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Phloem unloading in tobacco sink leaves: insensitivity to anoxia indicates a symplastic pathway

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Abstract. Phloem unloading in transition sink leaves of tobacco (*Nicotiana tabacum* L.) was analyzed by quantitative autoradiography. Detectable levels of labeled photoassimilates entered sink leaves approx. 1 h after source leaves were provided with $^{14}CO_2$. Samples of tissue were removed from sink leaves when label was first detected and further samples were taken at the end of an experimental phloem-unloading period. The amount of label in veins and in surrounding cells was determined by microdensitometry of autoradiographs using a microspectrophotometer. Photoassimilate unloaded from first-, second- and third-order veins but not from smaller veins. Import termination in individual veins was gradual. Import by the sink leaf was completely inhibited by exposing the sink leaf to anaerobic conditions, by placing the entire plant in the cold, or by steam-girdling the sink-leaf petiole. Phloem unloading was completely inhibited by cold; however, phloem unloading continued when the sink-leaf petiole was steam girdled or when the sink leaf was exposed to a N_2 atmosphere. Compartmental efflux-analysis indicated that only a small percentage of labeled nutrients was present in the free space after unloading from sink-leaf veins in a N_2 atmosphere. The results are consistent with passive symplastic transfer of photoassimilates from phloem to surrounding cells.

Key words: *Nicotiana* (phloem unloading) – Phloem unloading – Translocation (phloem unloading).

Introduction

There is considerable evidence that sink activity is important in the regulation of photoassimilate partitioning (for a recent discussion, see Gifford et al. 1984). Phloem unloading is the first step in the transfer of assimilates from the phloem to sink tissues and hence could be an important regulatory event. Two types of phloem unloading, apoplastic and symplastic, are distinguished on the basis of the cellular pathway of assimilate movement. In apoplastic unloading, photoassimilates enter the free space of the tissue after leaving the phloem and are subsequently taken up across the plasmalemma of recipient cells. A pathway including apoplastic transfer has been demonstrated in legume seeds (Offler and Patrick 1984; Wolswinkel and Ammerlaan 1985; Thorne 1986), corn kernels (Shannon et al. 1986), bean stems (Aloni et al. 1986), sugarcane stems (Glaziou and Gayler 1972), sugarbeet taproot tissue (Wyse 1979) and stems parasitized by *Cuscuta* (Wolswinkel 1986). In the symplastic pathway, photoassimilates are retained in the cytoplasm, passing from cell to cell through plasmodesmata. Symplastic unloading occurs in developing roots of pea (Dick and ap Rees 1975) and corn (Giaquinta et al. 1983). Structural and physiological evidence also points to a symplastic pathway in developing leaves (Turgeon et al. 1975; Giaquinta 1977; Gougler Schmalstig and Geiger 1985).

Detailed quantitative analysis of unloading has been undertaken in certain apoplastic systems where photoassimilates in the free space are available for sampling and where transport may be experimentally perturbed by the addition of inhibitors and growth regulators. In contrast, little is known about the regulation of symplastic phloem unloading since the pathway is not open to direct analysis or manipulation and the vascular bundles are usually arranged in a confusing three-dimensional pattern.

Sink leaves should be very suitable for the

Symbol: VI = ratio of ^{14}C in veins and interveinal tissue

study of symplastic unloading, if this proves to be the pathway, since they are thin, the phloem is relatively close to the surface, and the vascular bundles are arranged in a two-dimensional, reticulate network. In this paper a method is described for the analysis of phloem unloading in tobacco sink leaves and this method is used to study the effects of cold, steam girdling and anoxia on phloem unloading.

Material and methods

Plant material. Tobacco plants (*Nicotiana tabacum* L. cv. Maryland Mammoth) were grown in a greenhouse in a mixture of peatmoss, perlite and soil (2:1:1, by vol.) in 15-cm clay pots. Seeds were obtained from F.W. Snyder, U.S. Department of Agriculture, Beltsville, Md., USA. The plants were fertilized with controlled-release fertilizer (Osmocote 14/14/14; Sierra Chemical Co., Milpetas, Cal., USA). Daylength in the greenhouse was extended to 18 h with light from incandescent lamps (50 μmol photons $\cdot m^{-2} \cdot s^{-1}$). Plants were used for experimentation when eight to ten weeks old. Sink leaves were leaf plastochron index (LPI) 3.3–3.5 in age using a 2.0-cm leaf (0 LPI in age) as the index (Erickson and Michelini 1957). At this point in development sink leaves were undergoing the sink-source transition; they were 12.0–13.0 cm in length and the import termination boundary (Turgeon 1986) was 4–6 cm from the leaf tip.

