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Effect of Exogenously Supplied Foliar Potassium on Phloem Loading in *Beta vulgaris* L.[1]

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DIANE C. DOMAN[2] AND DONALD R. GEIGER
Department of Biology, University of Dayton, Dayton, Ohio 45469

ABSTRACT

The effect of foliar application of K^+ on processes associated with phloem loading was investigated in source leaves of sugar beet (*Beta vulgaris* L.). KCl was supplied exogenously at concentrations of up to 100 millimolar in the solution bathing the abraded upper epidermis of source leaves. K^+ added at concentrations below 30 millimolar generally promoted the rate of export of material derived from $^{14}CO_2$ but not from exogenously applied [^{14}C]sucrose. Paralleling promotion of export, the level of material derived from photosynthesis, which was released into the bathing solution, also increased in response to addition of K^+ to the free space. Net photosynthetic rate was not affected. K^+ at 5 and 15 millimolar concentrations did not stimulate uptake of [^{14}C]sucrose into source leaf discs.

The data suggest that the promotion of export rate by K^+ results primarily from an effect on the site regulating efflux of sucrose into the apoplast prior to loading into the minor veins, rather than on the loading site itself. A change in the level of sucrose in the free space appears to precede a change in export rate.

The presence of a relatively high concentration of K^+, in the range of 20 to 100 mM, in phloem exudate (10, 13, 27) suggests a possible role for this ion in transport, although little information is available implicating any specific mechanism. Amir and Reinhold (1) and Hartt (14, 15) observed a depression in translocation in K^+-deficient bean and sugarcane plants, respectively, before other deficiency symptoms became apparent. Work by Mengel and co-workers demonstrated a promotive effect of K^+ on exudation rate in *Ricinus communis* (24) and on translocation and yield in several plant species (12, 25). They suggested that the beneficial effect of K^+ results from its influence on phosphorylation, which increased the ATP status of the plant and therefore increased phloem loading.

A more direct effect of K^+ on sugar transport has been suggested by the results of experiments in which K^+ was supplied exogenously to plant tissue. Malek and Baker (23) reported stimulation of ^{14}C-sugar uptake into *R. communis* petioles following the addition of K^+ to the free space. They noted also that the addition of K^+ decreased the pH of Tris buffer without added sucrose, circulating in contact with the free space. This effect was attributed to K^+ stimulation of a plasmalemma pump mediating proton efflux linked with K^+ influx. It has been suggested that phloem loading of sucrose is coupled to the transport of protons along their electrochemical gradient (9). Under these circumstances, an increase in proton concentration in the free space caused by K^+ might be expected to promote sucrose uptake. However, the addition of K^+ to sucrose solutions bathing cotyledons of *R. communis* did not stimulate sucrose uptake (17, 19).

In an intact translocating plant there are several potential control points at which K^+ could affect export. According to the model of phloem loading presented by Geiger and co-workers (4, 5, 31), sucrose destined for translocation moves from its site of synthesis in the mesophyll cells to the region of the phloem where it enters the apoplast prior to being loaded into the se-cc[3] complex. The efflux site may be one of the points at which the rate of export is determined, with the efflux of assimilates being influenced by substances present in the apoplast.

The present study examined the effect of application of exogenous K^+ on translocation from sugar beet source leaves. Foliar application of K^+, at concentrations of up to 30 mM, enhanced the rate of export of material derived from photosynthesis, but not of exogenously supplied [^{14}C]sucrose. The rate of efflux of material derived from photosynthesis into solutions bathing source leaf tissue was also stimulated by K^+ addition while the uptake of [^{14}C]sucrose into leaf tissue was not affected. The results indicate that K^+ promotes export by increasing the efflux of assimilates into the apoplast prior to the phloem-loading step.

MATERIALS AND METHODS

Plant Material. Sugar beet plants (*Beta vulgaris* L., cv. US H20 or A-1 monogerm hybrid) were grown for 4 to 6 weeks by solution culture as previously described (7) or by sand culture in equal volumes of sand and Jiffy-Mix under the same light and temperature regime. The latter group was watered daily with the same mixture used for solution culture; K^+ was present at 4 mM concentration. Photon flux density was 300 μE m^{-2} s^{-1} at leaf level.

Translocation Experiments. The closed gas flow system for steady-state labeling of source leaves was similar to that used by Geiger and Swanson (8). Light from a metal halide lamp produced a photon flux density of 300 μE m^{-2} s^{-1} at leaf level. Plants were trimmed to a sink leaf/source leaf system at least 12 h prior to the experiment. The source leaf was abraded with a paste of 600-mesh aluminum oxide and distilled H_2O to facilitate entrance of the bathing solution into the leaf free space (31).

