, Sonnewald U, Willmitzer L (1990) Expression of a yeast-derived invertase in the cell wall of tobacco and *Arabidopsis* plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. EMBO J: 9: 3033–3044

Wimmers LE, Turgeon R (1991) Transfer cells and solute uptake in minor veins of *Pisum sativum* leaves. Planta 186: 2–12

Wylie RB (1939) Relations between tissue organization and vein distribution in dicotyledon leaves. Am J Bot 26: 219–225

Zimmermann MH, Ziegler H (1975) List of sugars and sugar alcohols in sieve-tube exudates. In: Zimmermann MH, Milburn JA (eds) Encyclopedia of plant physiology, NS, vol 1: Transport in plants 1: Phloem transport. Springer, New York. pp 480–503