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# The Effect of Leaf Temperature and Photorespiratory Conditions on Export of Sugars during Steady-State Photosynthesis in *Salvia splendens*[1]

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**Export and photosynthesis in leaves of *Salvia splendens* were measured concurrently during steady-state $^{14}CO_2$ labeling conditions. Under ambient $CO_2$ and $O_2$ conditions, photosynthesis and export rates were similar at 15 and 25°C, but both declined as leaf temperature was raised from 25 to 40°C. Suppressing photorespiration between 15 and 40°C by manipulating $CO_2$ and $O_2$ levels resulted in higher rates of leaf photosynthesis, total sugar synthesis, and export. There was a linear relationship between the rate of photosynthesis and the rate of export between 15 and 35°C. At these temperatures, 60 to 80% of the carbon fixed was readily exported with sucrose, raffinose, and stachyose, which together constituted over 90% of phloem mobile assimilates. Above 35°C, however, export during photosynthesis was inhibited both in photorespiratory conditions, which inhibited photosynthesis, and in nonphotorespiratory conditions, which did not inhibit photosynthesis. Sucrose and raffinose but not stachyose accumulated in the leaf at 40°C. When leaves were preincubated at 40°C and then cooled to 35°C, export recovered more slowly than photosynthesis. These data are consistent with the view that impairment of export processes, rather than photosynthetic processes associated with light trapping, carbon reduction, and sucrose synthesis, accounted for the marked reduction in export between 35 and 40°C. Taken together, the data indicated that temperature changes between 15 and 40°C had two effects on photosynthesis and concurrent export. At all temperatures, suppressing photorespiration increased both photosynthesis and export, but above 35°C, export processes were more directly inhibited independent of changes in photorespiration and photosynthesis.**

Temperature is one of the major environmental factors that affects many processes, including leaf photosynthesis, C partitioning in the leaf, allocation of C to developing sinks, and acquisition of assimilates among different sinks (Farrar, 1988). However, very little is known specifically about the manner in which temperature controls photosynthetic metabolism within the leaf lamina versus its effect on processes involved in the movement and export of photoassimilates from the leaf. There clearly exists "feed-forward" regulation of export processes as photosynthesis is altered by light, $CO_2$, $O_2$, and temperature. However, to understand possible "feedback" regulation of C fixation by end-product removal, we need to quantify export flux during a period of active photosynthesis. The objective of this study was to focus only on the effect that varying the source leaf temperature has on the rate of net $CO_2$ influx (i.e. photosynthesis) and the "concurrent" rate of C efflux from the same leaf (i.e. export).

In $C_3$ plants increasing leaf temperature above the growth temperature of the plant generally results in a reduction in leaf photosynthetic metabolism (Berry and Björkman, 1980). The reduction in net photosynthesis at elevated leaf temperatures is primarily a consequence of enhanced oxygenase activity of Rubisco (Ku and Edwards, 1977), due mainly to the lower $CO_2/O_2$ specificity of the Rubisco enzyme (Jordan and Ogren, 1984; Brooks and Farquhar, 1985). Elevated leaf temperatures also result in increased water loss and stomatal closure, and a reduction in the availability of $CO_2$. Under these conditions, photorespiratory loss of $CO_2$ and C cycling through the glycolate pathway (Zelitch, 1992; Tolbert, 1994) may increase, which can reduce ethylene-induced effects on membrane integrity (Grodzinski, 1984) and the extent of photoinhibition (Powles, 1984). Leaf temperatures above 35°C, for example, can result in thermal uncoupling of chloroplast thylakoids, inactivation of photosystems (Terzaghi et al., 1989; Oberhuber and Edwards, 1993), and inhibition of photophosphorylation (Berry and Björkman, 1980; Stidham et al., 1982; Havaux et al., 1991). The activity, activation, turnover, and assembly of components of the photosystems, as well as key enzymes of photosynthetic C metabolism, may also be affected as the leaf temperature is increased from 30 to 45°C (Berry and Björkman, 1980; Brooks and Farquhar, 1985; Hubbs and Roy, 1993; Ghosh et al., 1994).

