Photoassimilate translocation in the petiole of *Cyclamen* and *Primula* is independent of lateral retrieval

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Abstract

Phloem translocation of photoassimilates between source and sink is considered to be linked with active retrieval of sugars leaked to the vascular apoplast. This hypothesis was evaluated by studying photoassimilate movement in petioles of intact plants of *Cyclamen persicum* and *Primula obconica* in the presence of inhibitors affecting sucrose retrieval (PCMB, CCCP). Inhibitor solutions were applied by rinsing locally isolated petiole bundles or by injection into the petiole parenchyma. PCMB and CCCP reduced [¹⁴C]sucrose retrieval from the petiole apoplast by the vascular cells and altered the distribution pattern of ¹⁴C-photoassimilates within the petiole tissues. However, these treatments did not affect translocation through the petiole phloem. Evidence is provided that the reagents were present in the vascular apoplast surrounding the translocating phloem. It was concluded that assimilate movement in the petiole of *Cyclamen* and *Primula* was independent of apoplastic retrieval.

Key words: *Cyclamen*, *Primula*, phloem, transport, path, sucrose, retrieval.

Introduction

According to the pressure flow hypothesis (Münch, 1930), the driving force for phloem translocation arises from the osmotic difference between the source and sink ends of the phloem path. The osmotic gradient is established by loading sugars in the sieve tubes at the source end and their unloading at the sink end. This hypothesis has provoked a controversy over possible metabolic control along the phloem pathway. Local poisoning with strong metabolic inhibitors has yielded ambiguous results as to whether translocation is additionally energized (Willenbrink, 1957, 1966; Willenbrink and Schuster, 1978; Fensom, 1981). More recently, it has been proposed that phloem translocation is connected with active retrieval of sugars leaked to the vascular apoplast ("leak and pump" hypothesis; Baker, 1978; Willenbrink, 1980; Minchin and Thorpe, 1984; Delrot and Bonnemain, 1985). The occurrence (Kühl et al., 1997; Stadler et al., 1995) and activity (Sokolova et al., 1979; Daie, 1987; Grimm et al., 1990) of retrieval pumps located in the transport phloem (van Bel and van Rijen, 1994) has been demonstrated repeatedly. These pumps are assumed to support the phloem translocation by compensating for the lateral loss of photoassimilates from the sieve tubes. However, the extent of active retrieval as a prerequisite for phloem translocation is not well understood. The present paper explores this issue by monitoring phloem translocation through petioles of intact *Primula* and *Cyclamen* plants treated with strong inhibitors of sucrose retrieval.

Materials and methods

Plant material

*Cyclamen persicum* Mill. (Dresdner Zierpflanzen Steinle OHG, Weixdorf, Germany) and *Primula obconica* Hance (Zwaan

Abbreviations: Caps, 3-cyclohexylamino-1-propanesulphonic acid; CCCP, carbonylcyanide m-chlorophenylhydrazone; MES, 2-(N-morpholino)-ethanesulphonic acid; PCMB, p-chloromercuriibenzenesulphonic acid.
Pannevis, Kleve, Germany) were grown in a greenhouse (10–22°C, 50–80% relative humidity) for 6–12 months. For translocation experiments, plants were transferred to a climate chamber previous 14 h light of 165 μmol photons m⁻² s⁻¹ (22°C, 60% relative humidity) and 10 h darkness (12°C, 80% relative humidity). Acclimatization was carried out for 3 weeks.

Apart from one single source leaf exposed to ¹¹C- or ¹⁴C-labelled CO₂, all exporting leaves of the Cyclamen plants were removed 1 week before the translocation experiment.

Translocation experiments

The scheme of the translocation experiments is summarized in Fig. 1. The preparation of the petiole was carried out on the previous day. In one series of experiments, the abaxial side of the vascular bundle of the petiole was partially exposed (0.5 cm) by surgically removing the petiole parenchyma. This site was continuously rinsed with MES-Ca pH 5 (10 mM 2-(N-morpholino)ethanesulfonic acid, adjusted with NaOH to pH 5.0, and 1 mM CaSO₄) (Fig. 1A). In a second series of experiments, a small injection channel was prepared with a syringe needle between the position of the proportional detectors P3 and P4 and filled with 20 μl MES-Ca (Fig. 1B).

