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Sources of Sucrose Translocated from Illuminated Sugar Beet Source Leaves[1]

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ABSTRACT

A search for source leaf sucrose pools that differed in their relation to export was carried out in photosynthesizing leaves of *Beta vulgaris* L. The time course of depletion of [^{14}C]sucrose in a leaf in unlabeled CO_2 following steady state labeling provided evidence for two distinct sucrose pools. After the start of the light period, leaf blade sucrose remained constant although it exchanged between the two pools. Newly synthesized sucrose destined for export passed through one pool more rapidly than through the other. All of the leaf blade sucrose appeared to exchange with export sucrose. Modeling and regression analysis of [^{14}C]sucrose data provided a means for estimating the size of the two pools. From 20 to 40% of the sucrose was calculated to be present in the pool that provided the less direct path to export; this was likely vacuolar sucrose. The remainder of the sucrose in the blade was probably in the cytoplasm and veins. Added amounts of leaf blade sucrose, produced in response to elevated CO_2, appeared to be stored mainly in the vacuolar compartment.

Carbon exported from photosynthesizing sugar beet source leaves is derived from several sources. That which is translocated from photosynthesizing leaves comes largely from newly fixed carbon, whereas that exported at night comes mostly from starch formed during the previous light period (4). Changes in export rate accompany the transitions from one source of export carbon to another (4). At these times, export also may be supported by other sources of carbon such as accumulated sucrose. In some plants, source leaf sucrose is the most abundant form of carbon that is accumulated for later export (10–12). In other plants, including sugar beet, the storage role of source leaf sucrose is less clear although this function is certainly quantitatively less important than it is for starch (4, 12).

Available data indicate that most of the newly fixed carbon that is not converted to starch exits from chloroplasts and is synthesized into sucrose in the cytoplasm of cells fixing carbon by the C-3 pathway (20). In sugar beet leaves, a sizeable portion of this sucrose is loaded into the minor vein phloem and exported within a short time after synthesis (5, 9). Data from *Vicia faba* L. provide evidence that some newly synthesized sucrose is stored in the vacuoles of exporting leaves in this plant (2, 17). Histochemical analysis revealed that there are several kinetically distinct sucrose pools in exporting *Vicia* leaves (17). Kinetic analysis of cytoplasmic and vacuolar sucrose in palisade parenchyma demonstrated that the former turns over rapidly, whereas the vacuolar pool is less readily available for export and turns over slowly (2).

Studies in sugar beet show that source leaf sucrose is relatively constant throughout the day except during the initial part of the light period, when it accumulates (3, 4). This pattern is in contrast to that for barley where sucrose accumulates in photosynthesizing source leaves throughout the light period (10, 11). Photosynthesizing mesophyll protoplasts from barley leaves accumulate recent products of carbon fixation, including sucrose, in their vacuoles. Vacuolar sucrose increases at a rate similar to that of overall sucrose synthesis in the protoplasts (14). In *Vicia* leaves, for which kinetic data for sucrose export from various pools is available, it is not clear whether sucrose increases markedly during the light period. Clearly, exporting leaves differ markedly in partitioning and accumulation of carbohydrates (12).

The present study was undertaken to determine whether a portion of the sucrose in photosynthesizing sugar beet source leaves is relatively isolated from that which is rapidly exported, and if so, to characterize the size, kinetic behavior, and size variability of this pool as well as the extent to which it contributes to export during the light period. If this pool of sucrose is sizeable, it may prove to be significant in maintaining export during the transition periods between day and night (4) and following changes in environmental conditions.

Data from earlier studies with exporting sugar beet leaves led to the conclusion that nearly all of the sucrose in exporting source leaves is readily available for export (7–9) and quickly becomes equilibrated with newly fixed carbon (9). Recent studies (4) raised the possibility that a significant amount of storage sucrose may be present in sugar beet source leaves under certain conditions. In the present study, source leaf sucrose that is less readily mobile during the light period was examined by steady state labeling. The method was designed to increase entry of labeled carbon into pools which may be turning over slowly and to permit estimation of pool size. Contrary to earlier data (7–9), the results of the present study revealed that in sugar beet source leaves some of the sucrose was relatively isolated from, but exchangeable with, sucrose that is readily available for export. The amount of less available sucrose varied considerably among sugar beet plants but seemed large enough to be a significant source for export during transition periods.

MATERIALS AND METHODS

Plant Material. Sugar beet plants (*Beta vulgaris* L., Klein E type, multigerm) were raised in a mixture of sand and peat moss/vermiculite. Plants were watered twice daily with a nutrient solution described by Snyder and Carlson (22) and used when 5 to 6 weeks old.