The relationships between leaf temperature and regulation of the Calvin cycle via triose phosphate/Pi exchange and the resulting partitioning of C to starch and Suc within the leaf mesophyll tissue have been discussed by several groups (Weis, 1981; Kobza and Edwards, 1987; Stitt and Grosse, 1988; Sage et al., 1990). Inhibition of C export at low leaf temperature (0–5°C) may be related to changes in C partitioning and Pi exchange between the chloroplast and the cytosol in the mesophyll cells of the source leaf (Leegood and Furbank, 1986; Holaday et al., 1992; Mitchell and Madore, 1992). However, an increase in viscosity of the transport stream, phloem blockage, reduced phloem load-

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Abbreviations: GM, Geiger-Müller; IRGA, IR gas analyzer.

ing, and callose formation may affect C export from the leaf at low temperature (Webb, 1970; Canny, 1973; Giaquinta and Geiger, 1973; Geiger and Sovonick, 1975; Minorsky, 1985; Farrar, 1988; Robards and Lucas, 1990; van Bel, 1993).

The effect of temperature on physiological processes in the leaf is not restricted to leaf photosynthesis per se. At high temperatures the possibility of sieve-element impairment and inhibition of vein loading due, for example, to callose formation in sieve plates has been suggested (McNairn and Currier, 1968; McNairn, 1972; Geiger and Sovonick, 1975). In *Cucurbita melopepo,* which exports raffinose-series sugars, Webb (1970) noted that $^{14}$C export during a 15-s $^{14}CO_2$ pulse and a 1-h chase appeared to increase with an increase in leaf temperature from 0 to 25°C, was maximal between 25 and 35°C, decreased slightly at 45°C, and was inhibited at 55°C, although photosynthesis was still active. During these non-steady-state labeling experiments it was not possible to discriminate between the labeling of each of the transport sugars and the actual rate of C efflux (i.e. export) from the leaves.

In *Salvia splendens,* a $C_3$ plant, manipulating photorespiration by varying $O_2$ levels at 25°C did not change the relative proportion of Suc, raffinose, and stachyose (Madore and Grodzinski, 1984). However, at higher than ambient levels of $O_2$ (i.e. 21 kPa) photorespiration increased and $^{14}$C-labeled intermediates such as Gly and Ser were detected in the phloem (Madore and Grodzinski, 1984). These observations demonstrated that the photorespiratory C and N cycles (Tolbert, 1994) were not functioning in a closed manner and that phloem mobile assimilates change as the leaf is exposed to different photorespiratory conditions (Grodzinski et al., 1984; Ta et al., 1985; Givan et al., 1988; Sechley et al., 1992).

In this paper we report that when leaves of *Salvia* were exposed to different temperatures, the ratio of "concurrent" export to net $CO_2$ fixation was altered and the partitioning of $^{14}$C among the primary phloem mobile sugars (i.e. Suc, raffinose, and stachyose) varied. The data show the extent to which export during steady-state photosynthesis was sensitive to changes in photorespiration at each temperature between 15 and 40°C. The data help to define, independent of changes in sink strength, the upper leaf temperature at which vein loading and/or translocation rather than photosynthetic and photorespiratory metabolism of the leaf lamina may regulate C export in *Salvia.*

## MATERIALS AND METHODS

### Plant Material

Seeds of *Salvia splendens* (Stokes Seeds, St. Catharines, Ontario, Canada) were germinated and grown in PRO-MIX-BX (Les Tourbières Premier LTÉE, Rivière du Loup, Quebec, Canada) in 14-cm (1.4-L) pots in a research greenhouse. The plants were maintained at 25 ± 3°C day and 16 ± 1°C night temperatures. Plants were watered regularly and fertilized weekly with a nutrient solution of 14 mmol N $L^{-1}$, 6 mmol P $L^{-1}$, and 12 mmol K $L^{-1}$. Gas-exchange and C-export studies were conducted on the third or fourth leaves from the tops of flowering plants.