The ¹¹C-technique has been described elsewhere (Jahnke et al., 1989). In brief, ¹¹CO₂ was produced by the Compact Cyclotron of the Universität GH Essen (Radiologisches Zentrum). The leaf blade was exposed to a short pulse of ¹¹CO₂ for 1–2 min. Tracer profiles were recorded using scintillation detectors (S1–S3) mounted to the source, to the sink and to the reservoir of the rinsing solution. Additionally, tracer profiles were also obtained from proportional detectors, which were located 1.5 cm apart from the surgically exposed vascular bundle (Fig. 1A) and along the petiole at 1.5 cm intervals (Fig. 1B). During the ¹¹C-runs, the composition of the medium rinsing the surgically exposed vascular bundle was changed as shown in Figs 2 and 3. In another set of experiments 1 mM PCMBs and 0.1 mM CCCP were injected once in Primula and twice in Cyclamen (Fig. 5). Furthermore, 10 and 100 mM ammonia made up in Caps-Ca pH 11 (10 mM 3-cyclohexylamino-1-propanesulfonic acid, adjusted with NaOH to pH 11.0, and 1 mM CaSO₄) was applied to the petiole of Primula (Fig. 6). To examine inhibitor effects on the distribution pattern of ¹⁴C-assimilates in the Cyclamen petiole, a 5 min pulse of ¹⁴CO₂ (185 kBq) was fed to the leaf blade. ¹⁴CO₂ was released from [¹⁴C]Na₂CO₃ (1.67 GBq mmol⁻¹, Institut für Kernforschung, Dresden, Germany) in a small tube connected with the leaf chamber. Experiments were run for 3 h. During the second hour, 0.1 mM PCMBs or 0.2 mM CCCP was added to the rinsing solution (MES-Ca pH 5). During the third hour, MES-Ca pH 5 without inhibitors was applied. Afterwards, the petiole was divided into 6 × 2 cm segments (Fig. 1A). The vascular bundle was separated surgically from the surrounding petiole parenchyma and washed in cold MES-Ca pH 5 for 3 min. The radiolabel present in the washing solution and in the tissue was attributed to the vascular apoplast and symplast, respectively. Soluble fractions were obtained from methanol extraction (2 × 1 ml segment⁻¹, 24 h, 20°C). The residue corresponded to the insoluble proportion. Radioactivity was determined by liquid scintillation counting (see uptake experiments).

Calcoflour white (Sigma, St Louis, USA) solution (0.15 mg in 1 ml MES-Ca pH 5) was applied in the same way as the inhibitor solutions (see above). The apoplastic distribution of the fluorochrome was analysed microscopically after 15 min of incubation. [³H]inulin (MW = 5000, 37.8 kBq mL⁻¹, Radiochemical Centre, Amersham, UK) was provided in the rinsing solution to the surgically-exposed vascular bundle of the 4th petiole segment (Fig. 1A). After 3 h, the vascular bundle and the petiole parenchyma were separated and extracted in methanol. Radioactivity of the methanol extracts was determined by liquid scintillation counting.