Labeling with $^{14}CO_2$. The second and third leaves below the sink were used as sources. These leaves were individually enclosed in polyethylene bags and each was exposed for 5 min to 10^7 Bq $^{14}CO_2$ (1.9 GBq $\cdot$ mmol^{-1}) generated from $Na_2{}^{14}CO_3$ as described in Turgeon and Webb (1973). For both labeling and subsequent transport periods the plant was illuminated with a water-filtered 1000-W metal halide lamp (M1000/C/U metalarc; Sylvania, Danvers, Mass., USA) providing 400 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (photosynthetically active radiation) at the level of the leaves. Import to the sink leaf was detected with a Geiger-Müller (GM) counter (model RLM-2; Wm. B. Johnson, Research Park, Montville, N.J., USA), the output of which was monitored with a strip-chart recorder. Recorder readings were converted to absolute ^{14}C levels using a factor derived from separate experiments in which GM readings were obtained and total ^{14}C per unit area of leaf tissue was measured by scintillation counting (Turgeon 1984a).

Quantitative autoradiography. After various periods of import, as described in *Results*, sink-leaf tissue was excised and frozen immediately in powdered solid CO_2. To prevent curling and breakage the tissue was placed between pieces of stainless-steel screen before freezing, and held with paperclips. Frozen tissue was lyophilized as described in Turgeon (1984a) and compressed between polished steel plates in a vice. Compressed tissue was affixed to thin, flexible cardboard mounting sheets with double-backed tape and compressed again. The mounted tissue was placed in contact with X-ray film (XAR-5; Eastman-Kodak, Rochester, N.Y., USA) between two pieces of foam and clamped between two plywood sheets with large clips. This clamping arrangement resulted in uniform exposure even when a number of tissue pieces or whole leaves were autoradiographed on the same X-ray sheet. After 12 h to 3 d exposure the X-ray sheets were developed for 3.5 min at 23° C in Kodak GBX developer and fixed in Kodak GBX fixer for 5 min.

Pieces of X-ray sheet were glued to glass slides, exposed surface away from the slide, with cyanoacrylate adhesive. Slides were examined with a Zeiss (Oberkochen, FRG) research microscope equipped with a Leitz (Rockleigh, N.J., USA) spectrophotometer head with a Hamamatsu R928 photomultiplier tube (Hamamatsu Corp., Middlesex, N.Y., USA). The spectrophotometer was interfaced with an Apple IIe computer using an AI13 analog input system (Interactive Structures, Bala Cynwyd, Pa., USA). The microscope field of view was restricted with a series of circular apertures to spot sizes ranging from 31 μm to 883 μm diameter using a 4 × objective. Because of heterogeneity in silver-grain size the reliability of readings decreased with decreasing spot size. In the experiments reported here a spot size of 90 μm diameter was used; in scanning a uniform sample with this aperture the standard deviation of readings was 6% of the mean. Illumination was by an unfiltered tungsten lamp.

A standard curve of ^{14}C activity versus light transmission was generated using X-ray sheets which had been exposed to a ^{14}C-microscale (Amersham Corp., Arlington, Ill., USA). This standard curve was not linear over any portion of its range. Since samples in the same experimental series often differed considerably in activity it was necessary to use different exposure times to produce autoradiographs within the useful range of silver-grain density. Readings of microscale exposures ranging from 12 h to 12 d indicated that this procedure yielded reliable results and there was no evidence of reciprocity failure at long exposure times.

Use of a ^{14}C-microscale as a standard provided only a relative measure of radioactivity versus light transmission when constructing a standard curve. To establish an absolute scale for the standard curve, sink leaves containing uniformly distributed ^{14}C-assimilate were lyophilized, compressed, and autoradiographed as described above. Tissue samples were analyzed by scintillation counting and the amount of ^{14}C per unit area of compressed tissue was related to density readings from autoradiographs.

Compartmental efflux analysis. Sink leaves which had imported ^{14}C-assimilate were excised and immediately transferred to a cold room (4.5–5.5° C). The upper leaf surface was abraded for 1 min with dry carborundum. The distal third and proximal third of the leaf were removed and discarded and the remaining middle portion was rinsed twice in 30 ml precooled (4.5–5.5° C) 2-(N-morpholino)ethanesulfonic acid (Mes) buffer (25 mM containing 20 mM $CaCl_2$, adjusted to pH 5.5 with KOH) for a total of 30 s, blotted dry, and placed in a plastic dish containing 7.0 ml of the same buffer. The time between excision and placement in efflux buffer was approx. 2.5 min. At timed intervals the buffer was entirely removed and replaced. Each 7-ml sample was placed in a scintillation vial, dryed in a forced-air oven (85° C), and redissolved in 200 μl 80% (v/v) ethanol. Five milliliters of scintillation mixture (Turgeon 1984a) were added and radioactivity was counted. To measure soluble radioactivity remaining in the leaf, the lamina was extracted five times in 80% ethanol at 85° C and samples of the pooled extract were counted. Compartments were identified by the straight-edge regression technique (Walker and Pitman 1976).