### Net $CO_2$ Fixation

Steady-state gas-exchange measurements were conducted on attached intact leaves using an open-flow system similar to that described previously (Woodrow et al., 1988). The leaf cuvette, which was made from brass and chrome-plated to reduce problems associated with water absorption (Dixon and Grace, 1982; Jiao et al., 1996), consisted of an upper section with a glass window and a lower section, in which was mounted a GM detector (model EWGM, window area 6.8 cm, Bicron Corp., Newbury, OH). Both upper and lower sections of the leaf cuvette were milled so that they functioned as water-circulating jackets for leaf temperature control. Air temperature of the plant was maintained at 25 ± 1°C in a holding chamber while the temperature of the leaf being studied was varied between 15 to 40°C. Humidity of the gas entering the leaf cuvette was controlled by first passing the gas stream through a gas bubbler in a controlled-temperature water bath. During a period in which steady-state photosynthesis was measured, the leaf was fed $^{14}CO_2$, which was supplied by a precision syringe pump set at a constant delivery rate of 0.33 mL $min^{-1}$. During a typical 2-h feed period, $^{14}CO_2$ and $^{12}CO_2$ were constantly mixed in a 250-mL Erlenmeyer flask with a magnetic stirrer. The flow rate through the leaf cuvette was 620 mL $min^{-1}$. The specific activity of $^{14}CO_2$ was monitored by sampling 3 mL of the inlet gas at 15-min intervals. The $^{14}CO_2$ was trapped in 2.5 mL of ethanolamine:ethylene glycol monomethyl ether (1:2, v/v). The specific activity of the $^{14}CO_2$ ranged from 12 to 60 kBq $\mu mol^{-1}$ C, depending on the $CO_2$ concentrations. Levels of inlet $CO_2$ were maintained at ambient (40 Pa) and at high concentrations (90 and 180 Pa) by Wösthoff (Bochum, Germany) gas-mixing pumps, and the $O_2$ content of the feed gas was either 21 or 2 kPa (Madore and Grodzinski, 1984). PAR was supplied by high-pressure sodium lamps and maintained at 1000 $\mu mol\ m^{-2}\ s^{-1}$ at the leaf surface. Internal $CO_2$ concentration was calculated according to von Caemmerer and Farquhar (1981), where external $CO_2$ concentration was the average of $CO_2$ concentrations of the "inlet" and "outlet" port of the leaf cuvette.

### Rate of $^{14}$C Accumulation and Export

A GM detector was mounted in the lower half of the leaf cuvette (Jiao et al., 1996) to monitor $^{14}$C-photoassimilate accumulation in the source leaves in a manner similar to that described by Geiger and Fondy (1979). The export rate during the $^{14}CO_2$ feeding period was the difference between the rate of C fixation, which was estimated from IRGA (model MKIII, ADC Corp., Hoddesdon, UK) measurements, and the rate of $^{14}$C accumulation in the leaf, which was measured by the GM detector (Fig. 1A). The efficiency of the GM detector was corrected for each leaf studied by measuring the total radioactivity remaining in the leaf extract at the end of the experiment (see below). For *Salvia,* the values for concurrent export, namely that occurring during a period of steady-state $^{14}CO_2$ labeling, are those that were calculated between 90 and 120 min after commencing the feeding.

