Leaf Structure and Translocation in Sugar Beet

D. R. Geiger and D. A. Cataldo
Department of Biology, University of Dayton, Dayton, Ohio 45409
Received July 17, 1968.

Abstract. Anatomical and ultrastructural details of a translocating 10-cm leaf of sugar beet (Beta vulgaris L. var. Klein Wanzleben) were correlated with translocation rate data. The minor veins were found to be 13 times as extensive as the major veins and measure 70 cm/cm² leaf lamina. Measurements disclosed that a 33-μ length of minor vein services 29 mesophyll cells with the result that translocate moves an average of 73 μ or 2.2 cell diameters during transport from mesophyll cells to a minor vein. High-resolution, freeze-dry autoradiography revealed that assimilates accumulate in organelle-rich cells of the minor vein phloem. Correlation of phloem volume and loading rate for minor veins yielded an uptake rate of 735 μmoles of sucrose per g fresh weight of phloem. The arrangement and structural features of minor veins appeared to be consistent with the concept that vein loading precedes translocation.

In the search for the mechanism of translocation of organic compounds, various workers have proposed models based on active transport of materials by phloem. The proposed mechanisms can be divided into those in which the active driving process occurs all along the translocation path and those in which this process is centered in the phloem of the source regions such as the vein endings of the leaf blade (1, 8, 14, 15, 22, 25, 30, 34).

Although inhibitor studies offer some hope of distinguishing between the alternatives, to date this approach has failed to differentiate conclusively between the 2 groups of proposed mechanisms. Difficulties include the failure of the effects of chemical inhibitors to be sufficiently restricted to the point of application (15) and the production of chilling effects beyond the inhibition of metabolism (29).

Results of recent localized-chilling experiments suggest that in the phloem outside of source and sink regions, metabolism serves primarily to maintain structural organization while in source and possibly in sink regions it serves to move the translocate (10, 29). Findings from experiments employing low temperature treatment thus favor mechanisms which include active vein-loading in the source leaf (1, 3, 8, 25, 30, 33). From a histochemical standpoint, previous studies have suggested a possible active loading function for border parenchyma cells and companion cells in 1 case (30) and for companion and transfer cells (Übergangszellen) in another (1). In the present study, several recently devised techniques (6, 7, 18, 19) were used to locate cells which accumulate water-soluble organic compounds in a translocating leaf. In addition, anatomical measurements of a translocating leaf were correlated with translocation rate data to ascertain the feasibility of vein loading of minor veins as a means of driving translocation of organic compounds.

Materials and Methods

Plant Material. Sugar beet plants (Beta vulgaris L. var. Klein Wanzleben) were grown by solution culture in controlled environment cabinets as described earlier (11). Studies were performed on 10-cm leaves of 5 to 7-week-old plants pruned to a simplified translocation system (11, 12).

Measurement of Venation. To visualize the vein pattern, leaves were allowed to take up a 0.25% (w/v) water solution of acid fuchsin until the dye had reached the minor veins. Injected leaves were fixed in 80% ethanol, dehydrated, and then cleared in methyl salicylate. Extent of venation was measured in photographic enlargements of the cleared leaves.

Histological Procedures. Details of vascular tissue were studied in 2-μ thick sections of leaf tissue fixed in 3% (v/v) glutaraldehyde in pH 7.2 phosphate buffer or in 10% acrolein in tap water. Tissues were dehydrated in acetone and embedded in hydroxyethyl methacrylate by the method of Ruddell (26). Tissue for ultrastructural studies was prepared by fixing 1 mm² pieces of leaf in 3% (v/v) glutaraldehyde in pH 7.2 phosphate buffer for 1.5 hr at 4° or in 2% (w/v) KMnO₄ for 15 min at room temperature. Glutaraldehyde fixed tissues were washed for 1.5 to 3 hr in buffer, and postfixed in 2% (w/v) osmium tetroxide in pH 7.4 phosphate buffer for 2 hr at 4°. Sections were poststained in Reynolds' lead acetate.