The leaf chamber (Fig. 1) consisted of an inner chamber designed to hold solution on the abraded upper surface of the leaf and a larger outer chamber which was supplied with $^{14}CO_2$ from the gas flow system. The leaf was sealed between an aluminum bottom plate with an aperture slightly smaller than the blade and a Plexiglas top by caulking compound placed on both plates. A separate large chamber permitted an air-tight system without applying excessive pressure to the leaf edges during sealing. Solutions were added with two syringes connected to the solution

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[2] Present address: Department of Zoology, Arizona State University, Temple, Arizona 85281.

[3] Abbreviations: se-cc: sieve element-companion cell; Pipes: piperazine-*N*,*N*'-bis(2-ethanesulfonic acid); NPR: net photosynthetic carbon fixation rate.

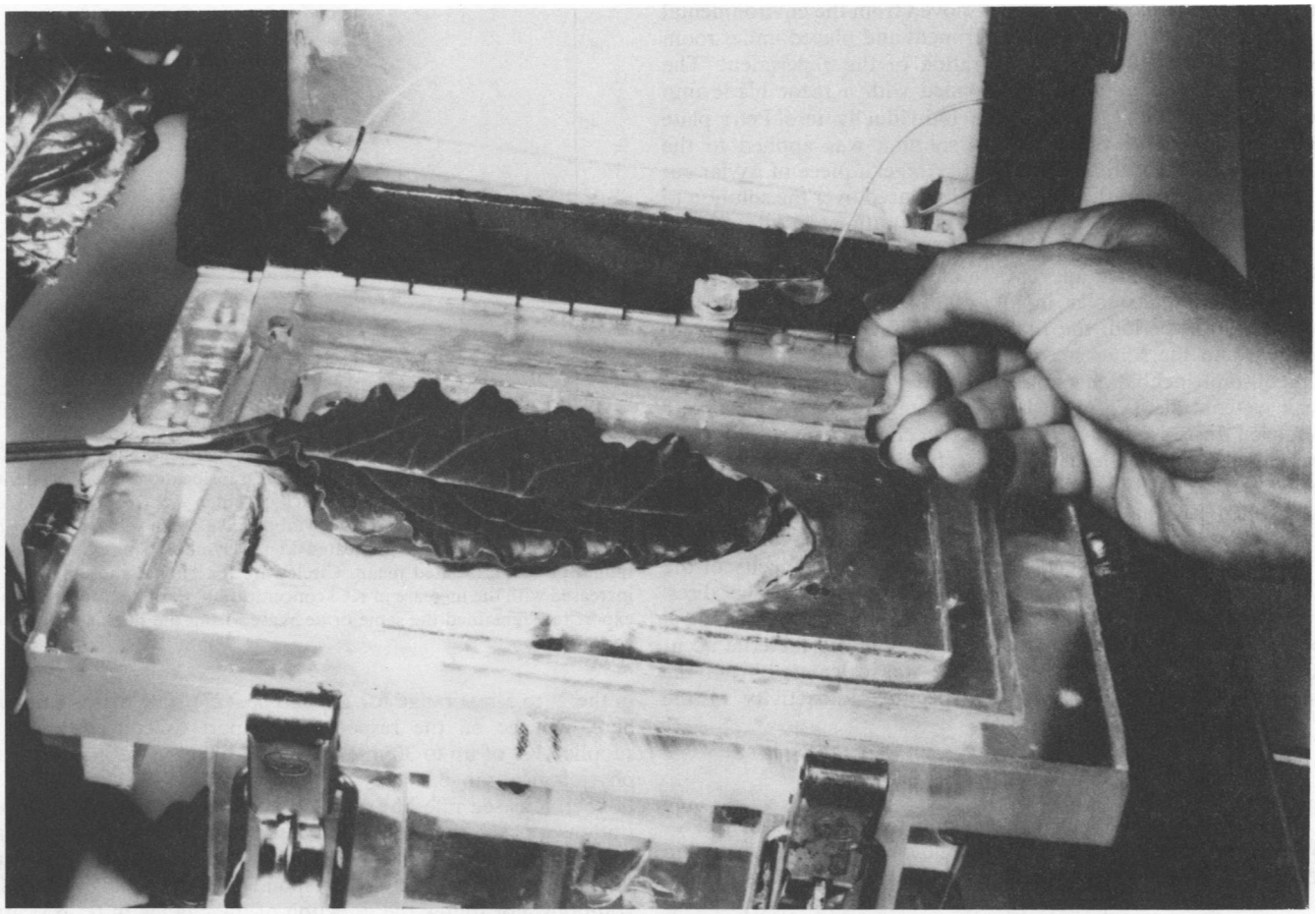

FIG. 1. Chamber for holding solution in place on upper leaf surface is shown in place inside labeling chamber. Small tubing leads to syringes on lid of labeling chamber. Aperture for lamina in base plate of solution chamber is 9 cm in length.

chamber by polyethylene tubing. The bathing solution used in all experiments contained 5 mM Pipes buffer and 1 mM $CaCl_2$, adjusted to pH 6.5 with Tris.