Labeling and Sampling. A source leaf was enclosed in a chamber through which was circulated air containing $^{14}CO_2$ of constant specific radioactivity. Concentration of CO_2 was controlled within

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a narrow range and labeling was carried out as described previously (6, 9). The blade to be sampled was allowed to photosynthesize under steady conditions in $^{14}CO_2$ for 4 to 6 h to allow pools which were turning over slowly to become more extensively labeled than is the case with pulse labeling and to allow source leaf sucrose as a whole to approach isotopic saturation (6, 9). At the time labeling was halted, sampling of a source leaf blade began and was continued at intervals for an additional 4 to 6 h. Four punches with a combined area of 0.6 cm^2 were removed, one from each quadrant of the lamina at each sampling time and processed for total and ^{14}C-labeled sucrose, hexoses, and starch (3). Care was taken that the areas sampled were not isolated from major veins by punches from a previous sample.

Carbohydrate Determinations. Sucrose, fructose, and glucose were assayed with a nonamplified enzymic assay in which the end product NADPH was measured spectrofluorometrically (13). Sucrose and fructose first were converted enzymically to glucose which was then assayed. The ^{14}C-labeled sugars were measured by liquid scintillation counting following separation by TLC (3). Starch was extracted and assayed spectrofluorometrically (13) following conversion to glucose by amyloglucosidase (18).

Data Analyses. Kinetic characteristics and sizes of sucrose pools present in exporting sugar beet source leaves were computed from [^{14}C]sucrose measurements obtained by steady-state labeling followed by a period in unlabeled CO_2. Loss of [^{14}C]sucrose from a photosynthesizing source leaf and accumulation of ^{14}C in a monitored sink leaf were measured during the chase period. Sucrose with varying degrees of availability for immediate export is contained in discrete compartments in the several layers of a leaf (2, 17). In order to permit estimation of pool parameters by modeling and regression methods, we reduced the description to a two-compartment model. The simple model avoided the many transfer coefficients with unknown values which result from a more explicit modeling of the intercellular compartmentation found in leaves (17). Description of the model and its mathematical analysis are given in detail in a later section.

The model chosen was similar to that used by Moorby and Jarman (15) and is shown in Figure 1. One compartment consists of relatively isolated sucrose that exchanges with the second compartment containing sucrose that is readily available for export. Sucrose in the veins of the source leaf was included with the rest of the pool of readily exported sucrose even though the former probably does not exchange with the less mobile pool. The error introduced by this aggregation was analyzed and found to be acceptable and similar in size to the uncertainty introduced by experimental error. The labeled sucrose content of the exporting source leaf, S^*, in the model is described by a two-term exponential function of the form:

$$S^*(t) = Ae^{-at} + Be^{-bt} \quad (1)$$

where a and b describe turnover of the faster and slower pools, respectively. A and B are related to the subdivision of the [^{14}C] sucrose in the leaf at the start of the chase period, but they do not

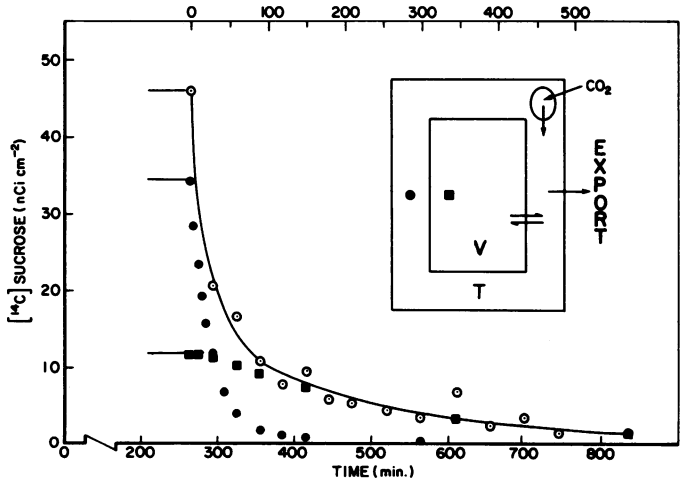

FIG. 1. Time courses of measured [^{14}C]sucrose content of a source leaf (⊙) and calculated content of pools that contribute [^{14}C]sucrose rapidly (●) and less rapidly (■) to export from photosynthesizing sugar beet source leaves. The measured data were fitted with the function $Y = 31.5\ EXP(-0.0413t) + 14.4\ EXP(-0.00394t)$, which was used to calculate the ^{14}C content of the two pools. Sucrose content of the source leaf was found to remain constant and the analysis supposes that the component pools did likewise under the conditions present.

