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Sucrose in the Free Space of Translocating Maize Leaf Bundles[1]

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ABSTRACT

Following exposure of portions of mature maize (*Zea mays* L.) leaf strips to $^{14}CO_2$, xylem exudate from the leaf strips contained [^{14}C]sucrose. Sucrose was the only sugar in the xylem exudate which was obtained from the cut surface of the leaf strips by reducing the external pressure. The sucrose found in the xylem exudate apparently was obtained from the free space of the vascular bundles, its concentration amounting up to 0.25%. When [^{14}C]glucose or [^{14}C]fructose was supplied in the dark to one end of a maize leaf strip, each was taken up by the xylem, and transported to the opposite end. Xylem exudate from such leaf strips contained ^{14}C-labeled sucrose in addition to the ^{14}C-labeled hexose. The results of this study support the view that sucrose is loaded into the companion cell-sieve tube complexes from the apoplast of the vascular bundles in the maize leaf.

Investigations by Geiger and co-workers (6, 14) have provided convincing evidence that phloem loading takes place from the apoplast of sugar beet leaves. These studies are supported by data from Kursanov and Brovchenko (12, 13), who found up to 20% of the total sugar and amino acid content of sugar beet leaves in the apoplast. Giaquinta (7, 8) has demonstrated that phloem loading can take place from the apoplast in 0.4-cm^2 discs of sugar beet leaves. Sucrose uptake was optimal at pH 5.0. Although it could be inhibited chemically, the sucrose apparently penetrated the plasmalemma in an unhydrolyzed form.

The concept of phloem loading via the apoplast has received additional support from recent ultrastructural and plasmolytic studies of the maize leaf by Evert *et al.* (5). Plasmodesmatal connections between bundle sheath cells or vascular parenchyma cells and the companion cell-sieve tube complexes were found to be scarce or rare in small and intermediate bundles. This contrasted sharply with the abundance of such connections between bundle sheath cells and vascular parenchyma cells, as well as between bundle sheath cells and mesophyll cells (4). Plasmolytic studies of photosynthesizing leaves revealed that the companion cell-sieve tube complexes had higher solute concentrations than neighboring vascular parenchyma cells (5). Judging from the distribution of the plasmodesmatal connections between the various cell types of the vascular bundles and from the solute concentrations of those cell types, it appears that sugar is actively accumulated from the apoplast by the companion cell-sieve tube complexes. If sugar is present in the apoplast or free space of the vascular bundles of the maize leaf, it should be possible to detect sugar in exudate obtained from the xylem of those bundles. The purpose of the present investigations was to test that assumption.

MATERIALS AND METHODS

Maize plants (*Zea mays* L. cv. Prior; Samen-Kröbel, Göttingen, Germany) grown in the greenhouse, and about 1 m tall, provided mature leaves from which flat strips of blade were obtained. The preparation of 25- to 30-cm-long and 1- to 2-cm-wide maize leaf strips was described previously (11).

Plant Material Collection of Xylem Exudate. Free space solutes were obtained from leaf strips by collecting xylem exudate in the following manner. One end of the leaf strip was inserted into a slit in a silicon rubber stopper, which then was pressed against the rim of a vacuum flask turned upside down (Fig. 1). With the opposite end of the leaf strip immersed in 1/20 strength Hoagland nutrient solution (3, p. 191), the pressure in the flask was reduced to about 50 torr with a water jet-pump. When this was done, droplets of xylem exudate accumulated at the cut ends of the vascular bundles in the range of 5 to 10 μl/min. Presumably this liquid represented contents drawn from the free space of the bundles. It was removed periodically with calibrated capillaries after switching to normal pressure.

Experiments Utilizing $^{14}CO_2$. In one set of experiments, leaf strips with both ends immersed in diluted Hoagland solution (1/20 N) were each labeled with 20 μCi of $^{14}CO_2$ at 22,000 lux ($3 \cdot 10^{-7}$ w·cm^{-2}) for 20 min over a 1-cm-long segment in the middle of the strip. This procedure was carried out with the Plexiglas device described previously (11). After an additional translocation period of 2.5 hr, xylem exudate was collected with the apparatus illustrated in Figure 1. Exudate was collected either from the portion of the leaf exposed to $^{14}CO_2$ (the feeding zone) or from a portion 3 cm basal to the feeding zone.