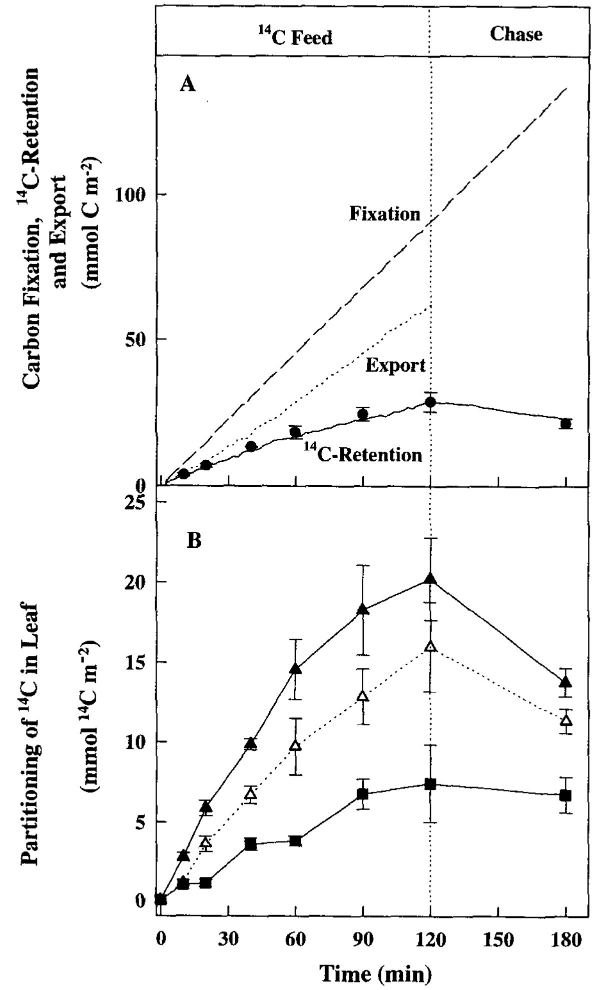

**Figure 1.** A, Total C "fixation," "$^{14}$C retention" in the source leaf, and "export" during a 2-h $^{14}CO_2$ feeding from the source leaf of *S. splendens* under 40 Pa $CO_2$, 21 kPa $O_2$, and 25°C. Cumulative net C fixation (dashed line) was calculated from IRGA data, whereas $^{14}$C retention in the leaf was measured both nondestructively by monitoring $^{14}$C with a GM detector continuously (solid line) and in a parallel set of leaves by destructive analysis (●). "Export" (dotted line) during steady-state $^{14}CO_2$ feeding was estimated as the difference between total fixation (dashed line) and $^{14}$C retention in the leaf (solid line). B, Partitioning of total $^{14}$C in the ethanol-soluble (▲) and total sugar fraction (△) and in starch (■) during steady-state $^{14}CO_2$ feeding and following a 1-h chase with $^{12}CO_2$ at 40 Pa and 21 kPa $O_2$ at 25°C. Each point is the average of at least four leaves on four different plants and error bars represent the SE.

### Extraction and Measurement of $^{14}$C Metabolites

Partitioning of $^{14}$C among the major photoassimilates of the leaf and petiole was determined after extraction in 80% ethanol:$H_2O$ (v/v) and separation of the extract into an ethanol-soluble and an insoluble fraction (Madore and Grodzinski, 1984). The ethanol-soluble extract was vacuum dried and partitioned into chloroform and water phases. The water phase was separated further into neutral, organic acid, and amino acid components using Bio-Rad AG50-X8 and AG1-X8 ion-exchange columns arranged in series. Sugars in the neutral fraction were analyzed using a Beckman $\mu$-Spherogel 7.5% cross-linked carbohydrate column at 85°C. Effluent was collected and the radioactivity in individual sugars was determined by liquid scintillation counting (Jiao et al., 1995). Free amino acids were derivatized with phenylthiohydantoin and analyzed with a Waters PicoTag column at 46°C, as described elsewhere (Côté et al., 1989). $^{14}$C-Labeled amino acids were analyzed simultaneously using an on-line Beckman 171 radioisotope detector with a 300-$\mu$L solid cell.

## RESULTS

### Export during Steady-State Photosynthesis

At 25°C and ambient conditions (40 Pa $CO_2$ and 21 kPa $O_2$), the $CO_2$ fixation rate was 12.5 $\mu$mol C m$^{-2}$ s$^{-1}$ (Fig. 1A). To achieve an equilibrium between the labeling of the export products and the $^{14}CO_2$ assimilated, $^{14}CO_2$ of constant specific activity was supplied to the leaf for at least 2 h, during which time steady-state photosynthesis was determined by IR gas analysis. The $^{14}$C remaining in the leaf (i.e. not exported from the feed area) was measured in two ways. First, the radioactivity of the leaf was monitored continuously with the GM detector (Fig. 1A, solid trace). Second, a series of $^{14}$C feedings of different duration (i.e. 10, 20, 40, 60, 90, and 120 min, and a further 60-min chase) were conducted and the leaves were extracted for analysis of total $^{14}$C remaining (Fig. 1A). These extractions also provided samples for studying $^{14}$C partitioning among the photoassimilates (Figs. 1B and 2) and values for total radioactivity in the leaf from which the efficiency of the GM detector was calculated. Figure 1A shows a close agreement between the destructive and nondestructive measurements of $^{14}$C retention in *Salvia* leaves. The differences between total C "fixation" and "$^{14}$C retention" in the leaf during the feed period were due to "export" of newly synthesized $^{14}$C-photoassimilates (the dotted line in Figure 1A). When export was blocked by heat girdling of the petiole, the value for C accumulation derived from $^{14}$C retention equaled total C fixation obtained from IR gas analysis (data not shown). In the light, $^{14}CO_2$ losses during photorespiration and dark respiration did not need to be measured to calculate export, since the IRGA only measured net $CO_2$ exchange by the leaf (see Geiger and Fondy, 1979; Geiger et al., 1988). The plot of export against time showed an initial lag with a linear rate of export after 45 min. A rate of export of 9.6 $\mu$mol C m$^{-2}$ s$^{-1}$ was calculated from 90 to 120 min (Fig. 1A). This value was similar to that obtained from longer feeding times. Thus, in *Salvia* leaves exposed to ambient $CO_2$ and $O_2$ levels at 25°C, about 77% of the C fixed during photosynthesis was leaving the leaf via the phloem stream and was not retained in storage pools.

