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Photosynthetic Carbon Metabolism and Translocation in Wild-Type and Starch-Deficient Mutant *Nicotiana sylvestris* L.[1]

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A high rate of daytime export of assimilated carbon from leaves of a starch-deficient mutant tobacco (*Nicotiana sylvestris* L.) was found to be a key factor that enabled shoots to grow at rates comparable to those in wild-type plants under a 14-h light period. Much of the newly fixed carbon that would be used for starch synthesis in leaves of wild-type plants was used instead for sucrose synthesis in the mutant. As a result, export doubled and accumulation of sucrose and hexoses increased markedly during the day in leaves of the mutant plants. The increased rate of export to sink leaves appeared to be responsible for the increase in the proportion of their growth that occurred during the day compared to wild-type plants. Daytime growth of source leaves also increased, presumably as a result of the increased accumulation of recently assimilated soluble carbon in the leaves. Even though starch accumulation did not occur in the leaves of mutant plants, nearly all the sugar that accumulated during the day was exported in the period of decreasing irradiance at the end of the diurnal light period. Changes in carbon allocation that occurred in leaves of wild-type and mutant plants near the end of the light period appeared to result from endogenous diurnal regulation associated with the day-night transition.

Diurnally regulated accumulation and mobilization of transitory starch are critical for providing a timely supply of carbon for growth and metabolism in plants that accumulate starch (Fondy and Geiger, 1982, 1985; Fondy et al., 1989). The effects of a lack of starch accumulation on carbon metabolism and growth have been studied extensively with an SDM of *Nicotiana sylvestris* L. in which starch accumulation is blocked (Hanson and McHale, 1988; Hanson, 1990; Goldschmidt and Huber, 1992; Huber and Hanson, 1992). Leaves of SDM plants had the same NCE rate as WT plants under a photoperiod with 14-h nights, but the daily leaf expansion was only 77% of that for the WT plants (Huber and Hanson, 1992). When the nights were shortened to 8 h, leaves of SDM tobacco plants grew at the same rate as those of the WT plants. These results demonstrate the need for a timely supply of carbon for supporting growth in sink tissues but also show the ability of SDM tobacco plants to adjust to the absence of starch accumulation when nights are shorter.

In addition to accumulation of alternative forms of assimilated carbon in leaves and shifts in the timing of growth of sink and source leaves (Hanson and McHale, 1988; Huber and Hanson, 1992), increased export is thought to be involved in the acclimation of SDM tobacco plants to blockage of starch accumulation. Huber and Hanson (1992) observed that increased daytime accumulation of neutral sugars, principally hexoses, in source leaves of SDM tobacco plants compensated for only about half of the carbon normally stored as starch in WT plants. From this shortfall the authors inferred that the daytime rate of export from leaves of SDM plants was somewhat higher than in WT plants. They proposed that accumulation of neutral sugars is the result of a limitation in the capacity for Suc movement into the phloem in leaves of the SDM tobacco, and, as a consequence, not all of the additional Suc synthesized in the leaf could be exported. However, the rate and time course of daytime export were not measured by these workers or by others; therefore, the actual extent to which export enables SDM plants to acclimate to the blockage of starch accumulation is a matter of speculation.

To gain an adequate understanding of the respective contributions of each of the significant processes to successful acclimation it is necessary to measure export. Quantitative assessment of the processes associated with acclimation enables us to understand to what extent, if any, they affect metabolism and growth. For instance, increased levels of soluble carbohydrate in source and sink leaves, which may result from changes in export rate, may depress photosynthesis or decrease the efficiency of carbon use by increasing respiration (Caspar et al., 1985). The form of carbon that is accumulated also can affect carbon metabolism. Plants, such as tobacco, that accumulate a considerable portion of the newly assimilated carbon as starch generally do not accumulate Suc (Huber, 1989). Huber (1989) found that hydrolysis of Suc by acid invertase impeded its accumulation in vacuoles of tobacco leaf cells and thereby caused increased accumulation of hexoses. When the hexoses re-enter the cytosol and are converted to Suc, the cycle is completed. This process, which likely is faster in leaves of SDM plants, may exact a metabolic cost that could contribute to reduced growth (Huber, 1989).

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Abbreviations: NCE, net carbon exchange; SDM, starch-deficient mutant; WT, wild-type.