[¹⁴C]sucrose uptake experiments

The procedure has been described by Grimm et al. (1990). Vascular bundles were isolated surgically from petioles and divided into pieces, 2–4 mm in length. Vascular segments were washed in MES-Ca pH 5 twice (2 × 10 min). Samples of the segments, with fresh weights (FW) of 80–120 mg, were incubated for 30 min in 1 mM (1.7 kBq) [U-¹⁴C] sucrose (0.885 GBq mmol⁻¹, UVVVR Prague, CZ) solution buffered at pH 5 with MES-Ca. Concentration-dependent uptake was determined between 0.1 and 300 mM sucrose. For inhibitor studies, 1 mM PCMBs or 0.1 mM CCCP in MES-Ca pH 5 was injected into the petiole parenchyma 1 h before the surgical isolation of the vascular bundle segments. The treated zone had a length of 3 cm (Fig. 5D). Alternatively, isolated segments were incubated in 0.1 mM PCMBs or 0.01 mM CCCP solutions buffered at pH 5 with MES-Ca during preincubation and radioactive incubation, each of 30 min duration (Table 1). Tissue samples were continuously shaken at 25°C under constant illumination (70 μmol photons m⁻² s⁻¹; R2-E14, Narva Berlin, Germany). [¹⁴C]sucrose uptake was stopped by removing the labelled solution and washing the tissue segments in cold MES-Ca pH 5 for 5 min. After methanol extraction, radioactivity of the extracts was determined in a cocktail of 4 g 2,5-diphenyloxazole and 0.24 g 1,4-bis(5-phenyloxazoyl)benzene in 11 toluene using a liquid scintillation counter (LS 6000 SC, Beckman Instruments, Fullerton, USA).

Results

Some characteristics of sucrose uptake by isolated vascular bundles of Cyclamen and Primula petioles are given in Table 1. For both species, the concentration-dependent sucrose uptake was found to be biphasic. A hyperbolic phase was evident at low (<50 mM) and a linear one at high (>50 mM) sucrose concentrations (data not shown). Kinetic constants of the saturable phase were determined following subtraction of the linear component. The latter was obtained from uptake in presence of CCCP, that abolished the saturable phase (Grimm et al., 1990). The apparent Vₘₐₓ values for vascular bundles of Primula was higher than that found for Cyclamen. In contrast, the apparent Kₘ values were almost identical (Table 1). The pH optimum of sucrose uptake by the Primula vascular bundles was slightly higher (Table 1). In both species, sucrose uptake was highly sensitive to the non-permeant thiol reagent, PCMBs, and to the protonophore, CCCP (Table 1). Both inhibitors were used in translocation experiments to examine possible effects of blocked sucrose retrieval on translocation through the vascular bundles. After exposing the leaf blade to a pulse of ¹¹CO₂, movement of ¹¹C-photoassimilates through the petioles was followed by scintillation detectors and proportional detectors (Fig. 1A, B).
Table 1. Comparison of sucrose uptake by vascular bundles isolated from petioles of Cyclamen persicum and Primula obconica

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<tr>
<th></th>
<th>Cyclamen persicum</th>
<th>Primula obconica</th>
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<tr>
<td>Apparent $K_m$</td>
<td>5.2 mM</td>
<td>12.2 mM</td>
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<tr>
<td>Apparent $V_{max}$</td>
<td>4.2 µmol g$^{-1}$ FM h$^{-1}$</td>
<td>5.6 µmol g$^{-1}$ FM h$^{-1}$</td>
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<tr>
<td>pH optimum</td>
<td>4.0-5.0</td>
<td>4.5-5.5</td>
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Percentage inhibition of uptake in the presence of:

<table>
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<tr>
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<th>Cyclamen persicum</th>
<th>Primula obconica</th>
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<tr>
<td>$10^{-4}$ M PCMBs</td>
<td>58%</td>
<td>68%</td>
</tr>
<tr>
<td>$10^{-3}$ M CCCP</td>
<td>73%</td>
<td>65%</td>
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Fig. 1. Set-up of the translocation experiments. (A) The leaf blade was exposed to a pulse of $^{11}$CO$_2$ or $^{14}$CO$_2$. Inhibitors (PCMBs, CCCP) were applied in a solution that rinsed the abaxial side of the vascular bundle, surgically exposed over a distance of 0.5 cm. $^{11}$C-label was monitored along the petiole by proportional detectors (P1 and P2) and in the leaf blade, the sink (bulb, developing buds, root) and collecting tube of the rinsing solution by scintillation detectors (S1, S2 and S3, respectively). $^{14}$C-label was determined in six 2 cm petiole segments, following leaf exposure to $^{14}$CO$_2$ by liquid scintillation counting. (B) The leaf blade was exposed to a pulse of $^{11}$CO$_2$. $20\mu$l buffer solution ($\pm$ inhibitors) was injected into the petiole parenchyma between detectors P3 and P4. $^{11}$C-label in the petiole was monitored by six proportional detectors (P1–P6), spaced at 1.5 cm intervals along the petiole.

