

Diurnal Pattern of Translocation and Carbohydrate Metabolism in Source Leaves of *Beta vulgaris* L.¹

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ABSTRACT

Transitions in carbohydrate metabolism and translocation rate were studied for evidence of control of export by the sugar beet (*Beta vulgaris* L. Klein E.) source leaf. Steady-state labeling was carried out for two consecutive 14-hour light periods and various quantities related to translocation were measured throughout two 24-hour periods. Starch accumulation following illumination was delayed. Near the end of the light period, starch stopped accumulating, whereas photosynthesis rate and sucrose level remained unchanged. At the beginning of the dark period there was a 75-minute delay before starch was mobilized. The rate of import to the developing sink leaves at night was similar to that during the day, whereas export decreased considerably at night.

Starch accumulation and degradation seemed to be initiated in response to the level of illumination. Cessation of starch accumulation before the end of the light period was initiated endogenously. Exogenous control appeared to be mediated by the level of sucrose in the source leaf while endogenous control seemed to be keyed to photoperiod or photosynthetic duration.

Allocation of materials for export from the source leaf is determined partly by carbohydrate metabolism in the source leaf, although sink metabolism also influences translocation (6). Involvement of the source leaf is evident from changes in translocation which occur soon after experimental treatment of the source leaf. For example, when net photosynthesis is increased, export increases proportionately, even though the demand generated by the sink remains unchanged (8, 10). When the supply of fixed carbon to a sink is lessened, there can be a change in partitioning among sinks rather than an increase in export from an untreated source leaf feeding the sink (4). These examples illustrate the importance of the source leaf in determining carbon available for export. Mechanisms by which source leaves control allocation of carbon among export and leaf pools are unclear.

Times of transition in carbohydrate metabolism and translocation rate are of interest because it is then that control mechanisms are likely to be most in evidence. This study presents the details of carbohydrate metabolism and translocation throughout 24-h periods. The data show that starch accumulation is delayed following illumination. Near the end of the light period, starch stopped accumulating; net mobilization of starch did not occur immediately at the beginning of the dark period. Changes in sucrose content were associated with these events. Diurnal changes

in carbohydrate metabolism appear to be decisive factors in controlling translocation.

MATERIALS AND METHODS

Plant Material. *Beta vulgaris* L., Klein E, a multigerm variety, was raised in 14 h light (35 nmol photons cm⁻² s⁻¹ PAR) and 10 h dark to an age of 6 to 8 weeks in a mixture of equal parts sand and Jiffy-Mix. Pots were watered through a drip-irrigation system with a nutrient solution described by Snyder and Carlson (11). Source leaves studied had characteristics similar to those described previously (4) except for a lower starch content at the end of the light period, averaging 0.38 mg cm⁻² leaf.

Labeling and Rate Determinations. Details concerning the selection of source leaves for study and the preparation for treatment have been described previously (4). Two source leaves on the same plant were enclosed in separate chambers connected in parallel in a closed system. Rates of net photosynthesis and of export were determined for one leaf during the light period. The carbohydrate content of the same leaf was determined during the dark period, whereas the rate of respiration was determined for the second leaf. For one set of experiments, the leaves photosynthesized for two 14-h light periods in ¹⁴CO₂ maintained at approximately 450 μl L⁻¹, with a specific radioactivity of 0.77 nCi μg⁻¹ C. Rates of export from the source leaf and import into a sink leaf were measured. Rates during the day were determined by a previously described method (5). Rate of export at night was determined from the rate of loss of ¹⁴C from the source leaf after taking loss by respiration into account. Import at night was measured from the rate of accumulation of ¹⁴C at a monitored sink leaf. In all cases it was assumed that after 2 d of steady-state labeling all pools contributing to transport, including starch, were at isotopic saturation.

For a second set of experiments the source leaves being studied photosynthesized in air for 3.5 h after lights were turned on, in ¹⁴CO₂ for 8 h and then again in air for 2.5 h. The displacement of ¹⁴C in starch and sucrose by ¹²C was followed in these plants.