The rate and timing of export from source leaves can affect both the timing of growth and the buildup of carbon in source and sink leaves. For instance, shifts in the timing of leaf growth, which were observed in SDM plants (Hanson and McHale, 1988; Huber and Hanson, 1992), likely are related to the timing of export from source leaves. Expansion of rapidly growing leaves of WT plants is several times greater at night than during the day, whereas that of leaves of SDM plants occurs almost equally during light and dark periods (Huber and Hanson, 1992). Low levels of export at night may deprive sinks of imported carbon, and the resulting deficiency of carbon may slow growth in SDM plants.

The present study was undertaken to measure export from leaves of SDM tobacco to: (a) compare the time course of export from source leaves throughout the day-night cycle in SDM and WT plants, (b) determine the relation of the time course of export to that of accumulation of Suc and of neutral sugars in leaves of SDM plants, (c) establish the relative contributions of daytime export and accumulation of soluble neutral sugars to growth of leaves of SDM plants at rates comparable to WT plants, and (d) examine regulation of export during the day-night transition in SDM plants.

MATERIALS AND METHODS

Plant Materials

Seeds of *Nicotiana sylvestris* (Spegazzini and Comes), both WT and F_3 plants of SDM NS 514 tobacco, isolated as "starchless" and containing a modified plastid phosphoglucomutase (Hanson and McHale, 1988), were a gift of Dr. Kenneth R. Hanson (Department of Biochemistry and Genetics, the Connecticut Agricultural Experiment Station, New Haven, CT). Seeds were germinated in Jiffy-mix (Jiffy Products, Bativia, IL) soil and after 2 weeks were transferred to containers with a 1:1 mixture of Jiffy-mix and sand and watered daily. Two weeks later the seedlings were transferred to 6-L plastic pots containing a mixture of Jiffy-mix:sand (1:1, v/v) and watered three times daily with a nutrient solution (Fondy and Geiger, 1982). Plants were maintained under a 14-h photoperiod at 25°C, 60% RH day and 20°C, 75% RH night. Lights were a combination of high-pressure metal halide, sodium vapor, and incandescent lamps that provided a stepped increase in irradiance with a midday maximum level of about 0.8 mmol quanta m^{-2} s^{-1} PAR at canopy level. Typically, 7- to 8-week-old plants were selected and leaves with an area of 2 to 2.5 dm^2 were used for the measurements.

Irradiance Regimes

Measurements of photosynthesis and translocation were made under a light regime in which irradiance was increased in the form of a sine function for the first 7 h and then was maintained at a photon irradiance 0.8 mmol m^{-2} s^{-1} for the remainder of the 14-h photoperiod. This regime allows regulation of physiological processes to be studied under steady irradiance where changes in light level are not a factor. For comparison purposes, similar measurements were made under a 14-h diurnal light regime in which irradiance was varied according to a sinusoidal wave function similar to that which occurs on a clear day (Fondy et al., 1989).

Carbon Exchange Measurement

Two leaves were enclosed in separate aluminum leaf chambers that were connected to an apparatus for controlling the CO_2 of the atmosphere circulating over the leaves (Geiger et al., 1988). The rate of CO_2 depletion in the circulating atmosphere was measured by an IRGA (LIRA 3000; Mine Safety Appliances, Pittsburgh, PA), and the level of CO_2 was maintained at a concentration of 350 ± 20 μL L^{-1} with a computer-controlled mass flow controller (Tylan, Carson, CA). RH was maintained at 70%, and leaf temperature was maintained at 24 ± 2°C. Data are generally reported from one of two replicate experiments rather than from computing an average.

The time course of nighttime respiration in source leaves of WT and SDM plants was determined by measuring the increase in CO_2 level produced by a respiring leaf in a leaf chamber that was part of an open gas-train. The flow of air was adjusted to keep the increase below 30 μL L^{-1} throughout the 10-h dark period.

Carbon Accumulation and Export Measurements

The amount of recently fixed carbon that was retained in a photosynthesizing source leaf was measured by following the amount of radioactive carbon accumulating by a steady-state labeling method during the 14-h photoperiod (Geiger and Fondy, 1979; Geiger et al., 1988). Labeling was carried out by maintaining an atmosphere with a constant level of CO_2 that contained $^{14}CO_2$ with a specific radioactivity of approximately 18 kBq mg^{-1} carbon. The specific radioactivity of the supplied $^{14}CO_2$ and the ^{14}C contained in sets of two 0.17-cm^2 leaf discs sampled from the leaf at specified intervals was used to calculate the amount of carbon accumulated per unit area of leaf. To measure labeled carbon accumulated in the leaf blade, leaf disc samples were dried, oxidized, and assayed for ^{14}C content by liquid scintillation counting (Geiger et al., 1988). A continuous time course of carbon accumulation was determined by fitting data for carbon contained in the individual leaf discs with the time-course curve for the accumulated radioactivity obtained by a Geiger-Müller detector located beneath the leaf.