Two- to 5 μl-samples of the xylem exudate were run by descending paper chromatography (Schleicher & Schüll 2043/b) utilizing isopropyl alcohol, 25% acetic acid, and water (3:1:1, v/v) as the solvent mixture. Glucose (Glc), fructose (Fru), and sucrose (Suc) were run as references on the same chromatogram as the xylem exudate. Labeled spots were localized by autoradiography on x-ray film and identified as sugars by spraying the chromatogram with a mixture of 0.3% 4-aminohippuric acid in 95% ethanol and 3% phthalic acid in water. The leaf area fed with $^{14}CO_2$ as well as a section of similar size 3-cm basal to the fed area was extracted twice with hot 80% ethanol and three times with hot 50% ethanol to obtain all labeled solutes. The combined extracts were vacuum-dried and taken up in a small amount of water. Acidic, basic, and neutral phases were separated on ion exchange columns (20 × 1.2 cm) filled with Dowex 50 and Dowex 1, respectively. The three phases were eluted with water, 4 N acetic acid, and 4 N NaOH, respectively. Only the neutral phase was separated by paper chromatography as described above.

Determination of Sugar Content in Xylem Exudate. Measurements of the sugar content from the xylem exudate of leaf blade bundles were carried out by the phenol-sulfuric acid method (2), which was adapted to small samples with low sugar concentrations. Optical density was read at 490 nm and sucrose was used to prepare the reference curve. Xylem exudate was obtained from leaf strips as well as from attached leaves.

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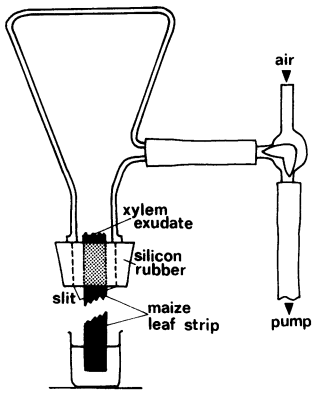

FIG. 1. Device for obtaining xylem exudate from cut leaf blade bundles. A water jet-pump or air can alternatively be switched to the vacuum flask by a three-way stopcock. The leaf strip is inserted through a slit in the silicon rubber stopper by pressing the stopper at right angles to the slit. The beaker contains ½0 N Hoagland nutrient solution or buffer. Emerging droplets are taken up with calibrated capillaries.

Experiments Utilizing [^{14}C]Glucose and [^{14}C]Fructose. In another set of experiments, the apical end of a leaf strip, taken from plants kept for 2 days in the dark, was placed in 1 ml of a 75 mM glucose solution to which 10 μCi of [U-^{14}C]glucose had been added. The other end of the leaf strip was immersed in diluted Hoagland solution. From previous experiments (10) it was known that the label from [^{14}C]glucose would migrate from one end of the leaf strip to the other within 2 hr. After 8 to 10 hr translocation time in the dark, xylem exudate was collected as described above. However, 2 cm of tissue were removed from the end of the leaf strip that was immersed in the [^{14}C]glucose solution and xylem exudate was obtained from the freshly cut end. The labeled components of this exudate were separated and identified by paper chromatography and localized by autoradiography as described above. The same experiment was conducted with 1 ml of a 75 mM fructose solution to which 10 μCi of [U-^{14}C]fructose had been added.

RESULTS

Labeled Compounds in Leaf Extracts after Photosynthesis with $^{14}CO_2$. When a 1-cm-long segment of a maize leaf strip is illuminated and placed in a stream of air containing $^{14}CO_2$, radioactive products of photosynthesis move to the basal end of the strip, especially when this end is darkened or kept in an atmosphere devoid of CO_2 (11). The autoradiograph of such a leaf strip exhibits heavy activity at the basal end of the strip after 2.5 hr translocation time (Fig. 2 "leaf strip"). During the present study the extracts of the 1-cm-long segments exposed to $^{14}CO_2$ contained several labeled solutes. Besides sucrose, glucose, and fructose, one oligosaccharide, and at least one smaller sugar molecule appeared labeled (Fig. 2 "extr. feed. zone" autoradiograph). Naturally, some amino and organic acids also were among labeled compounds of the extracts. For chromatography, basic and acid compounds were removed from the extracts by ion exchange resins. Results of chemical staining of sugars found in the extracts of the feeding zone are illustrated in Figure 2 far right ("extr. feed. zone"). Exactly the same sugars appeared in extracts of other regions of the leaf strip. Three cm away from the feeding zone (Fig. 2 "extr. 3 cm"), the extracts also contained sucrose, glucose, fructose, one oligosaccharide, and traces of at least one smaller molecule of the neutral fraction.