The system volume was increased from 1.5 to 10 L and the initial CO₂ concentration was lowered to about 165 μl L⁻¹ during the dark period to follow accumulation of respiratory CO₂. The CO₂ level in the closed system rose no more than 3-fold during the 10-h dark period.

Ratemeter data from GM detectors was acquired at 1-min intervals by a Bascom-Turner 8120 Recorder and stored on a floppy disk. Curve fittings, rate determinations and plots were done with the aid of a computer.

Determination of Carbohydrates. Four samples, with a total area of 0.65 cm², were removed at 30-min intervals from the leaf for which the rates of net photosynthesis and export were determined. Details of sampling and of nonamplified enzymic assay procedures for determination of starch and sugars have been described earlier (4).

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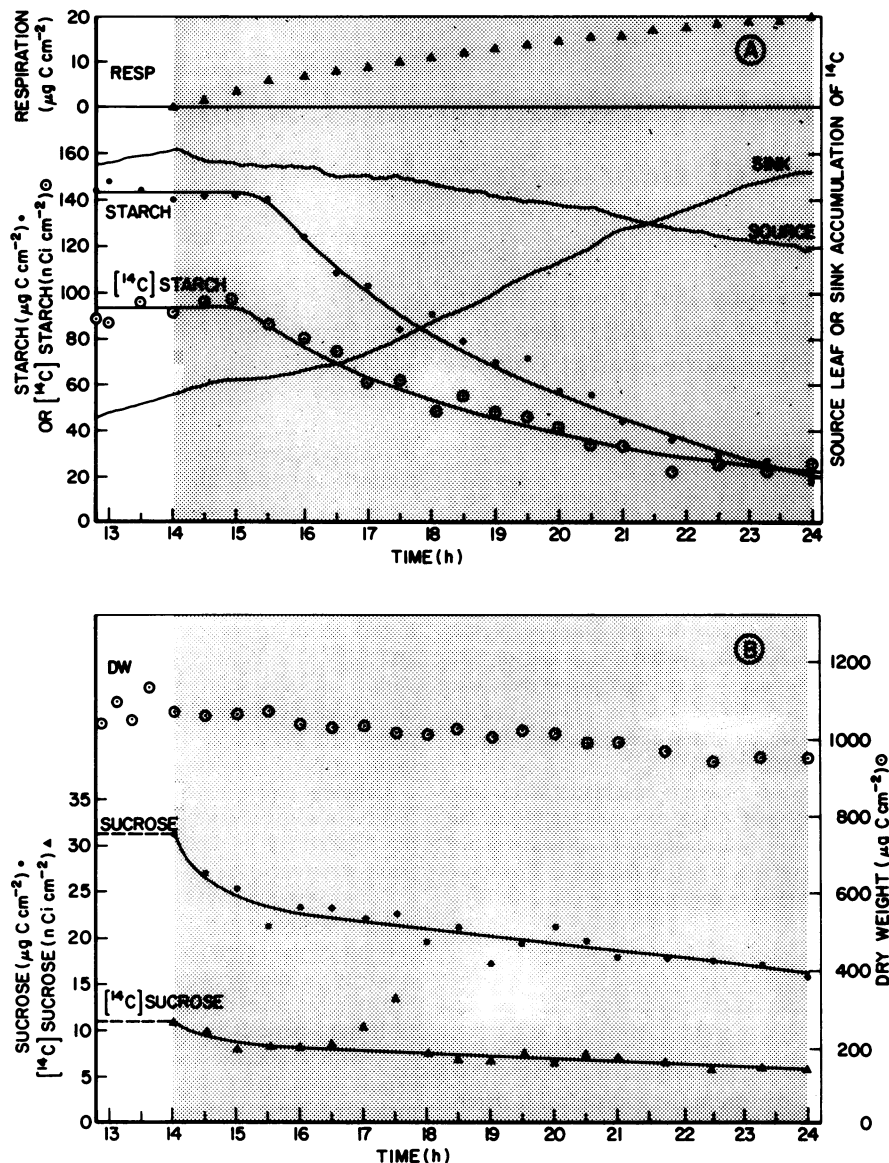


FIG. 1. Time courses for: A, respiration, accumulation of ^{14}C in source and sink leaves, starch; B, sucrose and dry weight (DW) during the last 11 h of a photoperiod. Duration of dark period (shaded area) was 10 h. Date for accumulation of ^{14}C are for one of four experiments and are without specific units. All other values are averages of three to five experiments.