In the first set of $^{11}$C-experiments, PCMBs and CCCP were added to a solution rinsing the surgically-exposed vascular bundles. Figure 2A shows translocation of $^{11}$C-photosynthates through a Cyclamen petiole while the surgically-exposed vascular bundle was washed with MES-Ca pH 5. Detector S1 monitored the export of $^{11}$C-photosynthates from the leaf blade and detector S2 the arrival of $^{11}$C in the sink consisting of the bulb, the developing buds and the root (Fig. 1A). The total radioactivity fixed in the leaf blade at the beginning of the $^{11}$C-run was set to 100% (cf. Jahnke et al., 1989). Export from the source commenced after a lag period of 20 min, import into the sink after 30 min. Within 2 h, the radioactivity in the sink did not exceed 30% of the fixed radioactivity (Fig. 2A). $^{11}$C-photosynthates leaked out from the vascular bundle into the rinsing medium and were monitored by detector S3. This efflux was very small (<0.1%; Fig. 2D–F) and was first detected 40 min after $^{11}$CO$_2$ exposure. A supplement of PCMB or CCCP added to the rinsing solution did not affect the slopes of $^{11}$C-profiles of the petiole proximal to the surgically exposed vascular bundle (Fig. 3B, C) in the rinsing medium (Fig. 2E, F), and at the sink (Fig. 2B, C). These experiments were carried out with (Fig. 2E, F) and without (Fig. 3B, C) 20 mM sucrose in the rinsing solution.

Calcofluor white (fluorescent marker) and $[^{3}H]$insulin (MW = 5000, apoplastic marker) were used to examine the spread of substances applied via the rinsing solution through the petiole apoplast. Within 15 min of application, the cell walls of sieve elements clearly were stained by Calcofluor white (image not shown). During 1 h of application, 53% of the $[^{3}H]$insulin insulin moved to the leaf blade, 24% to the bulb, 6% to the upper, 3% to the lower petiole segments, while the remaining 14% was retained at the application site (data not shown).

The distribution of $^{14}$C-photosynthates along the phloem path was analysed in a 3 h translocation experiment. The following changes were found. PCMBs significantly increased the proportion of radiolabel in the vascular apoplast (Fig. 4A). CCCP enhanced $^{14}$C-incorporation into insoluble constituents of the vascular bundle and the petiole parenchyma (Fig. 4B, C).

In another set of experiments, injection channels were prepared between the proportional detectors P3 and P4 (see Fig. 1B). Figure 5A–C shows $^{11}$C-translocation in the Primula petiole and Fig. 5E and F in the Cyclamen petiole. In the control experiment, MES-Ca pH 5 was injected during the $^{11}$C-run (Fig. 5A). Beside a small and transient depression of the record at the site of injection (detector P3), no effect on assimilate movement could be observed. A similar result was obtained in the Cyclamen control experiment (data not shown). Beside very weak disturbances, as indicated by the slopes of detectors P2 and P3 in Fig. 5E, the PCMBs treatment had no substantial effect on $^{11}$C-photoassimilate translocation in Primula and Cyclamen (Fig. 5B, E). CCCP treatment was followed by a reduced level of radioactivity registered at the position of detectors P3 and P4 in the vicinity of the injection site (Fig 5C). This was due to an expanding necrosis into the petiole parenchyma and a reduced lateral spread of radiolabel. However, since detectors P1 and P6 showed undisturbed tracer profiles, the long distance transport of $^{11}$C-photoassimilates continued unaffected.
Fig. 2. Temporal $^{13}$C-profiles in source leaf and sink organs of Cyclamen plants and in the solution, with or without PCMBS and CCCP, rinsing the petiole. The day before the experiment, the abaxial side of the vascular bundle of the petiole was surgically exposed over a distance of 0.5 cm by removing the petiole parenchyma. This site was continuously rinsed with MES-Ca pH 5 (cf. Fig. 1A). The experiments started with the application of an $^{13}$CO$_2$ pulse to the leaf blade. Changes to the composition of the rinsing solution are marked by the dotted vertical lines. (A, D) Rinsing with MES-Ca pH 5 (= control); (B, E) rinsing with MES-Ca pH 5 + 20 mM sucrose ± 2 mM PCMBS; (C, F) rinsing with MES-Ca pH 5 + 20 mM sucrose ± 0.1 mM CCCP. Radioactivity is given as percentage of the total $^{13}$C fixed by the leaf blade during the $^{13}$CO$_2$ pulse application as measured by scintillation detector S1 (leaf blade). Detector S2 monitored the sink (bulb, developing buds, root) (A–C). Detector S3 monitored the $^{13}$C-content in the rinsing solution (D–F). The position of the detectors is shown in Fig. 1A.