Uptake of [^{14}C] sucrose. The distal third and proximal third of a sink leaf were removed and discarded. The upper surface of the remaining third was gently abraded with carborundum. Discs (5.6 mm diameter) were cut with a cork borer, avoiding large veins, and were placed, after brief rinsing in Mes buffer, on a solution of [U-^{14}C] sucrose (10 mM, 10^5 Bq $\cdot$ ml^{-1}) in Mes buffer as described above. Radioactive sucrose was obtained from Amersham Corp. The discs were incubated at room

temperature in the dark, on a reciprocal shaker (100 shakes/min) for 90 min; they were either exposed to room atmosphere or to a continuous stream of N_2 bubbled through water which had been previously boiled and cooled to room temperature. At the end of the uptake period the discs were washed in five changes of buffer at 0–1° C for 30 min and total ^{14}C was determined by scintillation counting (Turgeon 1984a).

Chemicals. Chemicals were obtained from Sigma Chemical Co., St. Louis, Mo., USA.

Results

Termination of import. In tobacco (Turgeon and Webb 1973), as in other dicotyledonous species (see Turgeon 1986 for review), a developing leaf which is undergoing the sink-source transition stops importing at the tip before the base. The progressive decline in sink activity from the base to the tip of the leaf is best illustrated in Fig. 1. Samples of tissue were removed parallel to the midrib with a cork borer (5.6 mm diameter) and total ^{14}C was counted. The amount of imported ^{14}C decreased linearly from the leaf base to the transition point where import ceases.

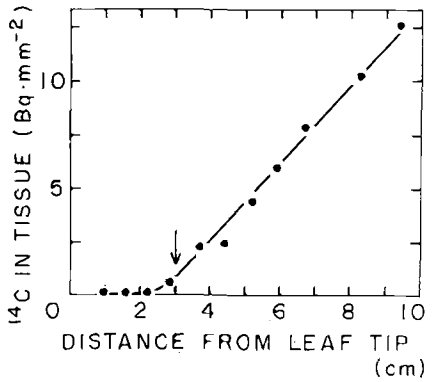

Fig. 1. Imported ^{14}C-labeled assimilate in a tobacco sink leaf measured at increasing distances from the leaf tip. The position of the import-termination boundary, as seen in the autoradiograph (not shown), is indicated by the *arrow*

Previous autoradiographic studies have demonstrated basipetal loss of import capacity but resolution has not been high enough to distinguish individual minor veins labeled with imported ^{14}C-labeled assimilate (Turgeon 1986). In this study, resolution was improved by compressing the lyophilized leaves, by shortening the translocation period so that most label remained in the veins, and by decreasing autoradiographic exposure time. Under these conditions the pattern of importing veins could be seen in detail (Figs. 2, 3).

Although import termination is usually described as basipetal in direction this is only one of two distinct developmental patterns in tobacco. The second pattern is termination of import from the distal toward the proximal veins; that is, from the smallest, most distant importing veins (third order) toward the largest vein (midrib). Note in Figs. 2 and 3 that, near the edge of the importing zone, the amount of label in third-order veins is substantially less than in the same size veins further from the transition area. There is also an obvious decline in the amount of label from the proximal to the distal regions of first- and second-order veins. This is most evident in first-order veins which import heavily at the base, near the midrib, but not at the tip. The result of this loss of import capacity in the proximal direction is a concentration of label in and near the largest veins.

As described earlier (Turgeon 1986), veins smaller than third order do not import photoassimilate in tobacco leaves. The lack of visible label in veins smaller than third order is not a consequence of problems of resolution since these minor veins are clearly resolved in mature leaf tissue