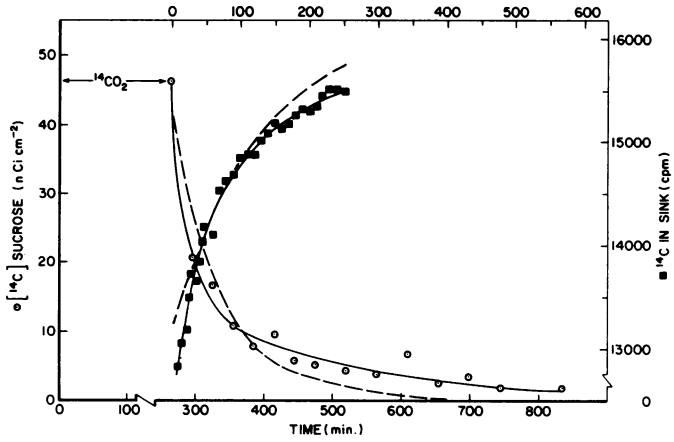

FIG. 2. Depletion of [^{14}C]sucrose from a sugar beet source leaf (⊙) and accumulation of exported ^{14}C in a sink leaf (■) following cessation of a 4.5-h period of steady-state labeling begun at time zero (top abscissa). Carbon dioxide specific radioactivity was 1.1 $\mu Ci\ mg^{-1}$ C. Data for arrival have been shifted to deduct the time required for sucrose to move from source to sink. Source leaf [^{14}C]sucrose data were fitted with the function given in Figure 1 (——). To demonstrate graphically the improvement of fit by the two-term function, the data were also least squares fit by a one-term exponential function. The resulting function obtained was $Y = 41.4\ EXP(-0.0138t)$ (- - -). The data for sink accumulation were fitted with the functions $Y = 16{,}100 - 1{,}510\ EXP(-0.2960t) - 1{,}710\ EXP(-0.00469t)$ (——) and $Y = 16{,}100 - 2{,}960\ EXP(-0.0081t)$ (- - -). The first term in each of the latter equations is the projected long term limit value for ^{14}C accumulated in the sink leaf.

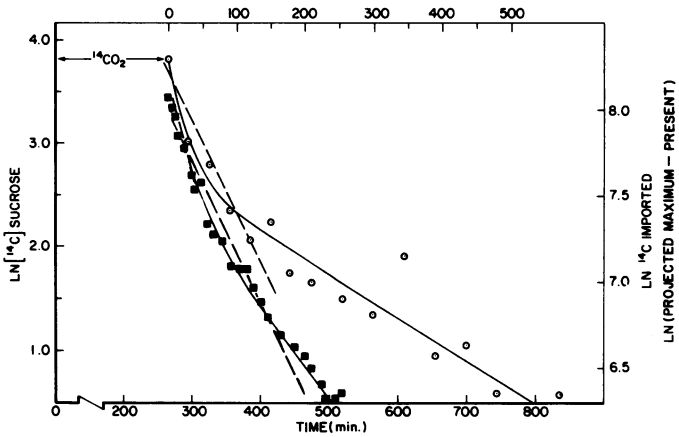

FIG. 3. Comparison of data for depletion of source leaf [^{14}C]sucrose and for accumulation in a sink leaf plotted as the logarithm of values from Figure 2. Key as in Figure 2. Data for arrival of ^{14}C in sink was transformed by subtracting the observed counts from 16,100, the projected maximum count rate, to facilitate comparison with source leaf data.

equal the pool size at $t = 0$ because of exchange between the pools. Mathematical details and derivations of equations used in analyzing pool parameters from regression analysis are contained in a later section.

Data for loss of [^{14}C]sucrose from the exporting source leaf and for ^{14}C accumulation monitored in a sink leaf were fitted by two-term exponential functions with nonlinear least squares methods. The sucrose content of the two pools of a source leaf described by the model was calculated by using the regression function, the measured specific radioactivity of the $^{14}CO_2$ supplied, and the average sucrose content of the source leaf lamina during the chase period. The latter must remain constant for the method to apply. Regression methods were used to fit the data because they do not introduce subjective bias into the curve fitting process (24).

RESULTS AND DISCUSSION

Turnover of [^{14}C]Sucrose. Depletion of [^{14}C]sucrose from a source leaf and accumulation of labeled carbon in a sink leaf during a chase period following 4.5 h of steady-state labeling are shown in Figure 2. Rapid turnover of sucrose destined for export led to a precipitous drop in source leaf [^{14}C]sucrose. After 500 min of photosynthesis in unlabeled CO_2, [^{14}C]sucrose was reduced to approximately 4% of the level found at the beginning of the chase period. A semilogarithmic treatment of these data (Fig. 3) revealed that they were well fitted by two-phase exponential functions. Similar patterns were observed for all five experiments of this type. Earlier pulse-chase experiments demonstrated this occurrence for export in *Lycopersicon esculentum* Mill., *Capsicum frutescens* L., and *Amaranthus caudatus* L. (15) and for *Vicia faba* L. (2, 17, 19). The kinetic parameters for accumulation in the sink leaf (Figs. 2 and 3) differ from those for export for unknown reasons. Possibly the intervening path modified the pattern generated in the source leaf, but the decline was advanced rather than being delayed as was expected. In spite of the difference, the pattern for export and sink leaf accumulation in sugar beets was consistent with a model in which sucrose synthesized from newly fixed carbon passes through either of two distinct pools prior to export.