After the source leaf was sealed in the chamber, a 10- to 12-ml volume of buffer was supplied to the abraded leaf surface. The experiment was started by replacing atmospheric CO_2 in the system with $^{14}CO_2$ at a concentration maintained between 500 and 700 μl/l. Translocation rate was monitored at the selected sink leaf with a GM tube and NPR was measured according to methods previously described (8). Approximately 1 h after the attainment of a steady rate of arrival, K^+ concentration was increased by dissolving the desired amount of KCl in a small amount of solution removed from the chamber and reinjecting the mixture. This procedure was repeated several times according to the particular experimental design. In some experiments, [^{14}C]sucrose sufficient to bring the concentration to 5 mM was added to the buffer bathing the leaf by withdrawing some solution and adding back the amended solution. Unlabeled CO_2 was maintained at air level in this type of experiment.

To assess better whether changes in the rate of translocation occurred as a result of treatments, a curve-fitting computer program was used. The error for a least-squares fit with a single second order polynomial was compared with those for a series of curve fits by two separate second order polynomials. The break point was sequentially moved along the time course curve at 5-min intervals. An *F*-statistic was computed to provide a basis for more objective determination whether or not a change in the translocation rate had been produced by a given treatment. Responses of translocation rate to treatments generally occurred within 30 to 50 min following the treatment.

Efflux of $^{14}CO_2$-derived Assimilates. The amount of labeled solute present in the solution bathing the translocating source leaf was monitored by sampling it at 10- to 15-min intervals. A volume of 1 to 1.5 ml of solution was collected in the syringe connected to the chamber near the base of the leaf and transferred to a small test tube. A 50-μl aliquot was placed in a liquid scintillation vial containing 40 μl of concentrated glacial acetic acid and the mixture was heated to free $^{14}CO_2$. The remaining buffer was replaced into the bathing solution by the syringe at the leaf tip. The activity in the solution was determined from the counts, corrected with factors for efficiency and sample volume.

The pattern of efflux of material derived from $^{14}CO_2$ out of pieces of source leaves was also investigated. Source leaves were labeled as in translocation experiments for 3 h. Then leaves were abraded and discs 1 cm in diameter were removed with a sharp cork borer. Discs were placed, abraded side down, on buffer in a glass-labeling chamber. The discs were allowed to photosynthesize in an atmosphere containing $^{14}CO_2$ at 500 to 700 μl/l for approximately 3 h prior to the addition of KCl to give a concentration of 15 mM. Samples of solution were removed and prepared for counting as described above.

In other experiments, source leaf discs were placed abraded side down, on filter paper moistened with buffer and labeled with $^{14}CO_2$ for 2.5 h. Ten ml of buffer was added to the chamber to wash ^{14}C-labeled solutes from the discs prior to their being placed into 2.5 ml of buffer containing 0, 15, or 50 mM KCl. The incubation dishes were kept in dim light for the 110-min efflux period. Solution was sampled for ^{14}C-solutes at 10-min intervals.

[^{14}C]Sucrose Uptake Studies. [^{14}C]Sucrose uptake studies were designed to investigate the effect of K^+ on sucrose accumulation

in source leaf tissue. Plants were removed from the environmental chamber 3 to 4 h prior to the experiment and placed under room light, 10 μE m^{-2} s^{-1}, for the duration of the experiment. The selected leaf was abraded and divided with a razor blade into several pieces which were placed individually into Petri plate moist chambers. A preincubation solution was applied to the abraded surface of the piece with a syringe; a piece of Mylar cut slightly smaller than the leaf piece was placed over the solution to hold the solution in place and reduce evaporation (3). All solutions contained the buffer described above and 0, 15, 50, or 75 mM KCl. Sorbitol was added in appropriate amounts to attain a value of 150 to 160 milliosmolar in all solutions. Following a 20-min preincubation period, the first solution was removed, the piece blotted, and the solution containing 2 mM [^{14}C]sucrose applied. The 30-min incubation period was ended by washing the piece with ice-cold distilled H_2O. Labeled sugars in the apoplast were removed during a 20-min period in two changes of solutions that were equivalent to the incubation solutions but contained unlabeled sucrose. Leaf discs were punched from the leaf pieces, rinsed in distilled H_2O, and placed in liquid scintillation vials. After two cycles of freezing and thawing, 0.5 ml of methyl cellusolve was added to each vial to increase the water-holding capacity of the fluor and enhance diffusion of soluble material from the leaf discs. Nine ml of fluor was added and the vials allowed to stand 12 h before being counted. The amount of ^{14}C-labeled material accumulated from uptake of [^{14}C]sucrose was calculated from the content of radioactivity and the specific radioactivity of the [^{14}C]sucrose.