Sucrose Was the Only Soluble Sugar in the Xylem Exudate of the Leaf Blade Bundles. In contrast to the diversity of labeled sugars in leaf extracts, the xylem exudate obtained with the device

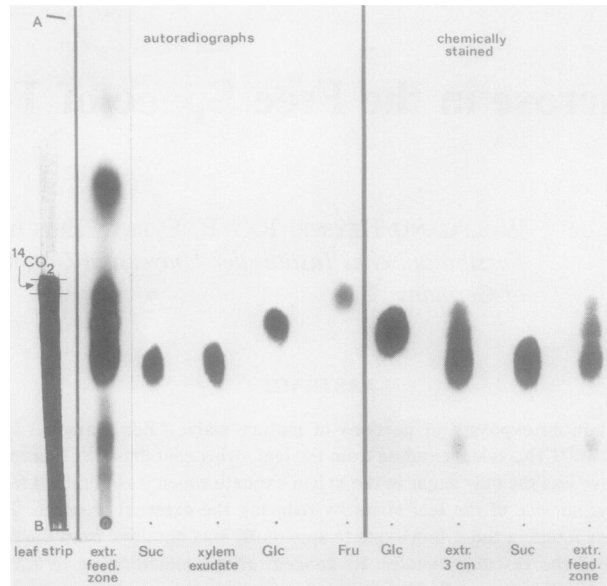

FIG. 2. Sugars of xylem exudate obtained after photosynthesis of $^{14}CO_2$. From left to right autoradiographs show: leaf strip with the feeding zone ($^{14}CO_2$); A, apical, B, basal end of the leaf. Neutral fraction of extract of the feeding zone separated by descending paper chromatography. Labeled sucrose (Suc) as reference, xylem exudate collected from the cut feeding zone, containing only sucrose, labeled glucose (Glc), and labeled fructose (Fru) as references. The chemically stained chromatogram shows from left to right: glucose (Glc), extract of leaf strip 3 cm basal to feeding zone, sucrose (Suc), and extract of feeding zone.

illustrated in Figure 1 apparently carried only one labeled sugar (Fig. 2 "xylem exudate"), which co-chromatographed with sucrose. Radioactivity of sucrose was highest in xylem exudate obtained from the feeding zone and rapidly decreased outside that zone. Sucrose was the only labeled sugar detected by paper chromatography in each of several samples of xylem exudate obtained from illuminated leaves.

Concentration of Sucrose in the Free Space. Since sucrose was the only sugar which could be detected in the xylem exudate by paper chromatography, its concentration was estimated photometrically using the phenol-sulfuric acid method. The sucrose concentration varied considerably depending upon pretreatment of the plant from which the leaf strip was taken. Xylem exudate obtained from leaf bundles of illuminated plants contained 0.3 to 0.5 mg of sucrose/ml. When the plant was darkened for 24 hr the sucrose content of the xylem exudate dropped to 0.05 to 0.1 mg/ml.

Much higher sucrose values were obtained from leaf strips of predarkened plants, when partially illuminated. The experimental conditions are illustrated in Figure 3. A leaf strip with both ends immersed in ½0 N Hoagland solution was exposed to mercury vapor light (22,000 lux). However, one half of the strip, either the basal or the apical, was kept dark. After 3 hr illumination the middle section of the strip was removed, and xylem exudate was collected from both freshly cut ends (arrow heads). The sucrose values obtained in several experiments were 0.4 to 0.6 mg/ml at the darkened side, and 0.8 to 1.1 mg/ml at the light side. When the exposure to light was extended to 6 hr, 0.7 to 0.9 mg/ml were obtained from the dark side and 2.2 to 2.5 mg/ml were recorded in the xylem exudate of the light side.

These values support the view that the sucrose concentration in the free space is dependent at least in part upon the rate of photosynthesis. In darkened parts of the leaf strips the increase in concentration of free space sucrose probably results from unloading of sucrose from the companion cell-sieve tube complexes in regions that effectively serve as sinks.

Conversion of Externally Applied ^{14}C-Hexoses to [^{14}C]Sucrose. Experiments were carried out using ^{14}C-labeled glucose or fructose applied to one end of maize leaf strips in the dark. As described previously (10, 11), during such experiments the tracer is taken up by the xylem and carried in the transpiration stream to the middle of the leaf strip, where it meets the solution moving from the other end of the strip. Once the tracer and solution have met, the tracer will not pass beyond that point unless it is taken up into the phloem.

Apparently glucose seems to be taken up into the phloem. At least its label can be detected by autoradiography at the far end of the leaf strip. After about 2 hr, activity was detected at the opposite end of the strip; and after 8 to 16 hr, the entire leaf strip was heavily labeled (10). Similar results were obtained with labeled fructose. Figure 4 shows, on the left, the autoradiograph of a leaf strip, the apical end (A) of which was immersed in 75 mM glucose solution containing uniformly labeled [^{14}C]glucose. After 8 hr the leaf strip was processed for autoradiography. Two cm of tissue were removed from the apical ends of similarly treated leaf strips, and the remaining portions were utilized to obtain xylem exudate with the apparatus illustrated in Figure 1.

When [^{14}C]glucose was supplied to the leaf strip the xylem exudate contained considerable amounts of labeled sucrose (Suc) in addition to labeled glucose (Glc). Similarly, when [^{14}C]fructose was supplied to the strip, both labeled fructose (Fru) and labeled sucrose were found in the xylem exudate. The relative quantities of both stained and labeled sugars can be estimated from the chromatograms in Figure 4.