The time course for the cumulative amount of recently fixed carbon that has been exported was calculated by subtracting data of the time course for carbon accumulated in the leaf from the time course of cumulative net carbon fixed (Geiger et al., 1988). Export rate was calculated by smoothing the data for the amount of carbon exported and taking the slope over a small time interval. Export of carbon derived from the unlabeled starch at the start of the photoperiod was not detected, but in the afternoon the method measured export of carbon derived both from labeled starch and from newly fixed carbon (Fondy et al., 1989).

Leaf Growth Measurements

To determine rates of leaf growth during the light period and at night, lengths of five sink leaves and areas of five source leaves were measured for 3 consecutive days on WT and SDM plants. Averages of these data from an individual leaf were calculated for the respective intervals. Because growth rate increased slightly on subsequent days, only averages are reported for these growth data.

Diurnal cycling of dry weight accumulation in leaves was measured in discs sampled at the beginning, middle, and end of the photoperiod from plants growing under the 14-h photoperiod. Discs were frozen, dried, and weighed to the nearest 10 μg. Measurements of dry weight and metabolite levels in leaf samples to construct a balance sheet of assimilated carbon requires careful interpretation. Carbon that contributes directly to expansion of the leaf will be lost, having been carried outside the original sample area as the leaf expands.

Allocation of Recently Fixed Carbon

The allocation of carbon in the source leaf during the light period was determined by measuring recovery of ^{14}C from the oxidized leaf discs and from analysis of the Suc, Glc, Fru, and malate and measurement of the carbon in the ethanol-soluble and -insoluble fractions of solutes. Radioactivity in the aliquots of the soluble fraction was measured by liquid scintillation counting. The insoluble residue was homogenized in KOH solution and digested with amyloglucosidase (Fondy and Geiger, 1985), and the suspension containing both soluble and insoluble material was counted.

The amounts of starch, Suc, Glc, Fru, and malate present in the leaf blade were measured in discs by enzymic assays following extraction of the leaf samples. Four discs with a total area of 0.68 cm^2 were removed from a leaf, the alcohol soluble contents were extracted, and the extract was assayed for carbohydrate as described in Fondy and Geiger (1985). Malate content was measured by the method of Lowry and Passonneau (1972).

Suc synthesis rate was considered to be the sum of the rate of export and the rates of foliar accumulation of Suc and hexoses derived from Suc (Geiger et al., 1988). This calculation is based on the simplifying assumptions that Glc and Fru accumulate as a result of the hydrolysis of Suc (Huber, 1989) and that Suc is the main component of the carbon being exported.

RESULTS

Photosynthesis

Time courses of NCE for leaves of WT and SDM plants were similar during the first 7 h of the light period (Fig. 1), increasing with irradiance levels during the first 5 h and