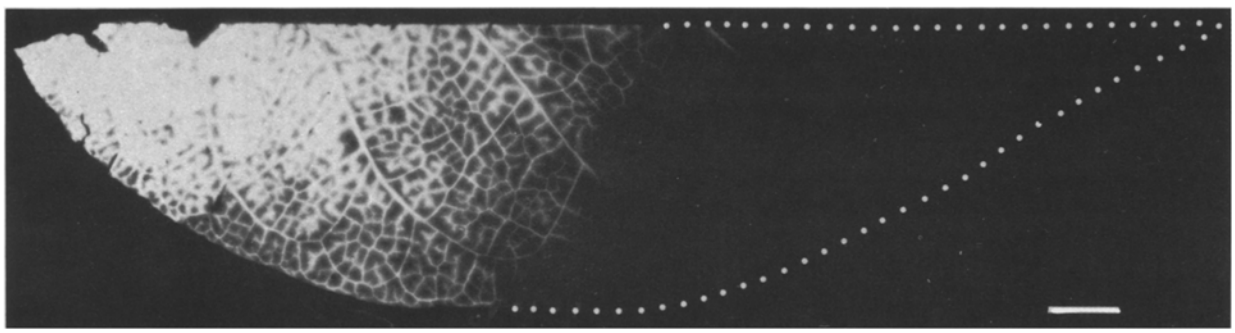

Fig. 2. Autoradiograph of one lateral half of a tobacco transition sink leaf which imported ^{14}C-labeled assimilate from source leaves. This leaf-half was excised and frozen when label was first detected in it, 1 h after the source leaves were labeled with $^{14}CO_2$. The upper half of the leaf (*right*) has stopped importing. The X-ray sheets was used as the photographic negative; white regions therefore indicate the presence of ^{14}C. Scale = 5.0 mm; ×1.9. The leaf tissue was reduced approx. 40% in area by lyophilization

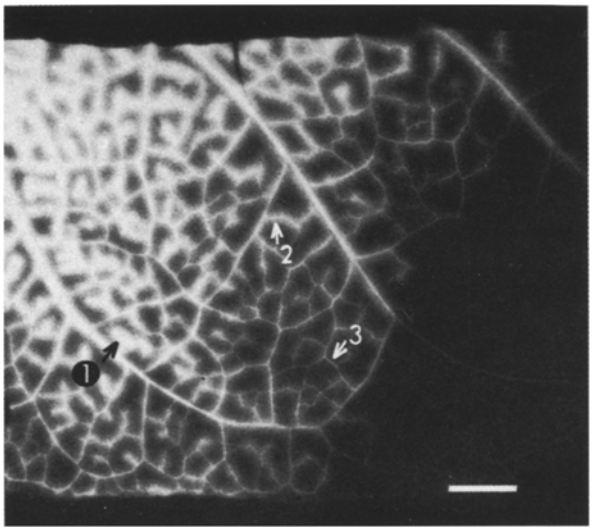

Fig. 3. Autoradiograph of one lateral half of a tobacco transition sink leaf at the import-termination boundary. Tissue to the *right* is no longer importing. The numbered veins were scanned by microdensitometry in the direction of the *arrows*. Scans are shown in **Fig. 4.** Scale = 3.0 mm; × 3.0. The leaf tissue was reduced approx. 40% in area by lyophilization

which is vein-loading (Turgeon 1984b). Also, the inability of minor veins to accumulate label cannot be explained on the basis of the short translocation period since the veins remained unlabeled when transport time was extended for an additional 15–45 min (autoradiographs not shown).

Quantitation of autoradiographs. A microspectrophotometer was used to analyze the distribution of silver grains in autoradiographs of sink leaves as described in *Material and methods*. Scans of the three numbered veins in Fig. 3 were made by measuring light transmission in successive spots of 90 μm diameter (Fig. 4). To reduce the error introduced by silver-grain heterogeneity, each vein was scanned three times and the readings were averaged. In autoradiographs it often appeared that the veins which were most weakly labeled were not unloading (Fig. 3). However, the shape of the curves obtained from these veins was approximately the same as that obtained from densely labeled veins (Fig. 4). These results indicate that the process which terminates import is gradual.

To analyze unloading with time, pieces of tissue (20–25 mm^2) were removed from a single attached leaf at intervals after imported ^{14}C was first detected with a Geiger-Müller monitor (Fig. 5). During this period ^{14}C-labeled photoassimilates were rapidly entering the sink leaf (Fig. 6). The first sample was taken furthest from the midrib and successive samples were removed in the direction of the midrib. Tissue at the edge of the leaf was avoided since it imports substantially less than that near the leaf center (Fig. 2). For each autoradiograph sample, ten microspectrophotometer readings were obtained from the densest part of the labeled veins and these readings were averaged. Ten readings from the most weakly labeled tissue