RESULTS

Effect of K^+ on Export. The effect of foliar application of K^+ on export of materials derived from photosynthesis was studied in a series of 15 experiments with two to four increments of KCl concentration per experiment. Figure 2 illustrates a typical response pattern of translocation rate when KCl is added to a source leaf in increasing concentrations. Two responses are evident: the lower concentrations of 5 and 15 mM KCl promote mass transfer rate, while the higher concentrations of 50 and 75 mM KCl cause a decline in the rate. New rates were obtained within 40 min following KCl addition and were maintained for the duration of the experimental period until the KCl concentration was changed.

It is assumed that addition of K^+ to the abraded upper surface of the source leaf increases the free space concentration of that ion. Concentrations of K^+ in xylem sap have been reported to be

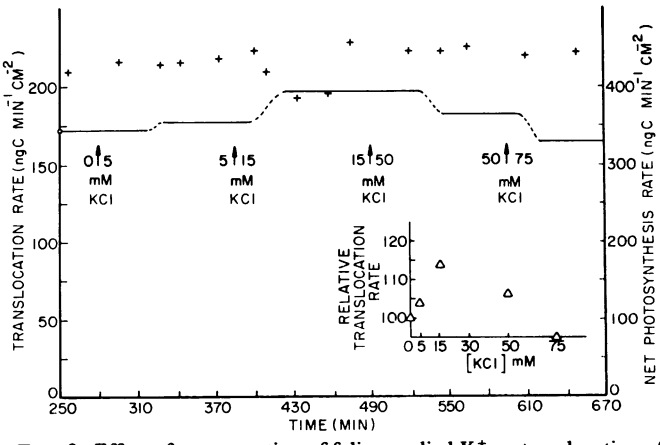

FIG. 2. Effect of concentration of foliar applied K^+ on translocation of labeled material derived from photosynthesis in $^{14}CO_2$ by a source leaf of sugar beet (——) NPR (+) was not affected by the stepwise changes in KCl concentration. Representative of 15 experiments of this type. Inset shows effect of KCl concentration on translocation rate expressed in relation to rate without KCl.

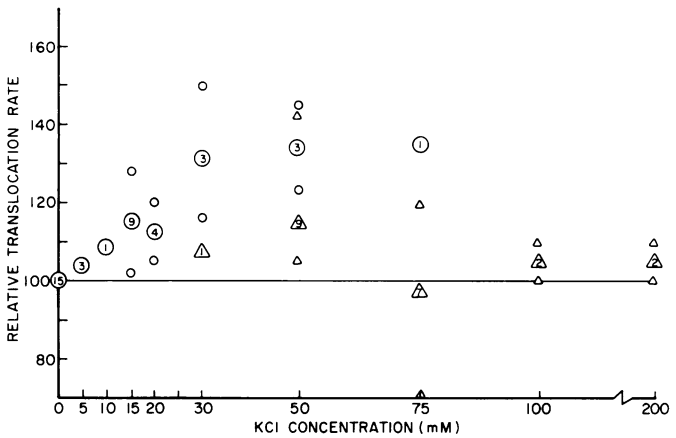

FIG. 3. Effect of concentration of KCl applied to abraded upper surface of a sugar beet leaf lamina on rate of translocation of $^{14}CO_2$-derived material. Data are from 15 plants, each of which was treated with at least two concentrations of KCl. Large symbols give mean relative export rate for the number of plants indicated in the symbol. Small symbols give range for the associated mean. Circles are for plants whose export rate increased with the increase in KCl concentration, triangles for those whose export rate remained the same or decreased from the rate at the previous concentration.

in the 2- to 5-mM range for *Lupinus* sp. (27). The major emphasis here, will be on the response of export to concentrations of supplied K^+ of up to 30 mM which are likely to be in or near the physiological range.

The increased rate of export observed at the lower K^+ concentrations was not the result of increased NPR as this was not altered by KCl additions. Slight variations in NPR occurred during the day, but generally did not correspond with the timing of the KCl additions, nor follow the direction of the change in translocation rate. Plants used in these studies exported approximately 40% of the carbon assimilated.

Plants differed somewhat in the response of export to foliarly applied K^+. Figure 3 is a composite of data from experiments on 15 plants. In general, the plants displayed a pattern of response similar to that in Figure 2, but the degrees of promotion and inhibition varied as did the concentration at which export rate first decreased. Addition of KCl to leaves at concentrations of up to and including 20 mM increased the export rate in all plants. The percentage of plants responding by enhanced export rate decreased as the K^+ concentration was increased above 20 mM.