Determination of Dry Weight. After materials soluble in 80% ethanol were extracted from the leaf discs, the latter were dried to constant weight at 80°C . The weight of the leaf tissue, designated as residue and assumed to be principally structural material and starch, was determined using a Cahn Electrobalance, Model 2000 RG.

RESULTS

The data presented here for a 10-h dark period complement our earlier report (4) on kinetics of starch accumulation and of export during a 14-h light period. Time courses for starch and sucrose content as well as DW² are compared with those for export, import and respiration in Figure 1. The paired leaves from which the data were collected had been in $^{14}\text{CO}_2$ of constant specific radioactivity for the two preceding light periods. During darkness, export can be determined by the disappearance of label from the source leaf

after taking loss of $^{14}\text{CO}_2$ into account. Import and export each passed through three similar phases: decline to a rate near zero, followed by resumption of export and import at a slow rate and then at a more rapid rate. Because of transport time between source and sink, import generally lagged behind export.

Carbohydrate levels were examined for changes concomitant with changes in translocation. Content of sucrose, unlike that of starch, began to decline immediately with the onset of darkness. The decrease in the sucrose level from 31.5 to $23 \mu\text{g C cm}^{-2}$ potentially made $8.5 \mu\text{g C cm}^{-2}$ leaf as sucrose available for export. Mobilization of starch began 75 to 90 min after the onset of darkness and continued throughout the dark period. The DW remained unchanged for a period and then declined, paralleling the pattern of starch mobilization. The time course of respiration followed the carbohydrate content of the leaf, declining as carbohydrates were used up.

Experimental evidence cited above suggests that starch, which is mobilized at night, fed into the sucrose pool. This idea was tested by following the displacement of ^{14}C in starch and sucrose by ^{12}C . Contribution of source leaf sucrose and starch pools to

² Abbreviations: DW, dry weight; NCE, net carbon exchange rate ($\mu\text{g C cm}^{-2} \text{ min}^{-1}$).

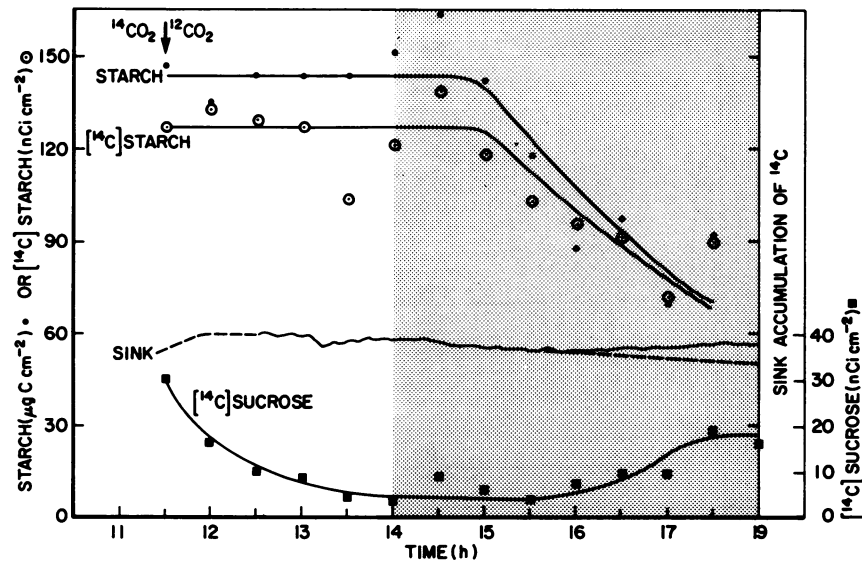


FIG. 2. Time courses for displacement by ^{12}C of ^{14}C in starch and sucrose of a source leaf and arrival of ^{14}C in a sink leaf. Duration of the ^{14}C -labeling period, which ended at 11.5 h, was 8 h. Dashed line after 15.5 h gives the slope of arrival of label in the absence of additional export of ^{14}C . Data are for one of three experiments.