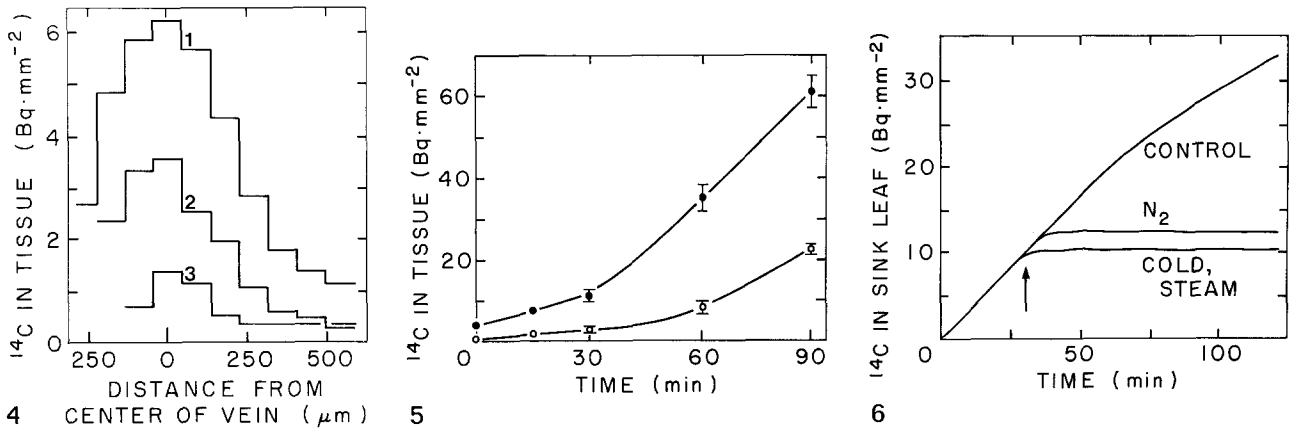

Fig. 4. Microdensitometry scans of three veins pictured in **Fig. 2**. Spot size is 90 μm

Fig. 5. Accumulation of imported ^{14}C in veins (•) and interveinal tissue (o) of a tobacco sink leaf. Source leaves were labeled with $^{14}CO_2$ and samples were taken beginning 10 min after ^{14}C was first detected in the sink leaf with a Geiger-Müller tube. Readings were obtained by microdensitometry of autoradiographs. Bars = SE

Fig. 6. Accumulation of ^{14}C in tobacco sink leaves, measured with a Geiger-Müller tube. The zero time point corresponds to the time when label was first detected in the leaf. In the experiments recorded here this time ranged from 59 to 76 min after the source leaves were labeled with $^{14}CO_2$. Thirty minutes after label was detected (*arrow*) the leaves were either chilled, subjected to an anaerobic atmosphere, or the petiole was steam-girdled

between the veins (interveinal tissue) were also averaged. With increasing time ^{14}C accumulated in both the veins and interveinal tissue (Fig. 5). The ratio between the amount of ^{14}C per unit area in the veins and interveinal tissues (V/I), decreased from 5.9 at zero time (10 min after label was first detected in the sink leaf with a Geiger-Müller tube) to 2.7 at 90 min. This decrease in V/I was expected since labeled photoassimilates accumulate in interveinal tissue while the amount of label entering the veins from the source leaves continuously declines. The results demonstrate that autoradiography, coupled with microdensitometry, provides a quantitative method for the spatial and temporal analysis of phloem transport and unloading in sink leaves.

Effects of steam-girdling, cold, and anoxia on import. To determine the effect of steam-girdling on import, source leaves were labeled with $^{14}CO_2$ and translocation into a sink leaf was monitored with a Geiger-Müller tube. Thirty minutes after label was first detected, the petiole of the sink leaf was steamed for 1 min at the point of attachment with the stem. Import to the sink leaf stopped immediately (Fig. 6).

Cold and anoxia also terminated import. To test the effect of low temperature the plant was moved to a cold room (4.5–5.5° C) 30 min after the detection of labeled translocate in the sink leaf. Import ceased immediately (Fig. 6). To test the effect of anoxia the sink leaf was enclosed, while remaining attached to the plant, in a plexiglass cuvette (1.2 l volume) and flushed with N_2 (1200 $cm^3 \cdot min^{-1}$) in the dark, as described in *Material and methods*. The inhibition of transport was not as rapid as with the cold or steam-girdling treatments, but within 12 min import ceased (Fig. 6).

Effects of steam-girdling, cold, and anoxia on phloem unloading. Phloem unloading was analyzed by comparing labeling patterns in the two lateral halves of a sink leaf. Source leaves were labeled with $^{14}CO_2$ and 30 min after label was first detected in the sink leaf with a Geiger-Müller tube, one lateral half of the lamina was excised and immediately frozen in preparation for autoradiography. The midrib and other leaf-half were left intact, attached to the plant. This remaining lamina tissue was excised and frozen after the experimental treatment. This procedure insured that control and experimental tissues were at the same stage of development. However, the two lateral halves of a tobacco leaf are served by different vascular bundles

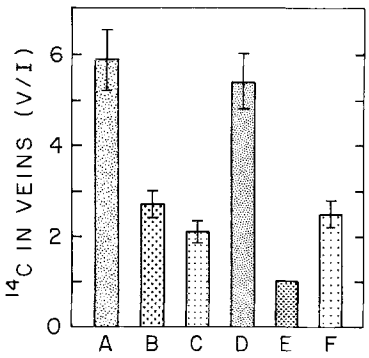

Fig. 7. Ratio of ^{14}C in veins and interveinal tissue (V/I) of tobacco sink leaves. Source leaves were labeled with $^{14}CO_2$ and the 90-min treatments were begun 30 min after label was first detected in the sink leaf. *A*, Lateral leaf-halves removed at the beginning of the treatment period; *B–F*, lateral leaf-halves removed after the treatment period; *B*, no experimental treatment; *C*, petiole of the sink leaf steamed for 1 min at the beginning of the treatment period; *D*, plant placed in a cold room (4.5–5.5° C) during the treatment period; *E*, sink leaf subjected to anoxia during the treatment period; *F*, sink leaf subjected to anoxia and sink-leaf petiole steamed at the beginning of the treatment period. Autoradiographs from treatments *A*, *D*, *E* and *F* are shown in Fig. 8. Bars = SE