Foliar application of Na^+ also produced promotion of translocation. When NaCl was applied at concentrations of 15 and 30 mM, it gave promotion of translocation rates by an average of 15 and 35%, respectively (data not shown), similar to the means for K^+. Addition of sorbitol to the buffer to increase osmolarity to the range present with added KCl did not promote translocation.

K^+ Effect on Uptake of Exogenous [^{14}C]Sucrose. Although data from experiments described above indicate that foliar application of K^+ increases the export of recently assimilated material it was not known whether the added K^+ acts directly on the minor vein loading system. Experiments were performed in which [^{14}C]sucrose was supplied to the free space along with various concentrations of KCl. Previous studies have shown that [^{14}C]-sucrose is loaded into the minor veins of exporting leaves and can be translocated at rates comparable to those for photosynthetically derived assimilates (3, 31). Figure 4 shows the effect of increasing concentrations of K^+ on export of [^{14}C]sucrose. The curves were tested for location of discontinuities along their time course but none of statistical significance were found. The same program readily identified K^+-induced changes in export rate of material derived from $^{14}CO_2$. The data show that K^+ did not promote phloem loading at concentrations up to and including 30 mM, the

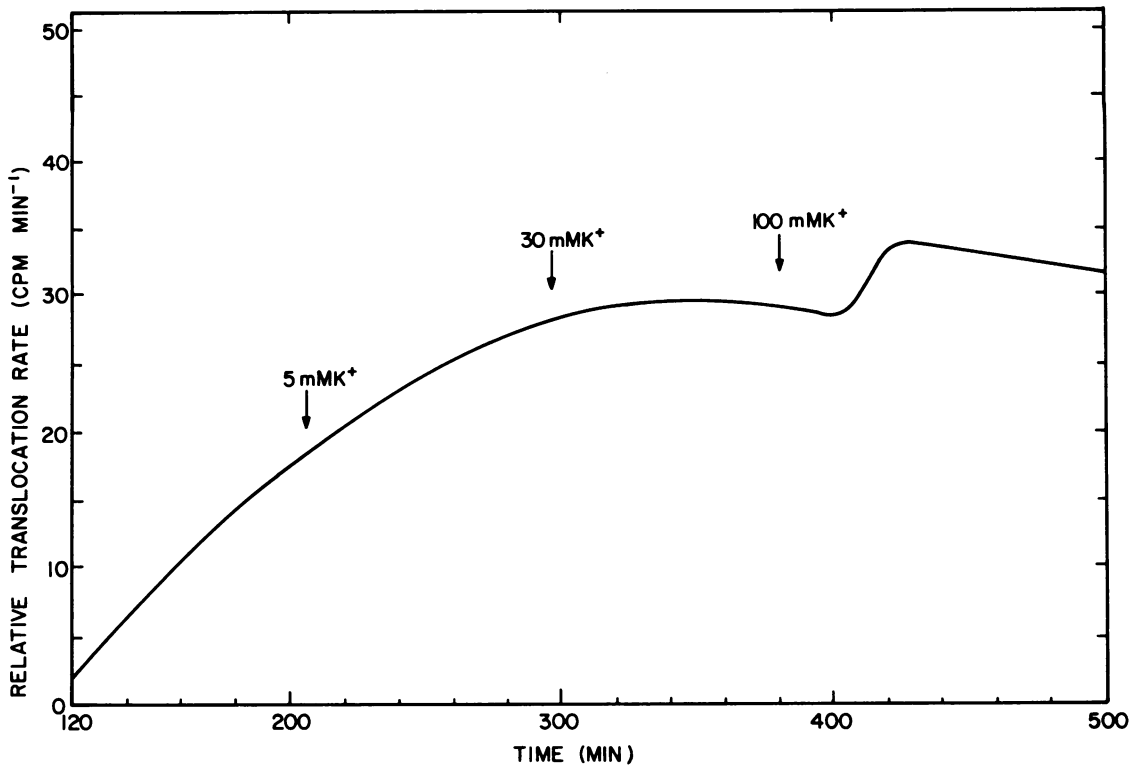

FIG. 4. Time course of export of ^{14}C-labeled material derived from [^{14}C]sucrose supplied to abraded upper surface of a sugar beet source leaf treated with 5, 30, and 100 mM KCl. Export followed a similar time course in two other experiments of this type as well as in control experiments with leaves not treated with KCl. Photon flux density was 300 $\mu E\ m^{-1}\ s^{-1}$.

concentration range in which the export of $^{14}CO_2$-derived material was stimulated.