The two-compartment model for source leaf sucrose pools that was used to analyze the data (Fig. 1) included the assumption that the quantity of sucrose present in both pools remained constant during the period of analysis. All of the leaves sampled in this study showed no substantial change in sucrose concentration throughout the period of sampling. This was consistent with the earlier finding that source leaf sucrose is nearly constant throughout a 14-h light period except for a 20 to 40% increase during the 1st h or so of the light period (4). For the plant described in Figures 2 and 3, the sucrose level in the sampled leaf during the 6.5-h chase period was steady at 43.9 ± 6.3 μg C cm^{-2} ($n = 15$). These data support the conclusion that under steady conditions sucrose was not accumulated except at the start of the light period. In this respect, sugar beet leaves differed from those of barley which accumulate sucrose throughout the day (10, 11). A major portion of the stored sucrose likely accumulates in the mesophyll vacuoles of the barley leaf (14). The pattern for source leaf sucrose level during the day was not reported for *Vicia* leaves used in the study of compartmentation mentioned earlier (2, 17).

With source leaf sucrose remaining constant (4), it is likely that neither of the sucrose pools of the sugar beet leaf blade changed after the beginning of the day under the conditions used in this study. Even if there were no change in pool size, decline in [^{14}C] sucrose in each of the pools, indicated by data in Figures 1 through 3, demonstrates that sucrose arising from current photosynthesis traversed either one or the other of the pools prior to being exported. It appears that these two pools constituted essentially all of the sucrose in the leaf lamina. After several hours of the second phase of [^{14}C]sucrose decline, only a few percent of the original labeled sucrose remained. If there were another pool of sucrose present but undetected it could have been only very slightly labeled even after 4 to 6 h of steady-state labeling. But the presence of a significant amount of unlabeled sucrose outside these two pools was unlikely because the specific radioactivity of sucrose at the end of labeling was always near that of the $^{14}CO_2$ supplied to the leaves. For the plant referred to in Figures 2 and 3, the regression function revealed that 46 nCi cm^{-2} was present in the source blade which contained 43.9 ± 6.3 μg C cm^{-2} blade. Based on these figures, sucrose specific radioactivity at the end of the steady-state labeling was 1.05 nCi μg^{-1} C or 95% of that of the CO_2 supplied.

Source Leaf Carbohydrate and Export. Analysis of source leaf starch sampled at 15 intervals throughout the 8.5-h chase period for the plant described in Figures 1 to 3 confirmed the earlier finding that starch does not contribute significantly to the synthesis of [^{14}C]sucrose in photosynthesizing sugar beet source leaves (3). During the chase period, [^{14}C]starch content in the leaf blade was 48 ± 7 nCi cm^{-2}. A regression line fitted through the [^{14}C]starch data indicated a decline of only 0.2 nCi cm^{-2} or 0.4% of the labeled starch, over the 8.5-h chase period. If starch turns over within the photosynthesizing chloroplast as some evidence indicates (23), the ^{14}C derived from mobilization of [^{14}C]starch did not contribute to synthesis of labeled sucrose in the mesophyll cytoplasm in our studies.

The decline of [^{14}C]sucrose is consistent with the model shown in Figure 1. Parameters of the regression equation fitted to the [^{14}C]sucrose decline data together with the specific radioactivity of the CO_2 supplied and the average sucrose content of the source leaf were used to determine the time courses of the [^{14}C]sucrose of each pool and their sucrose content. Details of derivation of the equations, assumptions, and methods are given in "Mathematical Analysis." Values for the plant described in Figures 1 to 3 are given in Table I. The sucrose pool less directly available to provide sucrose for export constituted from 18 to 42% of the total source blade sucrose in the four plants studied (Table II; Table III, day 1). The pool containing sucrose that is less directly available for export varied in size among plants but it appeared to constitute a sizeable store of source leaf carbon. This conclusion is at odds with an earlier set of studies of sucrose translocation by sugar beet source leaves which led to the conclusion that there is a single source leaf sucrose pool and that sucrose destined for export passes through this pool (8, 9). In the studies cited, failure of sucrose to reach isotopic saturation was considered to be a result of discrimination against ^{14}C isotope (7). It now appears that the effect was largely the result of labeling for too short a time to observe evidence of a second sucrose pool.