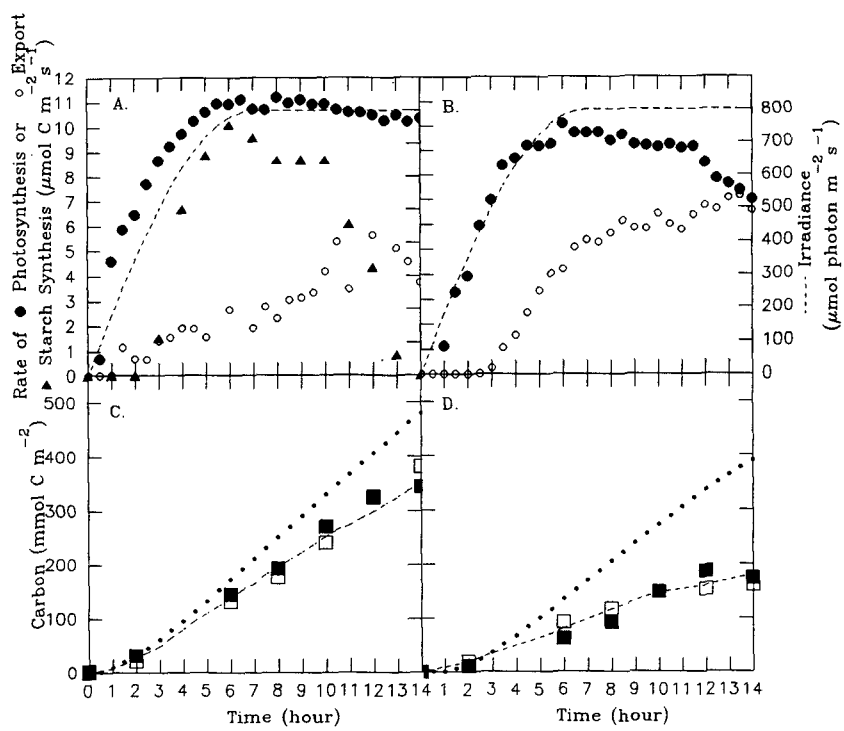

Figure 1. Photosynthesis, carbon accumulation, and carbon export in leaves of WT and SDM tobacco plants during a modified light regime in which irradiance was kept at the midday level during the second half of the light period. Data are from WT (A and C) and SDM plants (B and D). A and B, Rates of photosynthesis (●), export (○), and starch accumulation (▲) and level of irradiance (– – – –). C and D, Cumulative amount of assimilated carbon (·····) and labeled carbon accumulated in leaf assayed by oxidation of discs (■) and, by Geiger-Müller detector (– – – –), sum of the labeled carbon in the ethanol-soluble and -insoluble fractions (□).

leveling off at about 2 h prior to maximum irradiance (Fig. 1, A and B). Maximum NCE rates were 11 and 10 μmol carbon m^{-2} s^{-1}, respectively, in leaves of WT and SDM plants. Under a diurnal sinusoidal light regime the average values of NCE rates for leaves from three sets of WT and SDM plants were 10.8 and 9.7 μmol carbon m^{-2} s^{-1}, respectively, at maximum irradiance.

During the second half of the light regime, when irradiance remained at the midday level, NCE rates declined very slowly in both WT and SDM plants. In leaves of WT plants, the rate decreased to 90% of the maximum by the end of the light period, and in SDM plants the rate decreased to 75%. For the WT plant in Figure 1 a total of 480 mmol carbon m^{-2} was fixed during 14 h, whereas for the SDM plant a total of 395 mmol carbon m^{-2}, or 82% of the value for the WT plant, was fixed.

Export of Labeled Carbon

Export of recently fixed carbon from leaves of the WT plant increased with the NCE rate (Fig. 1A), attaining a level of 2.5 μmol carbon m^{-2} s^{-1} by 7 h, or 23% of the NCE rate. Under a modified light regime in which irradiance remained at the midday level rather than decreasing in the afternoon, the export rate gradually increased in both types of plants, slowly at first and then more rapidly during the last several hours of the light period. In the WT plant, export eventually increased to 5.5 μmol carbon m^{-2} s^{-1}, or 50% of the NCE rate, by 14 h. Results were similar in the two replicate experiments.

In the SDM plants, the export rate generally followed the NCE rate, attaining a rate of 6 μmol carbon m^{-2} s^{-1}, or 60% of the NCE, by 7 h, and thereafter the proportion of newly fixed carbon allocated to export increased (Fig. 1B). At about 11 h the export rate began to increase despite a marked decrease in the NCE rate, and by the end of the light period nearly all of the carbon that was fixed was exported immediately. At the midday maximum NCE rate, the export rate from the source leaf of the SDM plant (Fig. 1A) was approximately twice that for the WT plant (Fig. 1B). The WT plant exported a total of 130 mmol carbon m^{-2} during 14 h, whereas the SDM plant exported a total of 220 mmol carbon m^{-2}, or 170% of the value for the WT plant. Data for export were similar in the two replicate experiments.

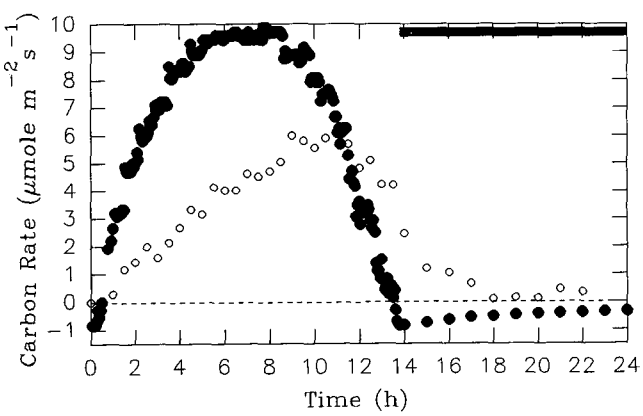

Figure 2. Photosynthesis and export of carbon in leaves of an SDM tobacco plant during a sinusoidal diurnal light regime. Rates of photosynthesis or respiration (●) and export (○) are shown for a light regime consisting of a 14-h photoperiod and a 10-h night.