In another approach to the investigation of a possible direct effect of KCl on the uptake system(s) of the source leaf, exogenous sucrose was applied to leaf pieces and the accumulation of [^{14}C]sucrose in the metabolic space was assayed. Sucrose uptake rates for sugar beet leaf discs incubated in buffered 2 mM [^{14}C]-sucrose containing various concentrations of KCl are shown in Table I. The proportion of label in each tissue was not determined in this experiment but accumulation into the minor veins of isolated sugar beet leaf discs has been demonstrated (9). The data in Table I show that there is no significant difference between uptake rates for discs incubated in 0, 5, or 15 mM KCl. This is in agreement with the results of the translocation experiment depicted in Figure 4. The addition of exogenous KCl up to a concentration of 30 mM neither stimulates phloem loading as measured by [^{14}C]sucrose arrival in the sink, nor increases sucrose uptake into the minor veins or mesophyll of the leaf tissue.

K^+ Effect on Efflux of Material Derived from Photosynthesis. To test whether an increase of free space K^+ concentration promotes transfer of sucrose into the free space prior to phloem loading, experiments were conducted in which accumulation of labeled material in sinks and efflux of labeled solute from the source leaf were monitored simultaneously. A summary of the pattern observed in seven experiments of this type is shown in Figure 5. The increase in efflux into the bathing solution occurs approximately 20 min prior to the increase in arrival of exported material in the monitored sink. In two other experiments in which addition of high concentrations of KCl caused a decline in export, a decrease in efflux into the bathing solution occurred prior to the decline in arrival in the sinks.

It is difficult to pinpoint the exact time at which label started to increase in relation to when the translocation rate increased. There are complications of identifying transport time to the sink region and the time lag in the sampling technique. No circulation system was used, and only manual stirring was employed, delaying immediate detection of change.

Table I. *Effect of KCl Concentration in Solution Bathing Abraded Upper Epidermis on [^{14}C]Sucrose Accumulation into Leaf Pieces of B. vulgaris L.*

KCl and 2 mM [^{14}C]sucrose were applied in 5 mM Pipes-Tris (pH 6.5) containing 1 mM $CaCl_2$ after 20-min preincubation in buffer at the chosen KCl concentration. Solutions were osmotically balanced with sorbitol. Leaf discs were punched from the pieces following a 30-min incubation period. Data are the average ± SE of 12 to 19 samples from three experiments, each normalized separately.

KCl Concentration	No. of Samples	Uptake Rates	
		Absolute	Relative[a]
mM		*nmol sucrose cm⁻²*	
0	19	6.39 ± 0.15	1.00 ± 0.02[a]
5	12	6.60 ± 0.22	1.01 ± 0.03[a]
15	13	6.63 ± 0.16	0.99 ± 0.02[a]
50	19	7.22 ± 0.25	1.14 ± 0.04[b]
75	19	7.41 ± 0.17	1.14 ± 0.02[b]

[a] Values with superscript *a* differ at the 1% level of significance from values with superscript *b*. Multiple comparison among the means was performed with Student Newman-Keuls test.

Interpretation of the results was based on a previous study of entry of sucrose into the free space prior to phloem loading (6). When solutions with no added sugar are used to serve as a trap the level of solute is considered to be a result of the rates of entry into and of uptake from the free space. The resulting equilibrium value likely reflects not only the rates for solute transfer in the phloem-loading region but for all leaf cells in contact with the solution. After isotopic equilibrium is attained for the major solutes entering and leaving the free space, a change in uptake or efflux by any group of cells should be reflected in the level of labeled solutes in the solution bathing the leaf tissue. The increases

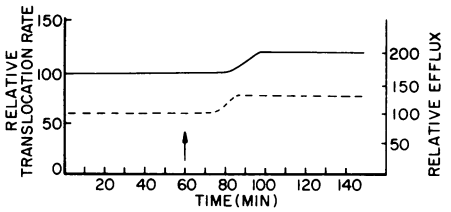

FIG. 5. Summary representation of time course of promotion of efflux of ^{14}C-labeled solute (- - -) and export of material derived from photosynthesis in $^{14}CO_2$ (——). Composite of data from seven experiments of this type showing response of addition of 15 mM KCl to the solution bathing a source leaf. Conditions similar to those for experiment shown in Figure 2.

in both efflux and translocation were of the same order; enhancement of export was similar to the values for promotion reported in Figure 3.

Another series of experiments was conducted in a nontranslocating system consisting of leaf discs photosynthesizing in $^{14}CO_2$ while floating on a buffered treatment solution. After a control period, 15 mM K^+ was added to the solution containing one of the two sets of discs (Fig. 6). Efflux was increased by approximately 50% over the control values within 50 min after treatment, an increase which is about twice the usual increase in export rate. The discs were excised several hours prior to the addition of KCl, so phloem loading had probably ceased. Oscillations in level of labeled solutes, probably triggered when the discs were placed on the buffer, were observed to have a period of approximately 60 min. The significance of these is not apparent. The solutes found in the solutions were mostly sucrose, glucose, and fructose but the probable presence of invertase activity precluded quantitative interpretation of the data.