To examine the effects of PCMBS and CCCP injection on sucrose retrieval, the vascular bundles were isolated from the treated zone. In both species $^{13}$C-sucrose uptake by the isolated vascular bundles was reduced significantly (Fig. 5D). The inhibition amounted to 20% in Primula and to 40% in Cyclamen. Access of injected material to the vascular apoplast was tested using Calcofluor white. Within 15 min, the cell walls of all vascular cells, including the sieve elements, were fluorescent (images not shown).

Figure 6 shows the response of $^{13}$C-photoassimilate translocation through Primula petioles following their
treatment with low proton concentrations (pH 11) or by ammonia. Injection of the buffer solution of pH 11 caused only slight perturbations at the site of application and on the upstream detector P1 (Fig. 6A), whereas the overall translocation was not affected. When the alkaline buffer was supplemented with ammonia, concentration dependent effects were observed. With 10 mM NH₃ (Fig. 6B), the increase of radioactivity in the petiole stopped temporarily at the site of injection (detectors 3 and 4). However, with 100 mM NH₃ (Fig. 6C), the tracer ascent clearly slowed above (detector P1) and stopped completely below (detector P6) the injection zone.

**Discussion**

Sucrose is the main transport sugar in *Cyclamen persicum* and *Primula obconica* (Rothe, 1994; Haupt, 1994). Characteristics of photoassimilate export from the *Cyclamen* leaf blade and translocation in the petiole were summarized by Grimm et al. (1995). *In vitro* measurement of sucrose uptake by isolated vascular bundles confirms the presence of a proton co-transport mechanism (Table 1 and for further details, see Grimm et al., 1990). However, the data did not provide any information on the distribution of the carrier within the vascular tissue. In petioles of *Heracleum*, sucrose carriers are present in the phloem as well as in the xylem parenchyma (Sokolova et al., 1979). In *Plantago*, a sucrose carrier protein (PnSUC2) is localized to the plasmalemmas of the companion cells (Stadler et al., 1995), whereas in *Solanium* a carrier was found in the plasmalemmas of the sieve elements (Kühn et al., 1997).

Whether the presence and activity of sucrose uptake, as measured using isolated vascular bundles, play any significant physiological role in phloem translocation is uncertain. The leak and pump hypothesis of phloem translocation depends on sucrose carriers for retrieval of sucrose back into the translocation stream. These carriers would need to be located on the plasmalemmas of the sieve elements or adjacent cells, symplastically connected with sieve tubes. To assess the interdependence of sucrose retrieval and translocation, phloem translocation, while manipulating retrieval of sucrose, was investigated. For this purpose, CCCP and PCMBS were selected as highly effective inhibitors of sucrose uptake (Table 1). PCMBS is a widely used, slowly penetrating thiol reagent affecting membrane transport of sucrose (Giaquinta, 1976; van Bel and Koops, 1985; Daie, 1987; Bourquin et al., 1990). A direct modification of sulphhydryl groups in the binding domain of the carrier protein was discussed (M'Batchi and Delrot, 1984). CCCP affects H⁺ sucrose co-transport by dissipating the proton motive force. Due to an inhibited uptake, both agents enhance net efflux from different tissues (Aloni et al., 1986; Hayes et al., 1987).