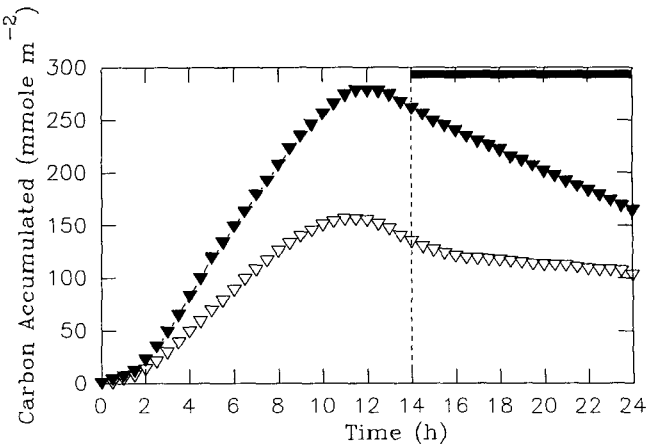

Figure 3. Net accumulation or loss of carbon in source leaves of carbon that was fixed during a sinusoidal diurnal light regime. The tracings show the amount of ^{14}C in a source leaf from WT (▼) and SDM (▽) plants as measured by a Geiger-Müller detector and expressed as total carbon.

When SDM plants were studied under a diurnal sinusoidal light regime instead of the modified regime, export of carbon that was fixed during the current light period reached a maximum rate of 6 μmol carbon m^{-2} s^{-1}, or 67% of the NCE rate, by about 10 h (Fig. 2). Export decreased to essentially zero by the beginning of the night, and the rate of carbon loss by respiration gradually decreased from 1 to 0.2 μmol carbon m^{-2} s^{-1} through the night.

Carbon accumulation occurred at a slower rate in leaves of SDM plants than in leaves of WT plants (Fig. 3). Given the similar rates of NCE in leaves of WT and SDM plants, the data indicate that the export rate was considerably higher in the SDM than in the WT plants. A net loss of carbon in both types of plants occurred during the last several hours of the light period when irradiance was low, indicating that accumulated carbon was used to support export. Carbon decrease slowed markedly by the end of the light period in leaves of SDM plants and remained very slow during the night, whereas, in WT plants, accumulated carbon continued to decrease at a significant rate throughout the night.

Carbon and Dry Weight Accumulation

Leaves of both WT and SDM plants accumulated dry weight per unit area during the day (Fig. 4) and lost essentially all of it during the night. In WT plants most of the dry weight increase during the day was due to accumulation of ethanol-insoluble residues, mainly in the form of starch (Fig. 5A). Only a small amount of the recently fixed carbon was accumulated in the ethanol-soluble fraction, mainly in the form of Suc and malate (Fig. 5C). The starch and ethanol-insoluble compounds that accumulated per unit

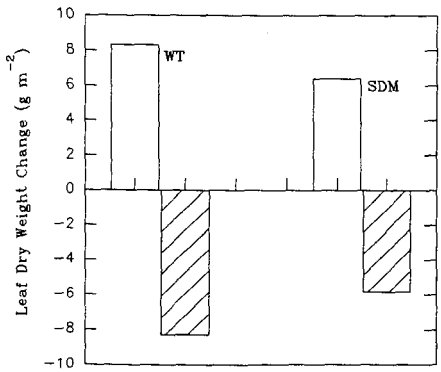

Figure 4. Daily increase in dry weight per area in leaves under a sinusoidal diurnal light regime. Accumulation during the day (open bar) and loss during the night (cross-hatched bar) is shown for both types of plants. Data are from six samples per day during a 3-d period. The coefficient of variation is 3.9% for WT and 5.7% for SDM plants.

area in source leaves of WT plants amounted to about 50% of the recently fixed carbon (Fig. 5A).

In leaves of the SDM plant, the dry weight gain during the 14-h d light period was smaller than in the leaves of WT (Fig. 4), likely because much of the recently fixed carbon was exported immediately. Most of the increase was in the form of soluble sugars and malate (Fig. 5D). Only 30% of recently fixed carbon accumulated per unit area of leaf in