In a second series of experiments using the nontranslocating disc system, efflux of material derived from $^{14}CO_2$ during a previous illumination period was followed during incubation of discs in K^+ solutions under dim light conditions. Table II gives data for the amount of labeled material in the treatment solutions, both in absolute terms and relative to that found for discs in 0 mM KCl. The relative increase in the efflux of labeled material compares favorably with the degree of enhancement of export in intact plants treated with 15 mM KCl and confirms the variability of effect on export seen for 50 mM KCl (Fig. 3). A difference in efflux can be detected within 5 min and efflux is nearly complete within 45 min after cessation of labeling and illumination.

Previous studies by Geiger (4) have shown that sucrose enters the free space in moving from mesophyll to minor veins and that the rate of export is dependent on the concentration of sucrose presented to the free space of translocating leaves. Loading and export of a fixed concentration of exogenously supplied sucrose were not promoted by the addition of K^+ (Fig. 4), but efflux of material derived from photosynthesis was increased by the addition of K^+. These results indicate that it is the increase of free space sucrose caused by K^+ treatment that increases phloem loading and export.

Possible Causes of Increased Efflux and Export. Labeled compounds in the discs used for experiments of the type reported in Figure 6 were examined. Most of the recovered ^{14}C-compounds remained in the discs (>96%); the partitioning into sucrose, total soluble and total insoluble material, was determined and compared for treated and untreated discs. Addition of 15 mM K^+ affected neither the rate of ^{14}C fixation, nor the amount of $^{14}CO_2$ incorporated into sucrose or insolubles including starch during the labeling period. We conclude that K^+ in the 0- to 30-mM range does not directly change synthesis patterns for sucrose or insolubles including starch in this nontranslocating system. Using isolated soybean mesophyll cells, Servaites and Schrader (29) also observed an increased efflux of sucrose into the incubation medium following the addition of up to 50 mM KCl. Their data indicated that increased efflux was accompanied by an alteration in sucrose/starch metabolism. In contrast, increased efflux from intact sugar beet leaf tissue appears to be the result of a change in membrane properties rather than in the internal concentration of sucrose available for efflux.

Increased efflux raises the apoplast level of assimilates, which in the translocating system would likely stimulate export rate. The increased export probably causes synthesis of sucrose to increase as well, possibly at the expense of the amount of assimilate entering the insoluble pool. We believe that increased sucrose synthesis is a result, rather than the cause, of K^+-enhanced export from sugar beet leaves.

Some evidence from previous studies is available for characterizing the nature of the efflux process. Rates of sucrose transport across membranes of the cells involved with the loading process were examined for indications that efflux is a result of passive permeation or facilitated diffusion. A mass transfer rate of 170 ng C min^{-1} cm^{-2} (Fig. 2), that is, 1.2 nmol sucrose min^{-1} cm^{-2} lamina will be used in the flux calculations. Assuming the area occupied by the se-cc complex to be 0.88 cm^2 cm^{-2} lamina (30), the flux across the membranes of the se-cc complex is 1,360 pmol sucrose min^{-1} cm^{-2} membrane. If there is a uniform exit of sucrose into the apoplast through all of the mesophyll cells prior to phloem loading, the flux is calculated to be 120 pmol sucrose cm^{-2} min^{-1} membrane, based on a mesophyll membrane area of 10 cm^2 cm^{-2}

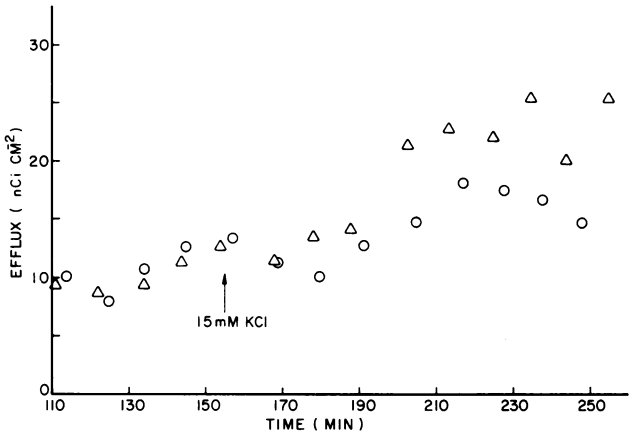

FIG. 6. Effect of KCl on time course of efflux from sugar beet source leaf discs of compounds derived from $^{14}CO_2$. Source leaves of intact plants were supplied with $^{14}CO_2$ for 3 h and leaf discs were removed and floated on buffer. At indicated time, KCl was added to concentration of 15 mM for half the discs (△) while the remainder served as a control (○). Data are similar for all three experiments of this type. A t test showed the difference between the groups with and without addition of K^+ to be significant at greater than the 99% level ($t = -6.57$, $df = 15$). Efflux increased by 50% after addition of K^+.