and it is common for one half of a sink leaf to receive substantially more label than the other. Therefore, absolute ^{14}C levels in the two leaf-halves could not be compared. Instead, the degree of vein unloading in each leaf-half was expressed as V/I. In effect, V/I is a numerical representation of relative vein densities seen in autoradiographs where the amount of label in interveinal tissue is equal to 1.0. When vein unloading is complete V/I = 1.0.

At the beginning of the experimental period, 30 min after the detection of label in the source leaf, V/I equaled 5.9 (Figs. 5, 7). Ninety minutes later, after continued import and unloading, V/I decreased to 2.7 (Figs. 5, 7). This decrease in V/I with time was partly the result of an accumulation of label in interveinal tissue, but was not a satisfactory measure of unloading in itself since ^{14}C continuously entered the veins during the unloading period. When import into the veins was restricted by steam-girdling the sink-leaf petiole at the beginning of the treatment period, V/I declined to 2.1 after 90 min (Fig. 7). This procedure reduced the effect of continued import of label into veins but introduced girdling as an additional variable.

To test the effect of cold on unloading, the plant was placed in a cold room (4.5–5.5° C) immediately after the control leaf-half was excised. Under these conditions V/I declined to only 5.4 after 90 min, a value which was not statistically different (95% confidence level) from the value of

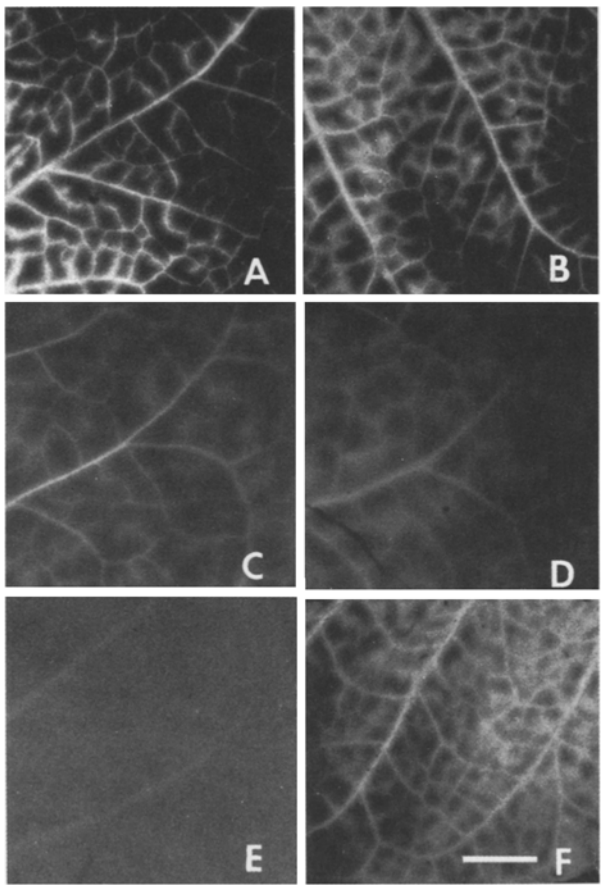

Fig. 8 A–F. Representative autoradiographs from inhibitory treatments. Treatments were begun, (zero time), 30 min after imported label was first detected in the sink leaf. **A** Zero time. **B** Plant placed in cold room (4.5–5.5° C) for 90 min. **C** Sink leaf subjected to anoxia for 30 min. **D** Sink leaf subjected to anoxia for 60 min. **E** Sink leaf subjected to anoxia for 90 min. **F** Sink-leaf petiole steamed at zero time and sink leaf subjected to anoxia for 90 min. Scale = 3.0 mm; × 3.2. Leaf tissue reduced approx. 40% in area by lyophilization

5.9 at the beginning of the treatment (Fig. 7). The values of V/I at the beginning and end of the cold-treatment period can be compared directly since cold completely blocked long-distance transport (Fig. 7). Under these conditions a decline in V/I is an accurate measure of unloading since V was not affected by newly imported label. The fact that V/I did not decline significantly during the 90-min experimental period indicates that cold inhibited phloem unloading. An autoradiograph of a cold-treated sink leaf is presented in Fig. 8B; the veins are clearly defined with few silver grains in the interveinal tissue.