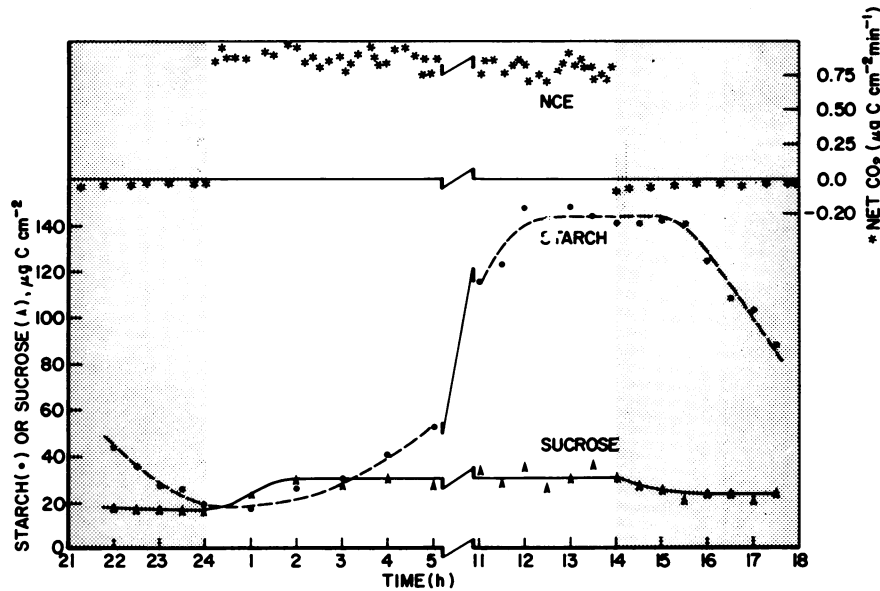


FIG. 3. Time courses for NCE and levels of starch and sucrose in the source leaf during periods of transition in carbohydrate metabolism. Values for NCE are from one of five sets of measurements. All other data are averages of values from three experiments.

translocation was verified by measuring arrival of displaced ^{14}C at a sink leaf. The increase in $[^{14}\text{C}]$ sucrose after 2 h of darkness followed the mobilization of starch evident after 1.5 h of darkness and resulted in renewed accumulation of ^{14}C in the sink leaf (Fig. 2).

NCE and levels of starch and sucrose were examined during transitions in carbohydrate metabolism to identify potential sources of carbon for export (Fig. 3). The following transition periods were examined: after the beginning of the light period, prior to the end of the light period and following the beginning of the dark period. When the light period began, the level of sucrose increased until starch accumulation began. Two hours before the light period ended, starch accumulation was no longer evident. Although the data in Figure 3 suggest that the level of sucrose did not change before the light period ended, there were indications from subsequent studies that, on occasion, the sucrose level may rise somewhat during this time. As the dark period began, sucrose declined for about 75 min, until starch mobilization began.

DISCUSSION

Data from this and a previous study (4) were examined to relate phases of carbohydrate metabolism with translocation during a 24-h period. As an aid in making these correlations, NCE, rates of export, and import, as well as amounts of starch, sugars and DW (Figs. 1 and 2 in reference 4 and Fig. 1) were assembled in Figure 4. Data in Figure 5 show the amount of material in various pools and rates of transfer into and out of these pools during the initial and subsequent phases of the light and dark cycles. These balance sheets showing carbon transfers and changes in pool size provide evidence for shifts in the source of carbon for export.

Light Period.

Initial Phase (0–90 Min). At the beginning of this 90-min period, NCE rose quickly to a steady rate of $0.85 \mu\text{g C cm}^{-2} \text{min}^{-1}$, and mainly fed into sucrose, with lesser amounts entering the starch and hexose pools (Fig. 5). Mobilization of starch continued up to the beginning of the light period; then sucrose synthesis from newly fixed carbon began.