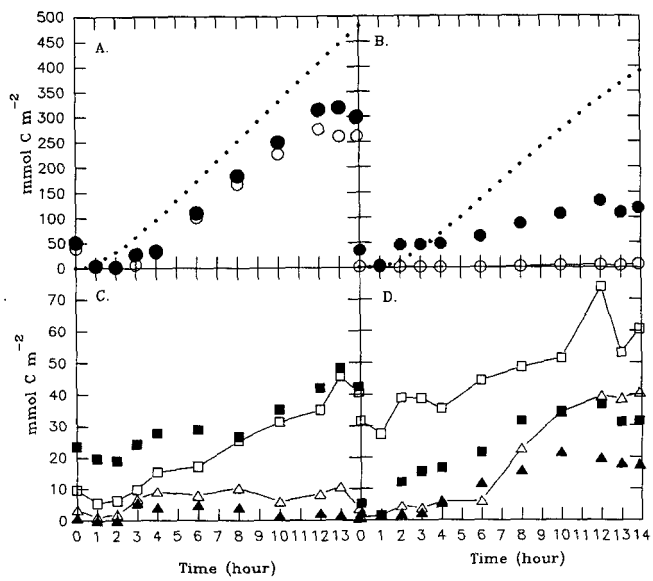

Figure 5. Levels of neutral sugars and malate in source leaves of WT and SDM tobacco plants during a modified light regime in which irradiance was kept at the midday level during the second half of the light period. Data are from WT plants (A and C) and SDM plants (B and D). A and B, Cumulative amount of assimilated carbon (·····). Diurnal change in starch content (○) and the sum of starch, Suc, Glc, Fru, and malate (●) were determined by enzymic assay. Curves for starch and for the sum were offset by subtracting the lowest values from values for each point. C and D, Accumulation of Suc (■), Glc (△), Fru (▲), and malate (□).

SDM plants during the daily light period, being distributed nearly equally between the ethanol-soluble fraction (Fig. 1D) and an unknown, nonstarch, ethanol-insoluble fraction. The dry weight per area that accumulated in leaves of SDM plants during the day was subsequently lost during the night (Fig. 4). Dry weight gain measured gravimetrically (Fig. 4) was in agreement with the accumulation of ethanol-soluble and -insoluble material measured by radiolabeling (Fig. 1, C and D).

Leaf Growth

Growth in the length of sink leaves and the area of source leaves was faster in SDM plants during the day (Fig. 6), whereas both sink and source leaves grew faster at night in WT plants than in SDM plants. It is not clear to what extent if any water potential changes were a factor in the timing of leaf expansion.

Starch, Sugars, and Malate

The amount of starch in leaves of WT plants decreased at the start of the light period while photosynthesis was low and then began to increase about 3 h after the start of the light (Fig. 5A). The starch synthesis rate increased to a maximum at midday and began to decrease at about 10 h (Fig. 1A), even though the photosynthesis rate remained high. Most of the starch was synthesized during 5 h in the middle of the day. In SDM plants starch was detectable but quite low (Fig. 5B).

The levels of Suc and malate in the WT plant showed a transient decrease at the start of the light period while photosynthesis was low (Fig. 5C). Malate accumulated throughout the light period, whereas Suc accumulated only after midday. In the SDM the levels of all of the sugars and malate were very low at the start of the light period (Figs. 5C and 7B). Suc and malate levels increased with irradiance throughout the first 10 h, whereas the Glc level began to increase only after 6 h (Fig. 5D).

Under the sinusoidal diurnal light period, as NCE decreased with decreasing irradiance from about 10 h on, an increasing proportion of export from leaves of the SDM

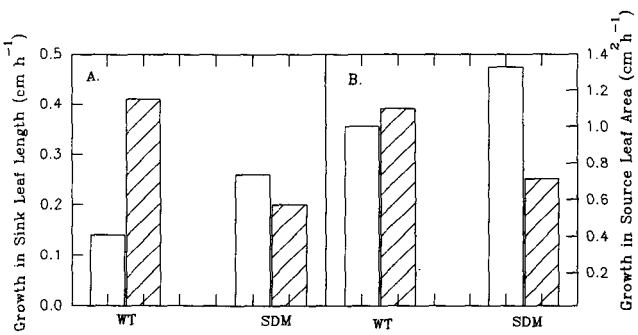

Figure 6. Daily growth in sink leaf length (A) and source leaf area (B) in WT and SDM tobacco leaves under a sinusoidal diurnal light regime. An increase in leaf length is shown for sink leaves (3–8 cm length) and leaf area for source leaves (15–28 cm length) during the day (open bar) and night (cross-hatched bar).