Phloem unloading continued in the absence of O_2 (Figs. 7, 8). At the beginning of the treatment period, after the control leaf-half was removed, the remaining attached sink leaf-half was enclosed in a cuvette and flushed with N_2 in the dark as described above for periods up to 90 min. Within 30 min, substantial unloading was detected (Fig. 8C). After 60 min in anaerobic conditions the pattern of third-order veins could still be discerned in autoradiographs (Fig. 8D), but in those leaves treated for 90 min the amount of label in the veins and interveinal tissue was the same and only larger (first-order) veins were visible (Fig. 8E). Microspectrophotometric analysis of autoradiographs of sink leaves exposed to N_2 for 90 min showed no peaks that could be identified as second- or third-order veins. Therefore V/I equaled 1.0.

Interestingly, phloem unloading did not proceed to completion in 90 min when petioles of sink leaves exposed to N_2 were steam-girdled (Fig. 7). After the 90-min treatment period veins were still visible in autoradiographs (Fig. 8F); V/I declined to 2.5, not to 1.0 as in sink leaves exposed to N_2 but not steam-girdled.

Compartmental efflux analysis. It is possible that, under anoxic conditions, labeled photoassimilates did not unload from the phloem into surrounding cells but rather leaked into and diffused through the apoplast. Compartmental analyses were conducted to determine where labeled compounds resided after N_2 treatments of up to 90 min. Thirty minutes after imported label was first detected in a sink leaf, the leaf blade was enclosed in a cuvette and exposed to N_2 for periods up to 90 min in the dark as described above. The sink leaf remained attached to the plant during N_2 treatment. After N_2 treatment the sink leaf was excised and immediately transferred to a cold room (4.5–5.5° C). The leaf was abraded and compartmental efflux analysis was conducted at 4.5–5.5° C as described in *Material and methods*. Three compartments were detected in all cases (Table 1). The present data do not permit identification of the compartments. The conventional description of a three-compartment system, based on half-time of exchange, is that it is composed of "free space" (most rapid exchange), "cytoplasm", and "vacuole" (slowest exchange). After each of the N_2 treatments the half-times of exchange of each of the three compartments were similar (1.5–2.3 min for most rapid exchange, 6.6–23 min for intermediate exchange, and >24 h for slowest exchange). However it is unlikely that the three compartments were the same after each treatment since most label was present in the veins before exposure to N_2 whereas after 30 min or 90 min of unloading a substantial proportional of the label had unloaded into interveinal tissue. Nevertheless,

Table 1. ^{14}C in sink leaf compartments as determined by efflux analysis. Source leaves were provided with $^{14}CO_2$ and attached sink leaves were exposed to N_2 atmosphere for varying periods of time after the arrival of label. Efflux analyses were conducted at 4° C at the end of the N_2 treatment. The half-time of exchange and the percentage of label in each compartment were determined by the stright-edge regression technique

N_2 treatment (min)	Compartment half-time	% label in compartment
0	1.5–2.0 min	1.3–1.6
	17–23 min	1.6–1.8
	>24 h	96.6–97.1
30	2.0 min	0.8–2.4
	14–20 min	2.8–4.8
	>24 h	94.4–94.8
90	1.2–2.3	0.3–2.0
	6.6–13.8	3.5–4.8
	>24 h	93.2–96.2

even though the identity of the compartments is unclear it is obvious that, whether the analyses were conducted at the beginning, during, or at the end of the unloading period, almost all the label was in the compartment with the slowest half-time of exchange and not in the free space. If labeled photoassimilates entered the free space under anaerobic conditions they could not be found in that compartment at the end of the treatment period.

To determine whether anaerobiosis inhibited uptake from the free space, sink-leaf tissue was incubated for 90 min, at 21° C, in the dark, in either room atmosphere or in a continuous stream of N_2. The amount of uptake under anaerobic conditions was 31.0% of that in air.

Discussion

In tobacco sink leaves, imported nutrient unloads from veins of the first, second and third orders. Smaller veins are used for loading and export (Turgeon 1984b) but not for import. At present we do not know why smaller veins are not part of the import pathway. It is possible that the phloem in veins smaller than this has not matured. It is also possible that a subpopulation of phloem cells is used for import (and perhaps later for export), but this subpopulation does not extend into veins smaller than third order.