The leakage of transported photoassimilates into the apoplast of the axial translocation path becomes obvious from the efflux of radiolabel into the rinsing medium (Fig. 2). According to the leak and pump hypothesis, an increased efflux is expected when retrieval pumps are blocked. However, neither PCMBS nor CCCP altered the flow of labelled photoassimilates into the rinsing medium. In addition, the presence of 20 mM sucrose in the rinsing solution should prevent binding of ¹⁴C-sucrose to retrieval carriers (Fig. 2E, F). Furthermore, no changes in phloem translocation through the petiole could be observed (Fig. 3B, C). Neither export from the leaf blade nor import into the sink was not affected significantly (Fig. 2B, C). One might argue that changes in net fluxes were not resolved by the ¹⁴C-technique or the exposure to the inhibitors was too short. Therefore, the assimilate distribution in the petiole after ¹⁴C-labelling was analysed (Fig. 4). The application was extended to 1 h followed by a chase of 1 h. Under these conditions an enhanced level of photoassimilates was found outside the transloca-
Fig. 5. Effect of PCMBS and CCCP on the temporal \(^{14}\)C-profiles of *Primula* (A–C) and *Cyclamen* (E–F) petioles in intact plants and sucrose uptake by isolated vascular bundles (D). The day before the experiment, small injection channels were prepared on the petiole between detectors P3 and P4 (cf. Fig. 1B). 20 µL MES-Ca pH 5 was injected. (A–C, E–F) The experiments started with the application of a \(^{14}\)CO\(_2\)-pulse to the leaf blade. 20 µL MES-Ca pH 5 (=control, A) + 1 mM PCMBS (B, E) or +0.1 mM CCCP (C, F) was injected during the \(^{14}\)C-runs. The first dotted line marks the start, the second one the end of the injection. In (E) and (F), injection was carried out twice. Radioactivity is given as 10^3 cps measured by the proportional detectors. The position of the detectors is shown in Fig. 1B. (D) The experiment started with the injection of 2 x 10^3 µL MES-Ca pH 5 (=control) + 1 mM PCMBS or +0.1 mM CCCP to the petiole of intact plants. After 1 h, the vascular bundle of the treated zone (3 cm) was surgically removed and cut into segments, 4 mm in length. The segments were incubated in an 1 mM \(^{14}\)C sucrose solution containing MES-Ca pH 5 for 30 min. After washing in MES-Ca pH 5, the amounts of sucrose taken up were measured. Significance to the control is indicated by *, bars describe standard errors of the means of 10 replicates per treatment; error probability = 5%.

Fig. 6. Temporal \(^{14}\)C-profiles in *Primula* petioles after application of Caps-Ca pH 11 and ammonia. The day before the experiment, small injection channels were prepared on the petiole between detectors P3 and P4 (cf. Fig. 1B). 20 µL MES-Ca pH 5 was injected. The experiments started with the application of a \(^{14}\)CO\(_2\) pulse to the leaf blade. 20 µL Caps-Ca pH 11 (=control, A) + 10 mM NH\(_3\) (B) or +100 mM NH\(_3\) (C) was injected during the \(^{14}\)C-runs. The first dotted line marks the start, the second one the end of the injection. Radioactivity is given as 10^3 cps measured by the proportional detectors. The position of the detectors is shown in Fig. 1B.
tion path. PCMBs raised the radiolabel levels in the vascular apoplast (Fig. 4A). CCCP enhanced the radiolabel incorporated into insoluble fractions of the vascular bundle and petiole parenchyma (Fig. 4B, C). The increase of labelled photoassimilates outside the translocation stream could be due to reduced reloading. However, the effects were obviously insignificant with respect to gross transport in the intact plant. Similar results were obtained using the injection technique providing high local inhibitor concentrations in the vascular apoplast of the petiole (Fig. 5). Although CCCP caused considerable damage to the petiole parenchyma, photoassimilate translocation was unaffected (Fig. 5C, F).

