Retention of xenobiotics along the phloem path

Eckhard Grimm1,*, Alexander Grube1, Siegfried Jahnke2, Stefanie Neumann1

1 Martin-Luther-Universität Halle-Wittenberg, Institut für Pflanzen- und Zellphysiologie, Am Kirchtor 1, D-06108 Halle (Saale), Germany
2 Universität GH Essen, Fachbereich 9 – Botanik/Pflanzenphysiologie, Universitätsstrasse 5, D-45117 Essen, Germany

Received: 11 May 1994 / Accepted: 23 November 1994

Abstract. Detached leaves of Cyclamen persicum Mill. can be used as a simple source-sink system. Phloem transport in the excised material was monitored by the noninvasive 11C-technique. Assimilate movement stopped immediately when the petiole was cut off. However, within 20 min a recovery of transport was observed. The translocation rate in the detached leaf was only 13% of that in the intact plant. 14C-Xenobiotics and [3H]sucrose were injected into the upper petiole parenchyma (source). They moved downstream by a symplastic route. The stump of the petiole was inserted into a buffer solution containing ethylenediaminetetraacetic acid (sink). After 3 h, the distribution of sucrose and xenobiotics was determined in five subsequent segments of the petiole (path). The retention coefficient (r) was calculated from the ratio of radioactivity in the vascular bundle to that in the petiole parenchyma. The distribution along the vascular path was given by a geometric progression, whereas its constant was the transport coefficient (q). Values of r and q corresponded with the degree of phloem mobility and ambimobility. Four groups of compounds were classified: (i) acidic substances with log Kow = -2 to -2.4 (Kow is the partition coefficient octanol/water) at pH 8 (pH of sieve tube sap), retained by ion trapping and exhibiting small lateral efflux (q > 0.7; maleic hydrazide, dalapon); (ii) acidic substances with log Kow = -0.7 to -0.8 at pH 8, retained by ion trapping and subjected to a moderate lateral efflux (0.7 > q > 0.5; 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, bromoxynil); (iii) nonionised substances retained by optimum permeability, exhibiting a considerable lateral leakage (q ≤ 0.5; glyphosate, amitrole); (iv) substances without basipetal transport in the phloem (atrazine, diuron). Retention of sucrose corresponded quantitatively with that shown in group (i). This classification was also supported by results of uptake and efflux experiments using the isolated conducting tissue. Theoretical translocation profiles were calculated from the determined transport coefficients (q).

Key words: Cyclamen – Leaf (detached) – Phloem transport – 11C-Photoassimilate – Sucrose – Xenobiotics

Introduction

The long-distance movement of biotic and xenobiotic substances in the phloem requires the uptake of a given compound into sieve tubes and its retention along the translocation path between source and sink (Devine 1989; Bromilow et al. 1991). Xenobiotics are taken up into vascular bundles by diffusion (Grimm et al. 1985, 1987, 1990). Numerous hypotheses have been formulated regarding the chemical properties of xenobiotics and long-distance transport in the phloem. According to the intermediate-diffusion theory, transport of xenobiotics in the phloem is thought to be associated with optimum membrane permeability (Tyree et al. 1979). It depends on the average velocity of translocation, the width of the sieve tubes, the dimension of the path and the feeding area. This model applies to the translocation of nondissociating substances, e.g. oxamyl, amitrole and glyphosate. On the other hand, acidic properties are essential prerequisites for movement in the phloem (weak-acid hypothesis). The introduction and removal of carboxyl groups is associated with the appearance and disappearance of mobility in the phloem, respectively. Acidic compounds undergo a sustained retention by the trapping of anions in the alkaline sieve-tube sap (Grimm et al. 1985; Neumann et al. 1985).

More recently, the mathematical model for calculation of the optimum permeability was extended to dissociating substances, thus unifying the weak-acid, carboxyl and intermediate-diffusion hypotheses of phloem transport (Hsu et al. 1988; Kleier 1988; Grayson and Kleier 1990; Hsu and Kleier 1990). Many xenobiotics without mobility in the phloem, including the bulk of xylem-mobile compounds, could enter sieve tubes. However, because of their physico-chemical properties, they are expected to
leak out from the translocation path near the application zone.

However, there is a lack of data on the lateral release from the translocation path. In the present study, an experimental system is described in which the longitudinal movement and the lateral escape of xenobiotics could be quantified. The approach allowed us to distinguish between different degrees of mobility in the phloem.