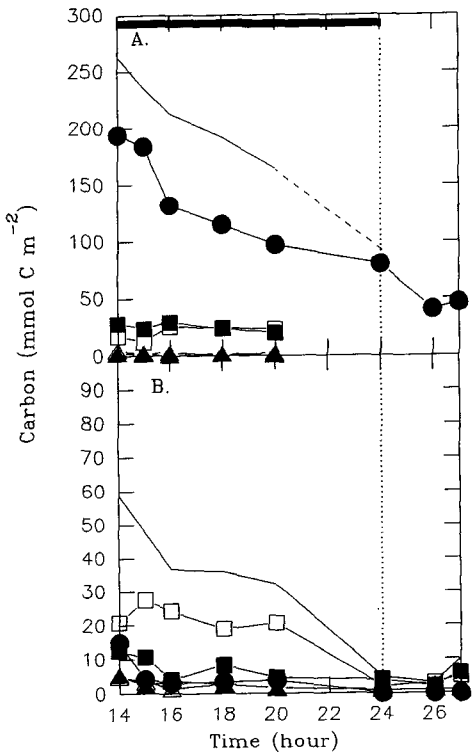

Figure 7. Carbon pool in leaves of WT and SDM tobacco plants during the night and the beginning of a sinusoidal diurnal light period. Data from WT (A) and SDM (B) plants show levels of starch (●), Suc (■), Glc (△), Fru (▲), malate (□), and the sum of these compounds (———). Data for the WT plant late in the experiment were not available (– – – –).

plant came from Suc that had accumulated earlier in the light period (Fig. 2). By the last hour of the light period essentially all of the 2 to 3 μmol carbon m^{-2} s^{-1} being exported came from the accumulated Suc. The pool of 30 mmol carbon m^{-2} (Fig. 5D) would support export at the latter rate for approximately 3 h. Data concerning the disappearance of carbon from an exporting source leaf at the end of the light period (Figs. 3 and 7A) indicate that export at night was minimal.

Source Leaf Carbon Pools and Export during Low Irradiance and Darkness

Under the sinusoidal diurnal light regime, starch decreased in source leaves of WT plants during a 12-h period consisting of the 10-h night and about 2 h into the following light period (Fig. 7A). The 160 mmol carbon m^{-2} decrease in starch during the 12-h period (Fig. 7) could support a maximum average export rate of about 4 μmol carbon m^{-2} s^{-1}. Under the light regime in which irradiance was maintained at the midday level, total irradiance was greater and a greater amount of starch accumulated by the end of the day.

Export of accumulated Suc in SDM plants under the sinusoidal light regime increased the export rate to a point that surpassed the NCE rate several hours before the end of the light period (Fig. 2). Export at night was minimal (Fig. 2). Of the decrease in leaf carbon at night (Fig. 2), about a third could be accounted for by respiration (Fig. 3). Malate and neutral sugars were already low by the end of the day and consequently showed relatively little decrease during the night (Fig. 7B).

Source Leaf Carbon Allocation at the End of a Day

Even though irradiance was maintained at the midday level, the rate of starch accumulation started to decrease at about 12 h and declined to near zero by the end of the day (Fig. 1A). The additional carbon contributed to increased export (Fig. 1A) and Suc accumulation (Fig. 5C). Although the SDM plant did not accumulate starch, export increased in spite of the decrease in NCE at the end of the day (Fig. 1B).

DISCUSSION

A high rate of daytime export from leaves of SDM plants appears to be a key factor in their successful acclimation to the blockage of starch accumulation. Although maximum NCE rates per unit leaf area were about 10% lower in SDM plants, long-term rates of leaf growth of SDM and WT plants of the same age growing under a 14-h photoperiod were not distinguishable by casual observation. The timing of growth and the amount of assimilated carbon that accumulated in the photosynthesizing leaves have been demonstrated to be factors associated with acclimation of SDM plants to blocked starch accumulation (Hanson and McHale, 1988; Huber and Hanson, 1992). The marked increase in daytime export (Figs. 1 and 2) gave evidence that both time course and rate of export from leaves also play a key role in acclimation.

In the absence of starch synthesis, much of the newly fixed carbon in leaves was diverted to synthesis of Suc, as reported previously by Huber and Hanson (1992). More than half of the Suc synthesized in SDM plants during the 14-h light period was exported immediately, whereas the remainder was accumulated as Suc and hexoses (Table I; Fig. 5). Twice the amount of Suc was exported from leaves of SDM plants during the day as was exported during the same time in WT plants (Table I). Under the sinusoidal light regime, the export rate not only increased with irradiance throughout the first half of the light period but continued to increase even as irradiance decreased (Fig. 2).

Table I. *Carbon budget for photosynthesis, export, and carbon accumulation in leaves of WT and SDM plants from 0 to 14 h maintained under steady irradiance after midday*

Data are from Figures 1 and 5. The ethanol-soluble amount is the sum of Suc, Glc, Fru, and malate.