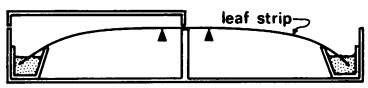

FIG. 3. Illustration of the experimental condition for darkening one half of a leaf strip (left) while exposing the other half to light (right). Both ends of the leaf strip are immersed in dilute Hoagland solution (stippled). After treatment the section of leaf strip between the two arrowheads was removed, and xylem exudate was collected from the freshly cut ends.

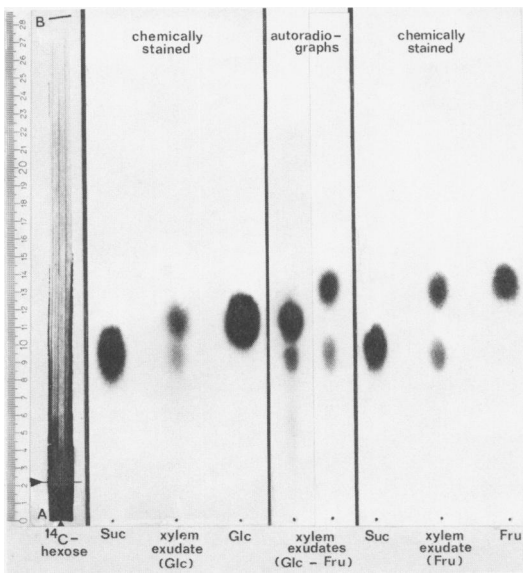

FIG. 4. Sugars of xylem exudates, obtained after feeding with ^{14}C-hexoses. From left to right: scale in cm, autoradiograph of a leaf strip that was supplied with [^{14}C]glucose at the apical (A) end for 8 hr. Arrowhead indicates region from which xylem exudates were obtained. B: basal end of leaf strip. Chemically stained chromatogram with xylem exudate obtained after glucose (Glc) supply. Lower spot represents newly formed sucrose which is given as reference at Suc. Autoradiographs of xylem exudates after application of [^{14}C]glucose (left) and [^{14}C]fructose (right). Chemically stained chromatogram with xylem exudate obtained after fructose (Fru) supply, sucrose (Suc), and fructose (Fru) as references.

DISCUSSION

The presence in the xylem exudate of only sucrose apparently confirms the assumption that sucrose is a normal constituent of the free space at sites of phloem loading. Furthermore, it supports the view of de Fekete and Vieweg (1) that invertase is not active in the free space of the bundle sheaths and vascular bundles of the maize leaf.

Following photosynthesis, the sucrose content of the xylem exudate obtained from maize leaves increases. When $^{14}CO_2$ is administered for photosynthesis, sucrose of the xylem exudate also appears ^{14}C-labeled. Sucrose of the free space of maize leaf bundles at least in part must be a product of photosynthesis. Depending on the duration of a preceding dark period, concentration of free space sucrose was found to vary between 0.05 and 0.5 mg/ml.

When $^{14}CO_2$ was fed to a 1-cm-long median portion of a leaf strip, the ^{14}C-labeled sucrose resulting from photosynthesis apparently entered the free space in that region and there was taken up by the companion cell-sieve tube complexes. Phloem transport then resulted in movement of the ^{14}C-labeled sucrose to the basal end of the leaf strip (Fig. 2). This sequence of events seems to be the only way to account for the movement of sucrose from illuminated to darkened parts of leaf strips (Fig. 3). Logically, when the sucrose content of the free space increases in the darkened parts of the leaf strip, that sucrose must have been transported there from the illuminated part of the leaf strip via the phloem. The sucrose then appears in the free space when unloaded from the phloem in the dark. In comparison with the low amounts of free space sucrose in attached maize leaves, sucrose concentrations in leaf strips may be much higher. This indicates that the free space of the maize leaf bundles has a much greater capacity for uptake of sucrose than normally required. It has not yet been demonstrated whether very high rates of photosynthesis can fill this capacity.

Giaquinta (8), using asymmetrically labeled sucrose [^{14}C]fructosyl-sucrose), has shown that sucrose is accumulated intact from the apoplast and is not hydrolyzed prior to phloem loading in *Beta vulgaris* leaves. Consequently, the conversion of externally supplied ^{14}C-hexoses (glucose or fructose) to [^{14}C]sucrose in the maize leaf strips was of particular interest. Since both the ^{14}C-hexoses and the labeled sucrose derived from them occurred in the free space, it seems most likely that the hexoses were converted into sucrose prior to vein loading. The site of this conversion is unknown, but the bundle sheath cells or vascular parenchyma cells are likely candidates.

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