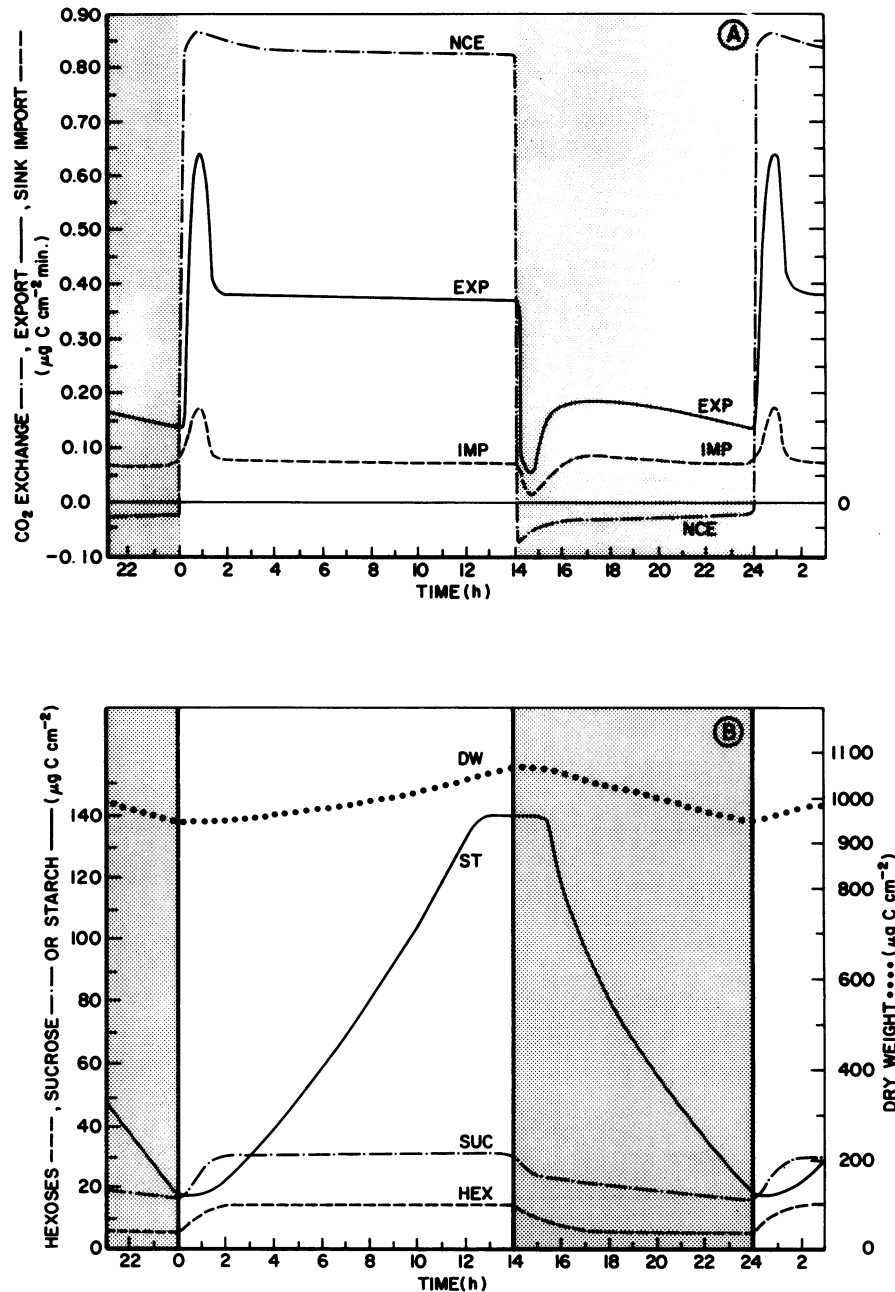


FIG. 4. Summary diagrams showing: A, NCE, export rate (EXP), import rate (IMP); B, amounts of starch (ST), sucrose (SUC), hexoses (HEX), and DW of leaf residue. Data for DW are from eight leaves for the day and five for the night. The rest of the data are from four experiments.