The present results do not allow an unequivocal conclusion to be drawn on the pathway of photoassimilate unloading. However, the data are most easily explained by passive movement from phloem to interveinal tissues exclusively through the symplast. This conclusion is based primarily on the observation that vein unloading was insensitive to anaerobiosis. If the phloem-unloading pathway involves an apoplastic component, sucrose would have to cross at least two membranes to enter interveinal cells and either or both of these transport steps could be energy dependent and thus sensitive to anoxia. The first step, efflux from the phloem into the apoplast, would probably occur with the sucrose concentration gradient and thus would not, in theory, require energy. In fact, it has been shown that anoxia increases efflux of sucrose from the phloem to the free space in tobacco source leaves (Turgeon 1984a). However, apoplastic phloem unloading is energy dependent in other systems (Thorne 1982; Patrick 1983; Wolswinkel and Ammerlaan 1983; Wolswinkel 1986). Therefore, it is difficult to predict whether transport of photoassimilate from the phloem, or adjacent cells, into the free space in tobacco sink leaves would require energy and be sensitive to anaerobiosis. However, assuming that labeled assimilates enter the tobacco sink-leaf apoplast under anaerobic conditions, subsequent uptake of sucrose into interveinal cells would be required and the present results demonstrate that this step is sensitive to anoxia. Therefore the data are not consistent with a pathway that involves transfer of photoassimilates to the apoplast and subsequent uptake by interveinal cells.

Another interpretation of the autoradiographic data is possible; namely, under anaerobic conditions, labeled photoassimilates leaked into the free space of the leaf and simply accumulated in the apoplast, thus giving the false appearance of unloading. If this were the case, compartmental efflux analyses should have detected label in the free space. However, these analyses indicated that, before, during and after the 90-min period required for photoassimilate to completely unload from the veins in an anaerobic environment, over 90% of the label was in a compartment with a half-time of exchange longer than 24 h, and not in the free space. It could be argued that label was not detected in the free space because it had already passed through this compartment on route to cells of the interveinal tissue. However, this is unlikely because N_2 treatments were begun when ^{14}C was rapidly entering the leaf (Fig. 6) and most of the label was still in the veins (Figs. 2, 3, 8). Another explanation for the small amount of label in the free space is that it entered the symplast during the compartmental analyses. Again, this is highly unlikely because the analyses were conducted at 4° C, a temperature which severely inhibits uptake of exogenous sucrose (data not shown).

Using a similar experimental approach Gougler Schmalstig and Geiger (1985) demonstrated that the sucrose-loading inhibitor *p*-chloromercuribenzene sulfonic acid (PCMBS) did not inhibit import of ^{14}C-assimilate into sink leaves of *Beta vulgaris*. They also suggested that their results were most compatible with symplastic unloading. Both our results and those of Gougler Schmalstig and Geiger (1985) are equivocal, however, because sucrose uptake was not completely prevented by the inhibitory treatments.

Unlike anaerobiosis, cold severely inhibited unloading. In this respect unloading is similar to long-distance phloem transport which is cold sensitive in tobacco. Cold could, of course, affect a wide range of processes involved in unloading. Although there is little information on the effect of cold on transport through plasmodesmata, the data which are available are consistent with cold inhibition (Minorsky 1985).

The fact that unloading continues in a N_2 atmosphere indicates that long-distance phloem transport and phloem unloading may be experimentally uncoupled. It is generally believed that long-distance transport is motivated by an osmotically driven mechanism which requires loading of assimilate at the source and removal of translocate at the sink. Import into sinks of various types is inhibited by cold or anaerobiosis (Geiger and Sovonick 1975; Thorne 1982) and in the soybean seed-coat system the inhibitory effect of anoxia on import has been narrowed to phloem unloading (Thorne 1982). However, the anoxia experiments reported here demonstrate that, if long-distance transport depends on unloading, the reverse is not necessarily true; unloading in tobacco sink leaves is possible in the absence of translocation. These results are supported by experiments in which the sink-leaf petiole was steam-girdled. Steaming caused transport into the sink leaf to stop immediately but did not prevent unloading.

It is possible that phloem unloading in an anaerobic atmosphere takes place at an unusually high rate because of relaxation of controls which normally restrict symplastic transport. This seems unlikely, however, when steam-girdling experiments are taken into consideration. Under anaerobic conditions, steam-girdling the petiole partially inhibited unloading. If N_2 causes relaxation of symplastic transport controls it should do so whether or not the petiole has been steamed. Another, more feasible, explanation of these results is that unloading is partly dependent on high turgor in the phloem and the trauma of steam-girdling caused a reduction or collapse of this turgor. Since anoxia did not inhibit import as quickly as steam-girdling, this treatment may have permitted the retention of phloem turgor for a long enough period to allow photoassimilates already present in the veins to unload.

Gougler Schmalstig and Geiger (1985) have pointed out that our knowledge of symplastic phloem unloading is rudimentary. Regulation of symplastic unloading could be achieved by structural changes in plasmodesmata (Turgeon 1984b) and-or by physiological changes which affect the concentration gradient of nutrients between phloem and recipient cells. To study symplastic unloading a system is needed in which intercellular transport from phloem to surrounding tissues can be quantitatively assessed. Vein unloading in tobacco sink leaves, monitored by quantitative autoradiography, appears to be such a system.

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