1 This research was supported in part by Grant GB-2470 from the National Science Foundation.
Fig. 1. (Upper left) Cleared 10-cm sugar beet leaf, xylem-injected with acid fuchsin, showing major venation. Fig. 2. (Upper right) Detail of small branches of major venation. White rectangle designates position of 0.5 mm² area shown in figures 3 and 4. Minor veins are not apparent at this magnification. × 2.6.

Fig. 3. (Lower left) Mesophyll cells overlying the minor veins shown in figure 4. × 125.

Fig. 4. (Lower right) Pattern of minor venation in 0.5 mm² area of 10-cm sugar beet leaf. × 125. c) connections of minor vein net with major veins; cc) presumed companion cell; ch) chloroplast; m) mesophyll cell; mv) minor vein; o) oxalic acid crystal; p) procambial cell; pd) plasmodesm; pp) phloem parenchyma or companion cell; s) sieve tube; sp) sieve plate; v) vacuole.
Freeze-dry Autoradiography. Details of steady-state labeling with $^{14}$CO$_2$ were described previously (12). Leaves were sampled after 2 or 4 hr of labeling at which time tissue activity was 30 to 50 μC/dm$^2$ leaf. Squares of leaf several millimeters on a side were quickly frozen with 8% (v/v) methylcyclohexane in isopentane at $-170$ to $-180^\circ$ and stored under powdered dry ice prior to dehydration by a modification of the method of Jensen (18). Moisture content of the air stream was maintained by equilibration with ice at $-35^\circ$. Dehydration was carried out at $-45^\circ$ while the trap was held at $-78^\circ$. Following 36 to 72 hr of drying, the tissue was vacuum infiltrated in 56% paraffin for 30 to 45 min. Sections 6 μ in thickness were pressed against a thin film of Ilford K-5 emulsion, in total darkness (18). Films of from 0.2 to 1 μ thickness were made by varying the speed of withdrawal of the coverglass from liquefied emulsion at 58° (19). Autoradiographic slides were sampled after periods of 3 to 15 days exposure at 4° and developed in Kodak Microdol X at 14° (18).

Results and Discussion

As in previous translocation rate studies (10, 11, 12, 13, 29), 10-cm sugar beet leaves were used as experimental material. Veins of the midrib and the first 3 or 4 branches were visibly injected with acid fuchsin (figs 1 and 2). Beyond these major veins, the vascular system is composed of a network of minor veins with relatively few elements (figs 3 and 4). A drawing of a representative pattern of minor venation in a 0.8 mm$^2$ area of lamina is shown in figure 5A. The minor vein net connects with a tertiary vein on 1 side and with quaternary branches on 2 other sides. Examples of paradermal and cross sections of a minor vein have been reconstructed from low magnification electron micrographs (figs 5-7).

To evaluate the relative accessibility of the leaf mesophyll to major and minor veins respectively, the extent of each of these classes of venation was measured. A value of 70 ± 10 cm of minor vein per cm$^2$ leaf was obtained from a sampling of 36 1-mm$^2$ fields. By comparison, the major veins measure 5.5 cm per cm$^2$ leaf. Consequently, the minor veins have more than 10 times greater accessibility to the mesophyll than the major veins. Calculations based on measurements from cleared and sectioned leaf material yielded an estimate of 3 × 10$^4$ mesophyll cells per cm$^2$ leaf lamina. Combining data for cell size, vein extent and cell population (table I), it was calculated that a minor vein length, corresponding to the diameter of an average mesophyll cell, receives translocate from approximately 29 mesophyll cells. By contrast, a similar length of major vein is accessible to about 370 cells. In a leaf 6.7 cell diameters thick, the cells serviced would be within an average lateral distance of 2.2 cell diameters or 73 μ of a minor vein. Wylie (32) found the average width of tissue bordering the minor veins in 66 species of dicotyledons to be 65 μ. A sampling of cross sections of 24 minor veins disclosed that the average perimeter of contact between the phloem in a minor vein and the adjacent mesophyll is