In the present study, the percent isotopic saturation was measured to be 90 to 97%. Taking the data for [^{14}C]sucrose content in the two pools at the end of steady-state labeling (Fig. 1) and the pool sizes (Table I), we can estimate the degree of isotopic saturation of sucrose in each pool. Using the values of 34 nCi cm^{-2} and 30 μg C cm^{-2} for the pool with the faster sucrose turnover and 11 nCi cm^{-2} and 14 μg C cm^{-2} for the pool with slower sucrose turnover, we arrived at values of 1.1 and 0.79 nCi μg^{-1} C for their respective specific radioactivities. The CO_2 supplied had a specific radioactivity of 1.1 nCi μg^{-1} C and, consequently, sucrose in the pool that contributed [^{14}C]sucrose to translocation more slowly was at 72% of isotopic saturation. Because this was so, entry of [^{14}C]sucrose from the pool with more rapid turnover to that with less rapid caused the specific radioactivity of the sucrose in the slower pool to increase slightly for a few minutes after the end of labeling before falling (because of the scale of Figure 1 this increase is not evident). This same entry initially slowed the decline of ^{14}C in the slower pool (Fig. 1). This pattern is seen also in Fisher and Outlaw (Ref. 2; Fig. 2A). The lack of isotopic saturation in this pool, even after several hours of steady-

Table I. *Range of Regression Equation Parameters and Estimates of Size of Source Leaf Sucrose Pools from these Parameters*

Fitted data are from the experiment shown in Figures 1 to 3. Regression analysis was carried out 10 times with varied initial estimates of parameters of the model equation.

	Parameters				Sucrose					
	A	*B*	*a*	*b*	Directly available		Less directly available		Total	
	nCi cm^{-2}		*min*$^{-1}$		*μgC cm*$^{-2}$	%	*μgC cm*$^{-2}$	%	*μgC cm*$^{-2}$	%
Average	29.1	16.4	0.0449	0.00469	29.9	68	14.0	32	43.9	100
Low	25.7	14.3	0.0413	0.00390	26.4		12.6			
High	31.5	20.2	0.0598	0.00630	31.3		17.5			
Range[a] (%)	(20)	(36)	(41)	(51)	(16)		(35)			

[a] Values are range as percent of average.

Table II. *Sucrose Content and Distribution between Sucrose Pools in Sugar Beet Source Leaves from Three Plants*

Sucrose content measured from parameters of regression equations.

Experiment	Sucrose Pools				
	Directly available		Less directly available		Total
	μgC cm^{-2}	%	*μgC cm*$^{-2}$	%	*μgC cm*$^{-2}$
1	30	68	14	32	44
2	23	58	17	42	40
3	23	82	5	18	28

state labeling, may explain, at least in part, the gradual increase in [^{14}C]sucrose observed in an earlier study of sugar beet (Ref. 9; Fig. 3). At that time the increase was attributed, probably mistakenly, to accumulating source leaf sucrose.

The slow exchange of sucrose in vacuoles with the cytoplasmic pool is supported by the slow sucrose transport across the tonoplast of vacuoles from castor bean endosperm observed by Nishimura and Beevers (16). They reported 62% of the cellular sucrose was present in the vacuoles after 5 h of incubation during isolation.

Changes in Size of Sucrose Pools. There is considerable difference in the patterns of allocation of newly fixed carbon to carbohydrate pools in photosynthesizing, exporting leaves among plants of different species (12) as well as in plants of the same species even when grown under similar conditions (4). One aspect of this difference, the distribution of sucrose between the two source leaf pools was seen in four plants raised and measured under similar conditions (Table II; Table III, day 1).

To determine how carbon allocation to the two source leaf sucrose pools might change in response to an increased level of accumulated sucrose, we measured distribution of sucrose in the same leaf at the same net carbon exchange rate on 2 successive d. On the 1st d, carbon fixation took place in air and, on the next, photosynthesis occurred for the first 7.5 h in an atmosphere with elevated CO_2, which resulted in increased leaf sucrose. Translocation rate adjusts rapidly to a new steady rate when the rate of net carbon exchange is lowered (1, 21) but not when it is raised (1). Sucrose level remains constant in the leaf when the rate of net carbon exchange is lowered (Table III; Sliwinski and Geiger, personal communication). As a consequence, the sucrose pools are considered to be at steady-state during the chase periods following labeling on both days.

The difference in [^{14}C]sucrose depletion under the two levels of leaf sucrose at the same carbon exchange rate is evident in Figure 4. Calculated sucrose pool sizes are given in Table III for leaves at the two levels of leaf blade sucrose. Most of the additional sucrose appeared to reside in the pool of less directly available sucrose. The sucrose that is more directly available for export is likely the sucrose in the cytoplasm of the mesophyll cells and in the minor vein phloem. The pool with slower sucrose turnover is likely the vacuolar pool. This interpretation of the identity of the model compartments is supported by the results of Fisher and Outlaw (2) and Outlaw *et al.* (17). Further studies are needed to characterize the role of the vacuole in sucrose storage in sugar beet, a plant that mainly accumulates starch during the light period (4).