To confirm that the best time period in which to compare steady-state photosynthesis with concurrent export was between 90 and 120 min of the feed, we measured the appearance of $^{14}$C label in the ethanol-insoluble fraction (starch) and the ethanol-soluble assimilates (Fig. 1B), which included the major translocated sugars Suc, raffinose, and stachyose (Fig. 2, A, C, and E, respectively). The specific

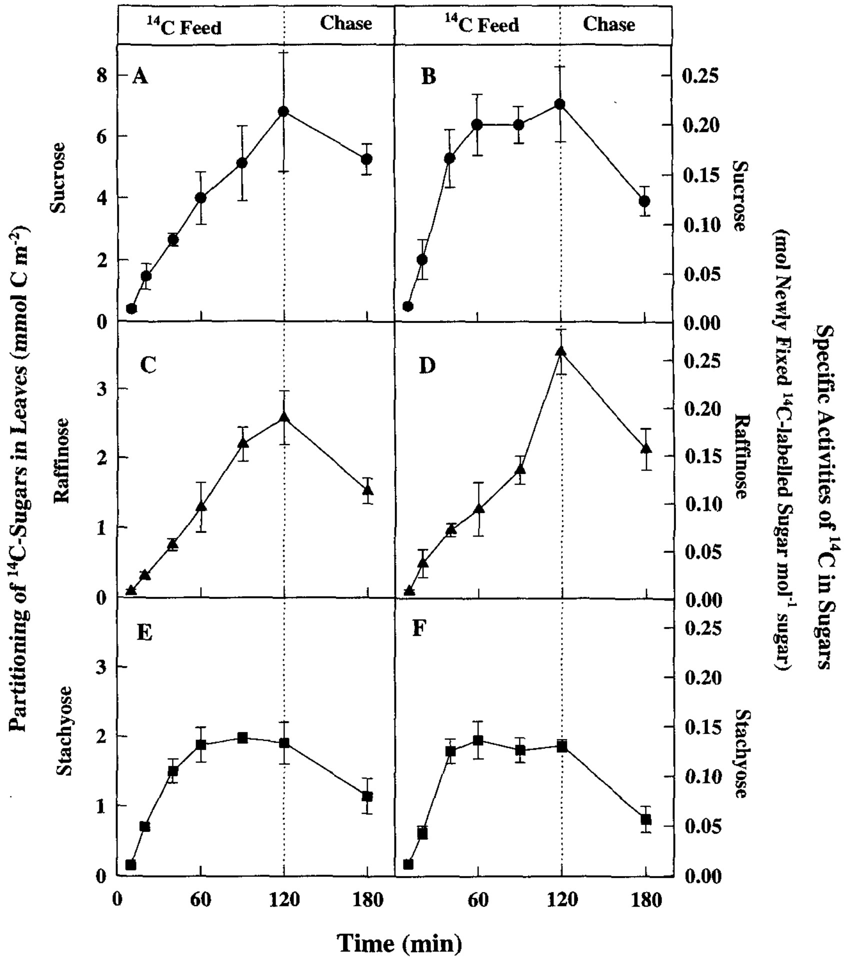

Figure 2. Partitioning of $^{14}$C in Suc (A and B), raffinose (C and D), and stachyose (E and F) in leaf tissue during a $^{14}$C feed and chase experiment with attached leaves of *Salvia* exposed to 40 Pa $CO_2$, 21 kPa $O_2$ at 25°C as described in Figure 1 and in "Materials and Methods."