Table II. *Effect of KCl Applied to Apoplast on Efflux in Darkness of $^{14}CO_2$-derived Assimilates from Source Leaf Discs of B. vulgaris L.*

Discs were labeled for 2.5 h with $^{14}CO_2$ and then floated in dim light on 5 mM Pipes-Tris (pH 6.5) containing 1 mM $CaCl_2$ and KCl. Efflux from 12 (experiment 1) or 10 (experiment 2) discs expressed as total amount of label cm^{-2} leaf in each solution at the conclusion of the 110-min efflux period.

Incubation Solution	Amount of Efflux			
	Experiment 1		Experiment 2	
mM	*pCi cm^{-2}*	*%*	*pCi cm^{-2}*	*%*
0 KCl	650	100	440	100
15 KCl	730	112	550	125
50 KCl	380	58	560	127

lamina (30). This flux calculated for mesophyll membranes is approximately 10^6 times as large as that reported for passive efflux of sucrose from whole cells (Hansen, cited in ref. 2). The efflux would be still higher if, as seems likely, it occurs by a facilitated process only from those cells in the immediate vicinity of the se-cc complex. The phloem parenchyma cells, located in close proximity to the se-cc complex, are likely sites for efflux (31). Osmotic studies indicate that the solute content in these cells is less than in the border parenchyma or mesophyll cells, and therefore, the osmotic gradient is in the right direction for symplastic movement of sugar from the mesophyll into the vicinity of the phloem (5). Gunning and Pate (11) have described transfer cells (B type), comparable to phloem parenchyma cells, which appear to be functionally adapted to promote efflux of assimilate into the apoplast in pea leaves. The concept of a localized facilitated efflux is further supported by the 5- to 10-fold discrepancy between the estimated range of sucrose concentration in the entire apoplast (6) and the K_j values for phloem loading of sucrose (31). Facilitated efflux would produce a localized higher concentration of sucrose at the loading site.

If efflux occurs by facilitated transport involving a carrier mechanism, K^+ may act at that site. Potassium may stimulate an ATPase involved in transporting sucrose into the free space in the vicinity of the se-cc complex in the minor veins. Alternatively, K^+ may cause a conformational change in membrane protein(s) which results in enhanced efflux or may act indirectly through displacement of Ca^{2+} from binding sites. Hawker and co-workers (16) have suggested that Ca^{2+} may play a role in regulating efflux into the apoplast. They reported that efflux of sucrose, but not of reducing sugars, was several times greater from sugar beet and bean leaf pieces incubated in K_2SO_4 than from pieces incubated in $CaSO_4$. Differences in starch and sucrose content of the tissues in the different incubation media were interpreted as being a consequence of the different efflux rates. No data are available in their work for efflux into solutions without metal ions. Displacement of Ca^{2+} by monovalent ions (21) or chelating agents (22) has been shown to affect carrier systems or membrane integrity or both in several plant systems. Further studies with increased levels of Ca^{2+} in the solution bathing source leaf tissue may help elucidate the mechanism of the K^+ effect on translocation.

A direct relationship between K^+ and the sugar uptake mechanism of the phloem has recently been proposed (23). Potassium has been shown to stimulate H^+ efflux from several plant tissues (17, 23, 26, 28). If, as in algal (20) and bacterial (18) systems, sugar uptake is energetically linked to the inward flux of protons along their electrochemical gradient, an increase in proton concentration in the free space might be expected to enhance loading. However, in the present study enhanced loading does not seem to be due to a direct effect on sucrose uptake. In addition, as Hutchings (17) pointed out, the influx of K^+ may actually compete with sucrose influx as an alternative way of dissipating the proton electrochemical gradient across the membrane. Such a depolarizing effect may explain the decrease in export seen at the higher concentrations of K^+ but appears not to effect export at the lower K^+ concentrations. The relationships between ion and sucrose fluxes across the membranes involved with sucrose efflux in the vicinity of the minor vein se-cc and with phloem loading need to be clarified by further study.

CONCLUSIONS

Several lines of evidence support the involvement of leaf free space in the transfer of sugars from their site of production to the se-cc complex (3–6, 9, 11, 27, 30, 31). The efflux sites, probably restricted to phloem parenchyma near the minor vein se-cc complexes, may act to regulate the level of assimilate released into the apoplast and consequently the rate of phloem loading and export. The present study shows that K^+, supplied exogenously to the apoplast of sugar beet leaves, stimulates export of photosynthetically derived assimilates by increasing efflux into the free space. We believe that the primary effect is on a membrane site involved with control of sucrose efflux. Further work is necessary to determine whether K^+ normally present in the apoplast functions in this regulatory manner.

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