Materials and methods

Plant material. Plants of Cyclamen persicum Mill. (seedlings from Dresdner Zierpflanzen Steinle OHG, Weixdorf, Germany) were grown in the greenhouse at 10–15°C.

For translocation experiments with xenobiotics, sucrose and inulin, fully expanded leaves were harvested by cutting off the petioles close to the bulb. The cut surface was immediately immersed in Mes-EDTA (10 mM 2-[N-morpholino]ethanesulfonic acid, adjusted with NaOH to pH 5.0, 1 mM CaSO4, 10 mM EDTA, disodium salt) and kept in the medium during the subsequent manipulations. For experimental use, the petiole was prepared as shown in Fig. 1. The first 2 cm of the petiole, below the leaf blade, remained intact for the injection of labelled compounds. The neighbouring 1-cm zone consisted of a vascular bridge, where the petiole parenchyma was thoroughly removed to uncover the vascular bundle over a length of 0.5 cm. The subsequent 5 cm of the petiole represented the translocation path. Both parts, path and bridge were wrapped in Parafilm M® (American Can Company, Greenwich, Conn., USA) to reduce transpiration. The petiole stump comprised 1.5 cm. It consisted of a 1-cm zone with peeled-off epidermis and outer layers of petiole parenchyma and the terminal 0.5 cm with the completely isolated vascular bundle. The stump was inserted into a plastic tube containing Mes-EDTA solution.

For translocation experiments with 14C-photosynthates, Cyclamen plants were transferred to a growth chamber [14 h light (60–100 μmol photons m−2 s−1) at 22°C and 10 h darkness at 12°C]. Apart from the single source leaf exposed to 14CO2, all exporting leaves were removed.

Translocation of 14CO2-derived assimilates. Carbon dioxide, labelled with the short-lived isotope 14CO2 (t1/2 = 20.4 min), was produced by the Compact Cyclotron of the Universität GH Essen (Radioisotopes Zentrum, Essen, Germany). The 14C-technique, including the pulse application of 14CO2, the detection of the 14C-tracer, as well as data acquisition and data analysis were as described by Jahnke et al. (1989). After a short feed of 14CO2 to the blade of the remaining source leaf, temporal profiles of radioactivity were measured at the leaf blade and at different sites on the petiole (Figs. 1, 2A, B). From the tracer profiles of the petiole, times were calculated when the slope on the increasing edge of the traces was 25% of the maximum slope. The obtained time differences corresponding to the known distance between the detectors (2 cm in the experiment of Fig. 2), were used to calculate speeds of translocation (van Oene 1994). When the petiole stump was cut off during an 14C-run, perturbations became obvious at the petiole detectors (see Fig. 2D). The times at which the bending of the tracer profiles was highest were used to calculate the speeds at which the perturbations were propagated along the petiole. They were obtained by setting the third derivative of the polynomial, fitted to the curves, to zero.

Translocation of xenobiotics, sucrose and inulin. For adaptation to the experimental conditions, the excised leaves were transferred to a moist chamber (see below) for 10–20 min. Afterwards, the solution bathing the petiole stump (sink medium) was replaced by a fresh one. The incubation was started by the injection of 10 μl of 10−2–10−4 M 14C-xenobiotics (3.3 kBq) and 10−3 M 3H]sucrose (13.3 kBq). The feeding solution was made up in Mes-buffer (10 mM Mes, 1 mM CaSO4, adjusted with NaOH to pH 5.0). The mixture was shaken for 2–3 h. The radioactivity in both fractions, separated by centrifugation, was measured by liquid scintillation counting.

Determination of partition coefficient (Kpw). The test compound was diluted in 3 ml Thorell-Stenhagen buffer pH 8 (see Rauscher et al. 1977) and mixed with 3 ml n-octanol (Merck, Darmstadt, Germany). The mixture was shaken for 2–3 h. The radioactivity in both fractions, separated by centrifugation, was measured by liquid scintillation counting. The partition coefficient (Kpw) is given by the ratio of radioactivity in the organic and the aqueous phases.

Measurements of 3H and 14C. Radioactivity was counted in a liquid scintillation spectrometer (Packard, Downers Grove, Ill., USA) using a cocktail of 4 g 2,5-diphenyloxazole (PPO) and 0.24 g 1,4-bis(5-phenyloxazolyl)benzene (POPOP) in 11 l toluene.