activity of the Suc pool (Fig. 2B) increased in the first hour of the feed but changed only slightly in the second hour. By the end of the chase period the specific activity was reduced from 0.21 to 0.13 mol [$^{14}$C]Suc mol$^{-1}$ of total Suc as the $^{14}$C pool was diluted, metabolized, and exported. Although Suc represented 41 to 46% of the total $^{14}$C-sugars, a larger amount of radioactivity was recovered in the combined raffinose and stachyose pools. During the feed, the radioactivity (Fig. 2C) and the specific activity (Fig. 2D) of raffinose increased, consistent with the dual role of raffinose and Suc (Fig. 2A) in storage and translocation. At the end of the feed period, label in raffinose represented about 20% of the total label in the sugars. In experiments in which the $^{14}CO_2$ feed period was extended to 4 h, the radioactivity in the raffinose pool increased (data not shown). In contrast, labeling of stachyose reached a steady level within the first hour of the feed period (Fig. 2E), at which time the label in stachyose represented approximately 27% of the label in the three transport sugars. Furthermore, there was essentially no change in the specific activity of [$^{14}$C]stachyose after 45 min (Fig. 2F), indicating that isotopic equilibrium was obtained and the flux of C into stachyose was equal to its efflux from the leaf during the second hour of the feed period. At the end of the feed period the radioactivity in starch represented about 24% of the $^{14}$C remaining in the leaf. During chase periods, although photosynthesis was constant (Fig. 1A) and the radioactivity in starch was unchanged in the light (Fig. 1B), the radioactivity in the transport sugars decreased markedly (Figs. 1B and 2).

## Varying Leaf Temperature

Steady-state photosynthetic rates (Fig. 3, A–C) and concurrent export rates (Fig. 3, D–F) were measured during the feed period when leaves were exposed to varying temperatures between 15 and 40°C and a variety of $CO_2$ and $O_2$ levels. At 40 Pa $CO_2$ and 21 kPa $O_2$, photosynthesis was optimal between 15 and 25°C but decreased at 35 and 40°C (Fig. 3A). Suppressing photorespiratory activity by reducing the $O_2$ to 2 kPa increased photosynthesis at each temperature but did not alter the general response of photosynthesis to temperature (Fig. 3A). When the $CO_2$ level was increased to 90 Pa, the optimum temperature for photosynthesis under both low and ambient $O_2$ levels increased, but photosynthesis was markedly inhibited at 40°C (Fig. 3B). Figure 3, A and B, indicate that at 35 and 40°C photosynthesis was $O_2$ sensitive (Sharkey, 1985). To establish an $O_2$-insensitive net photosynthetic rate at temperatures between 35 and 40°C, the level of $CO_2$ entering the leaf

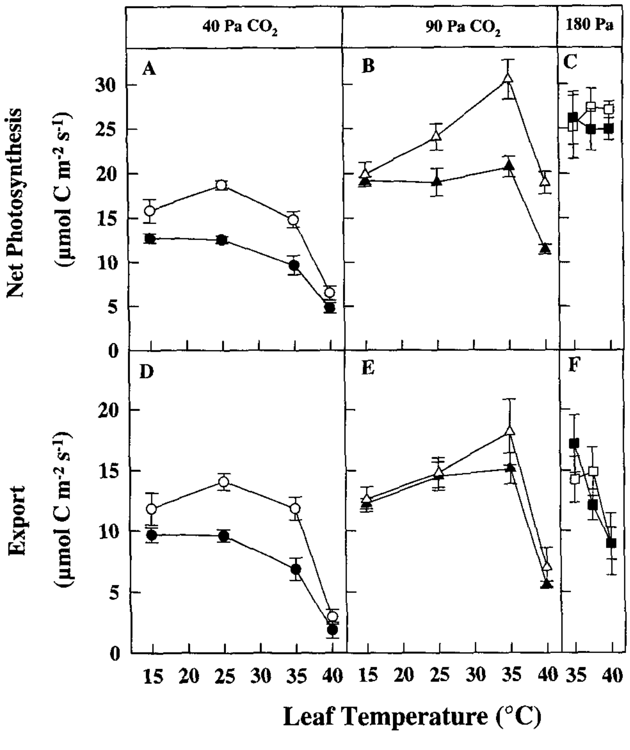

**Figure 3.** Effect of leaf temperature on net photosynthesis (A–C) and export (D–F) from leaves of *Salvia* exposed to three $CO_2$ concentrations (i.e. "inlet" air with 40 Pa $CO_2$ [●, ○]; 90 Pa $CO_2$ [▲, △]; or 180 Pa $CO_2$ [■, □]) and two "inlet" $O_2$ levels (21 kPa [closed symbols] or 2 kPa [open symbols]). Each point is an average of at least four leaves on four different plants. Each error bar represents the SE. All values of photosynthesis (A–C) and export during photosynthesis (D–F) were determined between 90 and 120 min of the $^{14}C$ feed period as outlined in Figure 1A and described in "Materials and Methods." The internal $CO_2$ concentration of leaves exposed to the three $CO_2$ levels (A, B, and C) were 20 to 30 Pa, 40 to 65 Pa, and 100 to 120 Pa, respectively (data not shown).

chamber was raised further to 180 Pa (Fig. 3C). At 180 Pa $CO_2$ photosynthesis rates remained high, even at 40°C.