Table I. Anatomical and Physiological Parameters for Minor Veins in Lamina of 10-cm Leaves of Beta vulgaris

<table>
<thead>
<tr>
<th>Extent of minor venation</th>
<th>No. of mesophyll cells serviced by a 33-μ length of minor vein</th>
<th>Surface area of phloem bundle at interface with mesophyll cells in leaf</th>
<th>Volume of minor vein phloem in a cm$^2$ of leaf</th>
<th>Rate of export of sucrose from leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm$^2$</td>
<td>cells/33-μ length</td>
<td>cm$^2$/cm$^2$ leaf</td>
<td>mm$^3$/cm$^2$ leaf</td>
<td>μg/cm$^2$ min</td>
</tr>
<tr>
<td>70 ± 10</td>
<td>29</td>
<td>0.49</td>
<td>0.31</td>
<td>1.3 ± 0.4</td>
</tr>
</tbody>
</table>

1 Average diameter of mesophyll cell is 33 ± 5 μ.  
2 Average perimeter of contact between phloem of minor vein cross section and mesophyll is 70 ± 11 μ.  
3 Average cross sectional area of minor vein phloem is 440 ± 95 μ$^2$.  
4 Data from Geiger and Swanson (12).

Table II. Distribution of $^{14}$C Between Various Categories of Compounds in the Lamina of a 10-cm Sugar Beet Leaf After Various Periods of Steady-State Labeling

<table>
<thead>
<tr>
<th>Labeling period</th>
<th>80 min</th>
<th>120 min</th>
<th>160 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 % Ethanol insoluble compounds</td>
<td>μg</td>
<td>%</td>
<td>μg</td>
<td>%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.7</td>
<td>27</td>
<td>5.6</td>
<td>20</td>
</tr>
<tr>
<td>Other soluble compounds</td>
<td>7.1</td>
<td>25</td>
<td>9.0</td>
<td>31</td>
</tr>
<tr>
<td>Total radioactivity in lamina</td>
<td>28.4</td>
<td>100</td>
<td>28.4</td>
<td>100</td>
</tr>
</tbody>
</table>

Details of method in Geiger and Swanson (13).
FIG. 5. (Upper) Diagram of minor venation from leaf area in figure 4. A) pattern of minor veins showing connection to branches of major veins; B) paradermal section through minor vein phloem; C) cross section through minor vein.

FIG. 6. (Lower left) Electron micrograph of cross section through phloem of minor vein similar to figure 5C. × 7500.

FIG. 7. (Lower right) Electron micrograph of paradermal section similar to figure 5B showing phloem parenchyma and sieve tubes. × 5800. Insert (rectangle) shows details of sieve pore with endoplasmic reticulum traversing the pore. × 31,000. See figure 4 for key.
Fig. 8. Freeze-dry autoradiograph of paradermal section of minor vein. A) (Upper) Phase contrast view showing concentration of silver grains over minor vein tissue. Some silver grains are dark and some are phase bright. B) (Lower) Brightfield view of same field as A showing pattern of silver grains at a slightly different level of focus. × 1750. See figure 4 for key.
70 ± 11 μ giving a combined surface of 0.49 cm² for minor vein phloem per cm² leaf (table I).

The greater accessibility and the higher surface to volume ratio of the minor veins is consistent with the thesis that they accumulate assimilate as part of the translocation process. Several workers (1,30) have proposed that parenchyma cells associated with the minor veins function in the transfer of assimilates into the sieve tubes (1,8,14,30) though they do not agree on the specific cell type involved. To investigate the possible transfer role of cells associated with leaf veins, we undertook a histoautoradiographic study to locate cells capable of accumulating assimilates in a translocating leaf. Sugar beet leaves were supplied with ¹⁴CΟ₂ under conditions which result in a high rate of translocation of labeled sugar (12). Previous studies have shown that during the first 100 min of steady-state labeling the sucrose in the leaf reaches isotopic saturation (12,13); thereafter, the spatial distribution of soluble compounds such as sucrose, which have reached their maximum specific activity, is accurately reflected in the distribution of radioactivity throughout the tissue. At the same time of sampling, sucrose constitutes 12 to 20 % of the label (table II); this proportion decreases with time as a result of accumulation of insoluble materials in the leaf.