Values for NCE and for the rate of accumulation of ¹⁴C by the source leaf revealed a temporary increase in export above the rate that was observed during the remainder of the light period (Fig. 4). Arrival of ¹⁴C in the monitored sink leaf revealed a similar temporary increase (Fig. 4). A number of factors likely contributed to this transient increase in export. Mobilization of starch supplied sucrose for export at least until the beginning of the light period. Carbon from photosynthesis was not allocated to starch during the initial phase of the light period; in addition, NCE showed a transient peak shortly after illumination began. Consistent with this additional export, DW did not increase as steeply during this phase as during the subsequent phase. This pattern of carbon metabolism is in keeping with the idea that sucrose must accumulate to a certain level before starch synthesis begins. Alternatively, the lag period may have been the result of a delay in synthesis of primer molecules or enzymes needed to enable starch

synthesis to compete with sucrose synthesis for fixed carbon. Questions which remain to be resolved include whether starch dissolution stopped, when starch synthesis began and why there was a delay in net starch accumulation. These and related issues are the subject of current research (1, 3, 7, 9).

Subsequent Phase (90–840 Min). During the remainder of the light period, NCE was constant or declined slightly. The levels of hexose and sucrose and the rates of export and import remained steady while starch and DW increased.

Although the DW per unit area increased in the light because of changes in the amount of starch and other, unidentified, materials, the accumulation of structural material per unit area was zero (Fig. 5). Increments in structural material added to the leaf resulted in additional area rather than in weight per unit area. A 0.65 cm² area of the leaf grows larger, but only 0.65 cm² of this larger area will be removed at the next sampling period. An