Value	WT	SDM
	mmol carbon m^{-2} (%)	
Cumulative NCE	475 (100)	385 (100)
Cumulative export	120 (25)	205 (53)
Ethanol soluble	60 (13)	120 (31)
Starch	250 (53)	Trace

As a result, the proportion of assimilated carbon increased until export greatly exceeded NCE by the end of the day. Little carbon remained to be exported at night in leaves of the SDM plants (Fig. 7). To compare these rates with those from WT plants, we calculated that nighttime export from leaves of WT plants occurred at a maximum rate of 3 to 4 μmol carbon m^{-2} s^{-1} (data from Figs. 3 and 7), close to the rate of 5.5 μmol carbon m^{-2} s^{-1} observed at the end of the day (Fig. 1A).

Accumulation of neutral sugars in leaves of SDM plants was nearly twice that seen in WT plants (Fig. 5, C and D). In the latter, sugars accumulated particularly during the second half of the day, mainly as Suc (Fig. 5C), whereas in SDM plants, sugars accumulated mainly as hexoses (Fig. 5D). The total amount of carbon exported and the amount of neutral sugars accumulated in leaves of SDM plants during the first 7 h of the light period were similar (Figs. 1 and 5), each about 45 mmol carbon m^{-2}. During the second half of the light period accumulation of neutral sugars amounted to only about 30 mmol carbon m^{-2}, whereas 155 mmol carbon m^{-2} were exported. Under the steady irradiance during the second half of the day, only a small amount of Suc and hexoses accumulated, whereas large amounts of Suc were exported. This pattern is opposite to what would be expected if the capacity for Suc movement into the phloem were limited, as proposed by Huber and Hanson (1992). Rather than a limit to the rate at which Suc can be exported by the phloem being the cause of the accumulation of sugars in leaves of SDM plants, the buildup appears to result from regulation of carbon allocation. The mechanism responsible for the increase in the rate of export from leaves of the SDM plants as the day progressed is unknown. It appears that Suc hydrolysis may be involved in regulating accumulation, because it was Glc and Fru, which do not enter the phloem readily, that accumulated during the latter half of the day.

Even in the near absence of starch accumulation, export of Suc from leaves of SDM plants was not limited markedly during the light period; therefore almost no carbon remained in Suc and hexoses at the end of the light period (Fig. 7B). Under the light regime in which irradiance was kept at the midday level, nearly half of the carbon assimilated was exported from leaves of SDM plants by the end of the light period (Table I). Under the sinusoidal light regime, nearly all of the carbon that accumulated as neutral sugars also was exported during the period of decreasing irradiance at the end of the day (Fig. 7B), leaving practically none for export at night.

The availability of carbon, which was greatly influenced by export and accumulation of sugars, likely was a major factor in the timing and extent of both source and sink leaf growth. The near doubling of export of recently assimilated carbon in SDM plants provided an increased daytime carbon supply to sink leaves, which likely increased the proportion of their growth that occurred during the day. The additional daytime supply of carbon to sinks was equivalent to about a third of the carbon accumulated as starch in source leaves of WT plants (Table I). Increased daytime sugar accumulation of exporting leaves likely caused a similar change in the pattern of source leaf growth (Fig. 6). The timing of leaf growth observed in the present study was similar to that reported by Huber and Hanson (1992). The almost complete depletion of accumulated source leaf sugars by the beginning of the night in SDM plants (Fig. 7B) not only slowed nighttime growth in this study but likely would adversely affect leaf growth when the nights are longer.

Even when irradiance remained at the midday level, starch synthesis practically ceased during the last several hours of the light period in leaves of WT plants (Fig. 1A). Most of the newly fixed carbon that was no longer being allocated to starch was exported in leaves of WT plants. This phenomenon, which has been described for leaves of sugar beet plants previously (Li et al., 1992), appeared to be related to the progressive transition from synthesizing Suc from newly assimilated carbon to making it from starch. Regulation of starch degradation appears to involve endogenous diurnal regulation of exit of hexose from the chloroplast by the Glc carrier in the chloroplast membrane (Li et al., 1992; Trethewey and ap Rees, 1994). A related shift in regulation of carbon allocation appeared to occur also in leaves of SDM plants near the end of the light period when irradiance was kept at the midday level (Fig. 1B). Coincident with increased rates of Suc synthesis and export near the end of the light period, the level of Glc-6-P in leaves of the SDM plants decreased markedly (data not shown). The changes in carbon metabolism that occurred near the end of the light period show that regulation of starch synthesis is related to starch degradation and that this regulation occurs even when starch synthesis is blocked.

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