The distribution of radioactivity in a paradermal section of freeze-dried leaf tissue, as revealed by high-resolution autoradiography, is shown in figures 8 to 10. The grain density in figures 8 to 10 indicates a greater amount of labeled material present in the phloem than in the cytoplasm of adjacent mesophyll cells. The sieve tubes are too small to be located with certainty, but the labeling appears to be greatest over the cells with the dense cytoplasm. Control autoradiographs (fig 11) show the absence of significant numbers of silver grains produced by chemical action or by background radiation. Because a large portion of the label in the leaf is in glycogen (13), protein and other insoluble compounds, autoradiographs of 80 % ethanol extracted tissue were prepared (fig 12). In contrast to the pattern for non-extracted tissue, there is a higher concentration of silver grains over the chloroplasts and cytoplasm of the mesophyll cells than over the minor veins in the extracted tissue. Extraction caused some loss of resolution as evidenced by the presence of grains at the periphery of the vacuoles and slightly beyond the edges of the tissue sections. These comparative autoradiographs indicate that a large portion of the radioactivity in the phloem is in ethanol-soluble compounds, presumably largely in sucrose.

This pattern of accumulation by organelle-rich cells resembles the previously reported distribution of energy-related compounds localized in cells postulated to function in vein loading during translocation (1,14,30). On the basis of phosphatase and tetrazolium reactions, Bauer implicated companion and transfer cells of the minor veins of Madia disstiflora and Vicia faba in the active loading of translocate (figs 10-13 of 1). He also found a lesser concentration of phosphatase and of formazan in the sieve tubes and little activity in the border parenchyma and mesophyll cells. The recent work of Gunning, Pate and Briarty (14) demonstrates an accumulation of insoluble materials in transfer cells of the minor veins following xylem injection with ³H-leucine solution. These workers also found acid phosphatase localized in the cell walls of the transfer cells.

An examination of the ultrastructure of the minor veins revealed specializations which may relate to the proposed vein loading process. Typically, a minor vein is composed of 1 to 3 files of xylem vessels and 4 to 12 files of phloem cells (fig 5C). In the light of vein loading hypotheses, 2 features of the phloem appear noteworthy. First is the abundance of mitochondria and other cytoplasmic organelles in the companion cells and parenchyma cells of the phloem (figs 6,7,13), which would be expected in cells involved in active transport. The second feature is the relatively large size of the organelle-rich companion cells in the minor veins (fig 13) as compared with similar cells found in the petiole (fig 14). This size relationship, previously noted by Morretes (23,24), may represent an additional adaptation for vein loading.

A number of calculations were made to correlate the structural features described above with translocation rate data. During photosynthesis under saturating light intensity, a 10-cm sugar beet leaf exports sucrose at the rate of 1.3 μg/min cm² or 7.8 mg/hr dm² leaf. On the assumption that loading of minor veins is an integral part of translocation, the entry of sugar into the minor vein phloem will occur at this same rate. The phloem of the minor veins occupies a volume of approximately 70 cm × 440 μ² (table I) or 3.1 × 10⁻⁴ cm³/cm² leaf; the estimated fresh weight of this phloem is 310 μg/cm² leaf. From this it follows that the sugar uptake rate would be 1.3 μg sucrose/min per 310 μg fr wt phloem tissue or 4.2 mg sucrose/min g fr wt phloem tissue. On a molar basis this is 735 μmol sucrose/hr g fr wt phloem in the minor veins. This rate of vein loading is equal to the rate of uptake of sugar from a 10 % sucrose solution reported by Weatherley for leaf disks of Atropa belladonna (31). In the latter case, nitrogen anaerobiosis reduced the uptake rate to 25 %, suggesting an active transport mechanism is responsible for a major part of the uptake. Another indication of the presence of an active transport mechanism is the high uptake rate by minor vein phloem of the sugar beet. In this regard, Bieleski (2) reported that phloem tissue excised from stems actively accumulates 9 to 16 μmol sucrose/hr g wt of tissue from a 10⁻¹ x sucrose solution, a concentration representative of the sugar content of photosynthesizing leaf tissue. The rate calculated for minor veins of the intact sugar beet leaf is 50 to 80 times the rate found by
Fig. 9, 10. (Upper) Phase contrast view of minor veins in a paradermal section of source leaf; identity of individual cell types difficult to discern. Similar to figure 8A. \( \times 950 \).