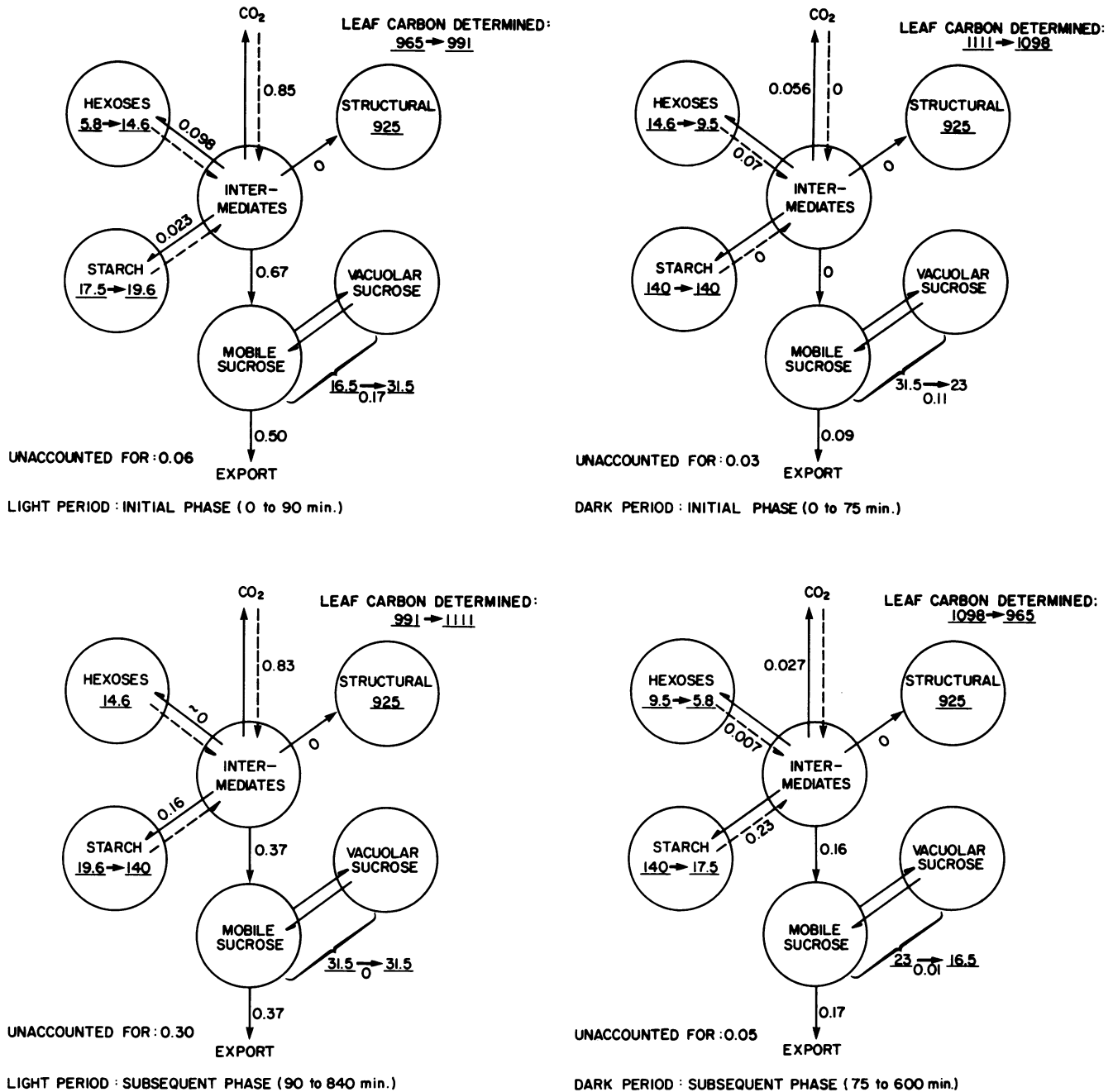


FIG. 5. Contents of and transfers among source leaf pools during phases of the diurnal cycle in sugar beet. Underlined numbers are amounts expressed as $\mu\text{g C cm}^{-2}$. Other numbers are rates expressed as $\mu\text{g C cm}^{-2} \text{ min}^{-1}$. Numbers associated with sucrose pools refer to total without distinction between cytoplasmic and vacuolar compartments. Amount of structural material was calculated from average dry weight values less starch and ethanol soluble materials. Unaccounted for material was computed from rates of input to intermediates minus rates of output.

estimate of the rate of growth for the entire leaf can be made as follows. Residual DW less starch yielded a structural DW of 925 $\mu\text{g C cm}^{-2}$ leaf after 14 h (Fig. 5). Under the growth conditions of this study, sugar beet leaves were found to increase in area and volume by 10 to 20% per 14-h day. Assuming the maximum growth increment, 185 $\mu\text{g C cm}^{-2}$ were deposited by the end of 14 h, at a rate of 0.22 $\mu\text{g C cm}^{-2} \text{ min}^{-1}$. This rate is comparable to that at which unaccounted for material was synthesized, 0.30 $\mu\text{g C cm}^{-2} \text{ min}^{-1}$ (Fig. 5), and is an estimate of the actual growth rate of the leaves studied.

During the last 60 min of the light period, the pattern of carbon allocation differed from that observed for the major part of the light period. Although NCE continued unchanged and DW continued to increase, net starch accumulation slowed down (Fig. 4).

Sucrose level stayed the same or increased somewhat but not in an amount equivalent to the decrease in starch synthesis. Hexose levels did not change. The observed decrease in net starch accumulation may represent an actual cessation of synthesis or the onset of mobilization which would offset synthesis. The latter explanation seems less likely because net starch mobilization was apparent only after 90 min in darkness. If net starch accumulation ceases in the light, it is a case of a transition in metabolism that is not triggered by an obvious change in the environment of the plant.

Low sucrose level was not likely to be involved in causing cessation of starch synthesis in this instance because sucrose level did not decrease. A closer look at changes which occur in protein, organic acids, carbohydrate, and other source leaf pools during

this phase may yield insights into endogenous control of which compounds contribute to export. This metabolic transition appears to anticipate or prepare for metabolism characteristic of the dark period.

Dark Period.