The experimental protocol for the experiment shown in Figure 4 is similar to the step-down experiments of Christy and Swanson

Table III. *Calculated Distribution of Sucrose between Two Pools in a Photosynthesizing Sugar Beet Source Leaf at Original and at Elevated Total Sucrose Content*

Photosynthesis rates were similar during both test periods (Fig. 4). Sucrose in the blade was steady on both days after the usual initial buildup. Sucrose content of the blade, measured during each sampling period, was found to be level at 16.4 μgC cm^{-2} (range 15.3–17.8; n = 11) on day 1 and at 35.8 μgC cm^{-2} (range 33.2–38.4; n = 11) on day 2. An 8- to 9-h pretreatment at usual or elevated CO_2 level was given at the beginning of the light period to establish sucrose level in the blade. Ranges reported in parentheses in the body of the table were calculated from the range of values for regression equation parameters in 10 trials.

Pretreatment CO_2 Concn.	Sucrose Pools				
	Directly available		Less directly available		Total
	μgC cm^{-2}	%	*μgC cm*$^{-2}$	%	*μgC cm*$^{-2}$
Day 1: 330 μl l^{-1}	9.9 (8.9–10.3)	60	6.5 (5.7–7.1)	40	16.4
Day 2: 750 μl l^{-1}	11.5 (11.1–12.3)	32	24.3 (23.5–24.7)	68	35.8

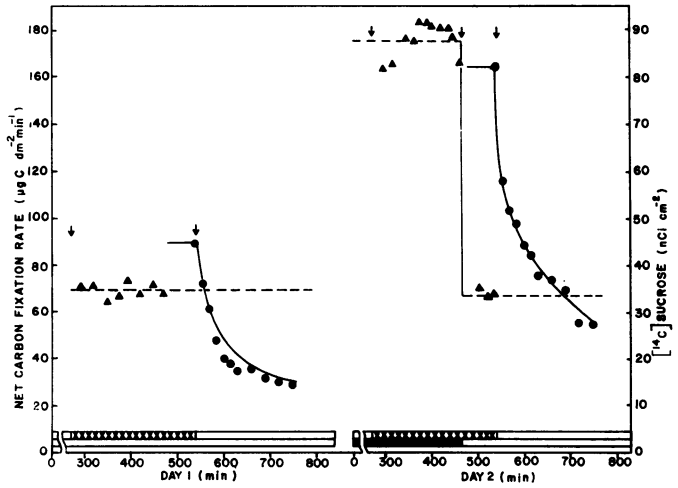

FIG. 4. Depletion of [^{14}C]sucrose from a sugar beet source leaf (●) during chase periods in air following 4 h of steady-state labeling in $^{14}CO_2$ on 2 successive d (labeling periods indicated by ×, top bar). Total sucrose content was steady throughout the labeling and chase periods on both days. Net carbon exchange rates (▲) were similar during each sampling period as was sucrose turnover (the level of sucrose was steady during each chase period). The CO_2 concentration around the leaf was maintained at an average level of 340 ± 10 $\mu l\ l^{-1}$ except for the first 8 h of the second day when the CO_2 concentration was maintained at 750 ± 15 $\mu l\ l^{-1}$ (shaded portion, bottom bar). Arrows indicate times at which CO_2 status was changed. Regression equations were $Y = 30.4\ EXP(-0.027t) + 15.4\ EXP(-0.0016t)$ for day 1 and $Y = 26.3\ EXP(-0.100t) + 55.9\ EXP(-0.00362t)$ for day 2.

(1). On the basis of their results, they hypothesized that the instantaneous translocation rate is directly dependent on the instantaneous concentration of the transport sucrose pool. The translocation rate of the plant was similar on both days during the chase period and was 50% higher at the elevated CO_2 level in the present study. Although the sucrose level was twice as high the 2nd d, consistent with the hypothesis, the sucrose in the more directly available pool was calculated to be similar during both chase periods. It is possible that sucrose from the cytoplasm moved into the vacuole when the rate of net carbon exchange was lowered by reducing CO_2 but this does not seem to be likely and is not supported by the calculations of sucrose pool sizes. The relationships between source leaf sucrose, translocation, and net carbon exchange rate are presently being studied. Recent data from our studies indicate that the relationship between leaf sucrose and translocation is more complex than stated in this hypothesis.

MATHEMATICAL ANALYSIS

Choice of Model. The model used for analysis of source leaf sucrose pools is shown in Figure 1. In the model that was originally developed, there was a third pool corresponding to sucrose in the sieve tubes and companion cells of the source leaf. This pool received sucrose from the rapidly mobile pool but, unlike the latter, did not exchange with the pool that was less readily available for export. The differential equations for this model can be routinely solved to give a three-term exponential function. Problems arise from this approach because the relationships between the six regression coefficients and the parameters of the model (pool sizes in particular) which we hoped to evaluate are exceedingly complex. Adding two more regression coefficients increased the amount of information that needed to be extracted from the fitting of a necessarily limited number of [^{14}C]sucrose values obtained during the chase period. Consequently, we chose to use the two-compartment model which combined the vein and readily mobile pools and thus lessened the SE of the estimate.