Along the vascular bundle of Cyclamen petiole an apoplastic sucrose gradient of 11–20 mM was found. The loss of translocated (non respired) photoassimilates amounted to 0.6% cm⁻¹ (Rothe, 1994). In contrast, the sink activity along the phloem path of Phaseolus stems is significantly higher. Minchin and Thorpe (1984; Minchin et al., 1984) postulated a buffering sucrose pool of 25–60 mM in the stem apoplast, which seems to be seasonally dependent. Concentrations of 3–12 mM (Patrick and Turvey, 1981) and 50–70 mM (Hayes et al., 1987) were found in winter and summer grown plants, respectively. Rinsing a zone of the peeled Phaseolus stem with 2 mM PCMBs caused an efflux of ¹¹C photosynthates from the phloem. The gross unloading (leakage without retrieval) amounted to 6% cm⁻¹, the net unloading (difference between leakage and retrieval into the phloem stream) amounted to 4% cm⁻¹ (Minchin and Thorpe, 1987). The higher sink activity in the Phaseolus stem might be caused by elongation and/or peeling off the epidermis over a length of 5 cm (Minchin and Thorpe, 1984, 1987). The lateral efflux from the translocation path and reloading might also be dependent on the strength of the main sink. The removal of the embryo in pea seeds and filling the cavity with a hypotonic medium caused a temporary standstill of the phloem unloading in the pod accompanied with transient accumulation of photoassimilates in the peduncle (van Oene, 1994).

A crucial point of the experiments presented here was the access of the supplied agents to the apoplast surrounding the translocating sieve tubes. Irrespective of the mode of application (rinsing or injection), the apoplastic marker Calcofluor White appeared in the cell walls of the sieve tubes within 15 min. Furthermore [³H]inulin moved from the rinsed petiole segment acropetally and to less extent basipetally. This spread could explain PCMBs- and CCCP-dependent effects on assimilate distribution outside the treated site (Fig. 4). In addition, the activity of sucrose uptake into the vascular bundles following the injection of inhibitors was analysed (Fig. 5D). In both Cyclamen and Primula vascular bundles, the amount of absorbed sucrose was significantly reduced. These results suggest that permeation of the applied inhibitors was not hindered.

Noticeably, even an extreme pH of 11 did not stop the translocation through the petiole, although retrieval is strongly inhibited under these alkaline conditions (cf. Grimm et al., 1990). The small bending on the tracer profiles of the acropetal detectors (P1, P3 and P4), but not of the distal one (P6), indicates that translocation was not or only slightly affected by alkalinization of the vascular apoplast (Fig. 6A). On the other hand, ammonia seriously hampered translocation: 10 mM NH₃ caused a transient inhibition of the lateral flow in the injection zone while perturbations at the more distal positions of the detectors P1 and P6 were very weak (Fig. 6B). Furthermore, the import into the sinks monitored by an additional scintillation detector (data not shown) was unaffected. However, 100 mM NH₃ stopped translocation completely (Fig. 6C). With both concentrations of NH₃, the response was prompt. One can expect a very rapid spreading of ammonia in the treated region, a diffusion through cell membranes, depolarization of membrane potential, and an alkalinization of the tissue causing partial disintegration of the cells (Fensom et al., 1990; Pickard and Minchin, 1992). In addition, this observation supports the view that access of the applied agents to the phloem stream is not a limiting step and hence does not explain the failure of the PCMBs- and CCCP-treatments to affect phloem.

In contrast to the leak and pump hypothesis, the results support the view that phloem translocation is independent of the reloading of photoassimilates released into the apoplast along the axial pathway. It has to be clarified how lateral sealing of the translocation path as demanded by the Münch hypothesis (1930) is influenced by sink strength, permeability of the sieve tube membranes and the speed of the translocation stream.

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