Initial Phase (0–75 Min). During the first 75 min of the dark period, starch level was constant, DW declined slightly; sucrose and hexose began to fall immediately. Prior to starch breakdown, hexoses declined at $0.07 \mu\text{g C cm}^{-2} \text{min}^{-1}$, an amount sufficient to support dark respiration which proceeded at $0.056 \mu\text{g C cm}^{-2} \text{min}^{-1}$. The depletion of sugars during this phase probably contributed to the gradual decline in the rate of respiration. During the initial decrease in translocation, export, which occurred at $0.09 \mu\text{g C cm}^{-2} \text{min}^{-1}$, was supported by the decline of source leaf sucrose which fell at an average rate of $0.11 \mu\text{g C cm}^{-2} \text{min}^{-1}$ (Figs. 1 and 5). In the absence of starch mobilization, a lower level of sucrose was established and export and import declined to near zero. Delay in starch mobilization, like the delay in starch synthesis at the beginning of the light period, could be dependent on completion of preparatory events or on a change in the level of sucrose or on some factor related to sucrose decline (7).

Subsequent Phase (75–600 Min). The liberation of sugar from starch slowed the decline in respiration rate and in levels of sucrose and hexose. Almost all of the decline in DW, $0.196 \mu\text{g C cm}^{-2} \text{min}^{-1}$, was accounted for by the decline in starch, $0.205 \mu\text{g C cm}^{-2} \text{min}^{-1}$. Starch degradation was rapid enough to support those processes which deplete leaf carbon, specifically export and respiration (Fig. 5). Export accelerated upon the initiation of starch mobilization, reached a maximum, and then slowly declined; export at night was approximately 40% of the daytime rate. The pattern of import was similar to that of export but the rate of import into the monitored sink at night was comparable to that for import observed during the light period.

This suggests that a mechanism exists for assuring that a developing sink leaf receives a steady supply of nutrients over a 24-h period. The work of Swanson and Hoddinott (12) suggests that such a mechanism exists. They found that in bean plants during the light period the absolute rate of translocation to a sink leaf is constant irrespective of whether the sink leaf is illuminated or darkened.

Data from some studies may be interpreted to support continuous turnover of starch, even in the light (8). We observed little or no turnover as evidenced by the delay in starch breakdown (Figs. 1–3) and by the lag in contribution of labeled starch to sucrose and translocation (Fig. 2). In intact leaves, starch degradation in the light appears to be zero or insignificant except possibly at low NCE.

Unlike at the end of the day, at the end of the night there was no evidence for a transition in metabolism which would anticipate the impending environmental change. Rather, these metabolic preparations likely occurred during the lag phase at the beginning of the light period (Fig. 3 and Ref. 9). Events that prepare for carbon metabolism in the light probably were completed too quickly to explain the entire lag in starch accumulation. This interpretation favors the existence of a mechanism which requires sucrose to accumulate to a certain level before starch synthesis is initiated (2).

CONCLUSION

This study shows that some changes in carbohydrate metabolism were initiated exogenously by environmental signals. Starch

accumulation, as well as starch degradation, seemed to be initiated by the presence or absence of light. There were other changes in carbohydrate metabolism which were not directly initiated by environmental factors but were subject to endogenous control; for example, cessation of starch accumulation before the end of the light period. Exogenous control factors seemed to be mediated in part by the level of sucrose in the leaf. Endogenous control is probably related to photoperiod or photosynthetic duration, as discussed by Chatterton and Silvius (1).

During the span of time from near the end of the day to sometime after the beginning of the night period, a number of events occurred which provided evidence for a major shift in source leaf metabolism. There seemed to have been a change in allocation of the products of photosynthesis near the end of the light period to be more aligned with the nature of metabolism at night. This transition in metabolism disrupted translocation briefly at the beginning of the dark period. The absence of a high rate of starch turnover during the day seemed to contribute to this disruption.

Although there was a considerable shift in source leaf metabolism associated with the transition from day to night, sink leaf metabolism likely was more uniform throughout the 24-h period. In line with this more uniform metabolic state, the rates of import of ^{14}C to the developing sink leaf were more similar during day and night than the rate of export. The latter decreased considerably at night. A steady supply of imported material to developing sink leaves over a 24-h period seems to be an adaptive advantage, contributing to metabolic efficiency during development.

The information obtained provides background for future investigations to determine effects of changes in translocation, imposed by treatment of sink and path, on export and on metabolism of the source leaf. The data also provide background for a study of mechanisms for control of export by exogenously triggered changes or by endogenous shifts in metabolism of the source leaf.

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