Fig. 11. (Lower left) Freeze-dry autoradiograph of a paradermal section of unlabeled control tissue. \( \times 950 \).

Fig. 12. (Lower right) Autoradiograph of 80\% (v/v) ethanol-extracted freeze-dried tissue showing labeling pattern from insoluble materials. \( \times 950 \). See figure 4 for key.
Fig. 13. (Upper left) Electron micrograph of cross section of minor vein of leaf showing relationship between mesophyll cell, phloem parenchyma cell and sieve tube. × 22,000.

Fig. 14. (Upper right) Ultrastructure of phloem in cross section of petiole. Note relative sizes of phloem parenchyma cells and sieve tubes in minor vein of leaf (figs 6, 7, 13) and in petiole. × 5800.

Fig. 15. (Lower left) Plasmodesmata between sieve tube and companion cell in cross section of minor vein. × 20,000.

Fig. 16. (Lower right) Cytoplasmic connections between mesophyll cell, phloem parenchyma cell, and sieve tube. × 17,400. See figure 4 for key.
Bieleski for phloem excised from the stem. This difference in rates is consistent with the relatively greater abundance of organelle-rich cells in the phloem of the translocating leaf than in the petiole. In the leaf lamina, the minor vein phloem consists of 80 to 90 %, by volume, of organelle-rich parenchyma cells while in the path or petiole portion of the leaf these cells occupy 20 to 30 % of the volume. Consequently, the surface to volume ratio is more conducive to sieve tube loading in the minor veins than in the petiole (figs 14, 15).

Although there is evidence favoring accumulation of assimilates by the phloem parenchyma cells of the minor veins prior to translocation, many of the details of the model are tentative and remain to be examined. From studies of chloroplasts isolated by non-aqueous methods, it appears that sucrose is first produced in the chloroplasts (4, 28) and quickly moves into the cytoplasm (28). Evidence for the intercellular movement of sucrose in the free space via the cell walls and intercellular spaces has been presented by Hawker (16) and by Kriedemann (20). Several modes of entry of sugar into the phloem have been suggested as a result of various experiments. Some studies give evidence for the hydrolysis of sucrose prior to its accumulation in cells (5, 27) while others indicate that phosphorylation precedes entry (22); still others indicate no hydrolysis prior to uptake (17, 21). The contribution of cell surface structures in the minor veins is not clear. Cell wall protuberances, postulated as active in vein loading (14), were not observed in sugar beet. Numerous plasmodesmata were observed in the walls of both mesophyll and phloem cells (fig 16) but their importance in vein loading is conjectural (9).

The present study demonstrates that organelle-rich minor vein cells of the translocating leaf accumulate assimilates. The structural features of these cells and their relationship to the mesophyll cells is consistent with the concept that parenchyma cells of the minor veins actively accumulate sugar prior to its entry into the sieve tubes. Correlation of physiological and structural measurements revealed no major inconsistencies with respect to vein-loading theories.

Acknowledgments

The authors are grateful to Drs. M. Arifi Hayat of the University of Dayton, and Robert M. Giesy of the Ohio State University for their assistance in the electron microscope study and to the Ohio State University College of Biology for the generous use of their electron microscope facilities.

We also thank Miss Sharon McCloskey and Miss Mary Anthony for their competent technical assistance.

Literature Cited