Solution of Differential Equations. In writing the differential equations that describe transfer of radioactivity, we made no assumptions about attainment of isotopic saturation in either pool. As noted above, we observed that sucrose was constant during the chase period and we assumed that distribution between the pools did not change. For simplicity of reference, the rapidly mobile sucrose pool is referred to as the transport pool and the less readily available sucrose as the storage pool. In the model, r is the rate of mass exchange between transfer and storage pools, k is the rate constant for export from the transport pool. Total leaf sucrose S is the sum of the transport sucrose T and the storage sucrose V. An asterisk denotes the radioactive sucrose in a pool. The equation describing the change of radioactive sucrose in the storage pool is:

$$\dot{V}^* = r\left(\frac{T^*}{T} - \frac{V^*}{V}\right) \tag{2}$$

and the equation for change of radioactive sucrose in the transport pool is:

$$\dot{T}^* = r\left(\frac{V^*}{V} - \frac{T^*}{T}\right) - kT^* \tag{3}$$

The change in total radioactive sucrose is given by:

$$\dot{S}^* = \dot{V}^* + \dot{T}^* = -kT^* \tag{4}$$

To solve for S^*, we rewrite equations (2) and (3)

$$\dot{V}^* = \frac{r}{T}T^* - \frac{r}{V}V^* \tag{5}$$

$$\dot{T}^* = -\left(\frac{r}{T} + k\right)T^* + \frac{r}{V}V^* \tag{6}$$

To simplify notation, let $x = V^*$, $y = T^*$, $\alpha = r/V$ and $\beta = r/T$. Then:

$$\dot{x} = \beta y - \alpha x \tag{7}$$

$$\dot{y} = -(\beta + k)y + \alpha x \tag{8}$$

Differentiate Eq. 8, use Eq. 7 to substitute for $\dot{x}$ and arrange in standard form:

$$\ddot{y} + (\alpha + \beta + k)\dot{y} + \alpha k y = 0 \tag{9}$$

The characteristic equation has two roots both of which are negative. To simplify notation, let the roots be $-r_1$ and $-r_2$ where $r_1 > r_2 > 0$, and C_1 and C_2 constants depending on the initial conditions. The solution can then be written:

$$T^* = C_1 e^{-r_1 t} + C_2 e^{-r_2 t} \tag{10}$$

To solve for S^*, return to Eq. 4 and substitute for T^*:

$$\dot{S}^* = -k(C_1 e^{-r_1 t} + C_2 e^{-r_2 t}) \tag{11}$$

Integrating gives:

$$S^* = \frac{kC_1 e^{-r_1 t}}{r_1} + \frac{kC_2 e^{-r_2 t}}{r_2} + C_3 \tag{12}$$

As t approaches infinity, $S^*(t)$ must approach zero and hence $C_3 = 0$; so:

$$S^* = \frac{kC_1 e^{-r_1 t}}{r_1} + \frac{kC_2 e^{-r_2 t}}{r_2} \tag{13}$$

Eq. 13, which enables us to evaluate pool parameters in the model from the regression equation, is a function of the form of Eq. 1. Relating Eq. 13 to Eq. 1 reveals the identifications $A = kC_1/r_1$, $B = kC_2/r_2$, $a = r_1$ and $b = r_2$. We now need to develop equations

relating the six unknown pool parameters—[V, T, k, r, $V^*(0)$, and $T^*(0)$]—to the parameters derived from the regression analysis and other known quantities. Sucrose in the source leaf was at steady-state; from sucrose assay an average value of source leaf sucrose per unit area of leaf, $\bar{S}$, was computed. Thus, since total sucrose consists of transport and storage sucrose:

$$\bar{S} = V + T \tag{14}$$

At $t = 0$, from Eq. 1:

$$S^*(0) = A + B = T^*(0) + V^*(0) \tag{15}$$

Multiplying the roots of the quadratic used in obtaining the solution from Eq. 9, we get:

$$R_1 = r_1 r_2 = ab = \alpha k \tag{16}$$

Adding the roots we obtain:

$$R_2 = r_1 + r_2 = a + b = \alpha + \beta + k \tag{17}$$

Substituting for α and β, we obtain:

$$R_1 = \frac{r}{V} k \tag{18}$$

$$R_2 = \frac{r}{V} + \frac{r}{T} + k \tag{19}$$

From the measurements of CO_2 and $^{14}CO_2$, we know the specific radioactivity σ. We assume that the sucrose in the transport pool is at isotopic saturation at the initiation of the chase period and so has the same specific radioactivity as the CO_2 supplied. Therefore:

$$\sigma = \frac{T^*(0)}{T} \tag{20}$$

At time $t = 0$, export of ^{14}C from the leaf, τ, is given by

$$\tau = kT^*(0) \tag{21}$$

By differentiating Eq. 1 and evaluating at $t = 0$, we obtain:

$$\dot{S}^*(0) = -aA - bB \tag{22}$$

In the same way, from Eq. 4:

$$\dot{S}^*(0) = -kT^*(0) = -\tau \tag{23}$$

Therefore:

$$\tau = aA + bB \tag{24}$$

With these relationships, we are ready to write the equations which will evaluate the pool parameters. To do this, we combine Eqs. 14, 15, and 18–21 to obtain a set of six equations relating the model parameters r, k, $T^*(0)$, $V^*(0)$, T, and V to values which can be obtained from the coefficients a, b, A, and B of the regression equation and $\bar{S}$ and σ. We then solve this system of six equations simultaneously to obtain:

$$T = \frac{\tau^2}{\sigma\tau R_2 - \sigma^2 R_1 \bar{S}} \tag{25}$$

$$k = \frac{\tau}{\sigma T} \tag{26}$$

$$T^*(0) = \sigma T \tag{27}$$

$$V = \bar{S} - T \tag{28}$$

$$V^*(0) = S^*(0) - T^*(0) \tag{29}$$

$$r = \frac{R_1 V}{k} \tag{30}$$

To evaluate these equations, $S^*(0)$, τ, R_1, and R_2 must be evaluated from the regression equation fit to the source leaf [^{14}C]sucrose data. These values are obtained by use of Eq. 15 for $S^*(0)$, Eq. 24 for τ, Eq. 16 for R_1, and Eq. 17 for R_2. In addition, σ must be obtained from the specific radioactivity of the $^{14}CO_2$ supplied and $\bar{S}$ from the average amount of sucrose per unit area of source leaf.

Simulation of Data. The above method of estimating pool parameters was tested on data generated by simulation with a two-compartment model. Supplying of $^{14}CO_2$ was simulated to occur until the transport pool was at greater than 99% of isotopic saturation. The supply of ^{14}C was removed and the [^{14}C]sucrose content of the source leaf was generated during a chase period. The nonlinear regression fit was exact and the pool parameters were estimated to within less than 0.1% error, confirming the method.

We used simulation of data by a three-compartment model to test the error introduced by fitting such a system with a regression equation from a two-compartment model. Systems with transport sucrose to storage sucrose in ratios ranging from 30:70 to 70:30 were tested. Distribution of transport sucrose between cytoplasm and vein was varied between 1:3 and 3:1, the transport storage ratio range that is believed to exist in the present study. We have little data for selecting the cytoplasm/vein distribution ratio. Pool values were selected according to the desired ratio, and the decline of [^{14}C]sucrose during a 5-h chase period was simulated. The latter data was then fit with a two-term exponential function. The pool sizes of transport and storage sucrose were calculated and compared with those used in the original simulation. With the 70:30 ratio, the error ranged from 15% for the transport pool at a 1:3 cytoplasm:vein ratio to 1.6% for the same pool at the 3:1 ratio. At the 30:70 ratio, the error had a range of 6.7% for the transport sucrose at the 3:1 ratio to 0.8% for the storage pool at the 1:3 cytoplasm to vein ratio. This degree of error was judged to be acceptable in view of the level of experimental error in the measured quantities.

Nonlinear Regression Analysis. To perform the least-squares regression fitting of data for [^{14}C]sucrose in the source leaf blade at intervals during the chase period, we used a routine supplied for a Univac 90/80 computer. The user supplied data to be fit, along with initial estimates of the four parameters of the model equation and size of increments to be used in the regression search. Output included values for the four regression equation parameters and a multiple regression coefficient. Each attempt to fit the data was repeated ten times with estimates that were systematically varied. Initial values which provided a good fit as judged by the multiple regression coefficient resulted in convergence to similar regression parameters. The results of ten trials are summarized in Table I along with the range of pool size estimates for data from Figures 1 to 3.

Time Course of [^{14}C]Sucrose in Storage and Transport Pools. Solutions of the differential equations used to describe flow of labeled sucrose within pools of a source leaf can be used to determine the time course of [^{14}C]sucrose in the transport and storage pools of the source leaf. By using Eq. 10 and the identifications associated with Eq. 13, we can write:

$$T^* = \frac{Aae^{-at}}{k} + \frac{Bbe^{-bt}}{k} \tag{31}$$

Based on the model relationship that total [^{14}C]sucrose is the sum of that contained in the transport and storage pools, we obtain from Eqs. 13 and 31:

$$V^* = \frac{A(k-a)e^{-at}}{k} + \frac{B(k-b)e^{-bt}}{k} \tag{32}$$

These equations were used to compute data for the time courses of [^{14}C]sucrose in the transport and storage pools (Fig. 1).

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