The data in Figure 3, D through F, demonstrate several major features of the interaction of $CO_2$, $O_2$, and temperature on export during steady-state photosynthesis. First, the lowest rate of export was observed under ambient conditions at very high temperatures when photosynthesis was low due to high photorespiration (Fig. 3D). Second, at ambient $CO_2$ levels, export was increased between 15 and 35°C when photorespiration was suppressed by reducing $O_2$ to 2 kPa (Fig. 3D), consistent with the increase in photosynthesis (Fig. 3A). Third, export was greatest at 35°C during $CO_2$ enrichment (Fig. 3, E and F) when the photosynthetic rates were higher (Fig. 3, B and C) than they were at ambient $CO_2$ levels (Fig 3A). Fourth, during $CO_2$ enrichment at 90 Pa (Fig. 3B), photosynthesis was more sensitive to $O_2$ at elevated leaf temperatures than was export (Fig. 3E). Fifth, export was inhibited at 40°C regardless of the $CO_2$ and $O_2$ levels (Fig. 3, D–F). For example, even though the rate of photosynthesis was high at 180 Pa (Fig. 3C), export was still inhibited (Fig. 3F). When export was expressed as a percentage of photosynthesis (Fig. 4A), there clearly was, at 40°C, a marked reduction in the rate of C efflux from the leaf relative to the influx of $CO_2$ via photosynthesis under all gassing conditions studied.

In this paper we stress the need to calculate export rates during photosynthesis when steady-state $^{14}CO_2$ labeling conditions are present (Figs. 3, D–F, and 4A). Many calculations of export have also been obtained by first labeling the leaf and then analyzing the amount of radioactivity remaining in the leaf following a chase period of minutes, hours, and in some cases, even days (see Webb, 1970; Shishido et al., 1987; Farrar, 1988; Moing et al., 1992, and refs. therein). Figure 4B is presented here for comparison with Figure 4A. When $^{14}C$ efflux during the first 10 min of the chase was calculated, regardless of the $CO_2$ and $O_2$ levels tested, only 10 to 20% of the label appeared to be exported between 15 and 40°C (Fig. 4B). The differences in export that were due to temperature (Fig. 4A) were not clearly demonstrated from measurements of the disappearance of $^{14}C$ during the chase (Fig. 4B). The IRGA data confirm that photosynthesis was constant during the chase period (Fig. 1A). The loss of $^{14}CO_2$ to the $^{12}CO_2$ air stream was adjusted. However, the partitioning of label among the sugars changed rapidly when the switch from a $^{14}CO_2$ stream was initiated, creating an unreliable condition for estimating turnover and export of the $^{14}C$ photoassimilates during photosynthesis. In all instances in this paper,

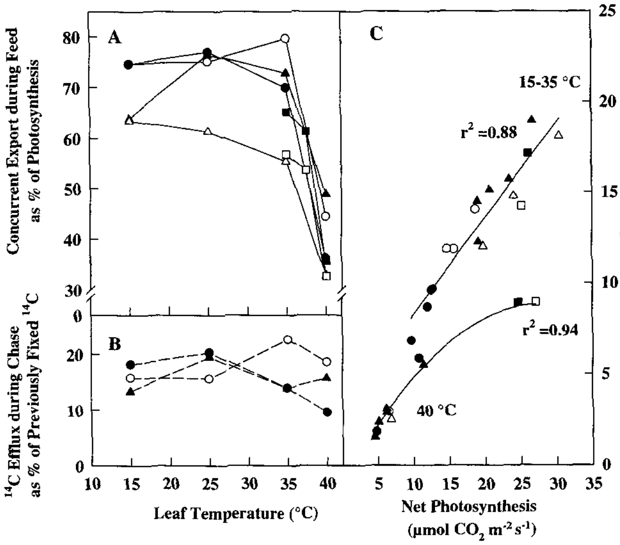

**Figure 4.** A, Effect of leaf temperature on concurrent export as a percentage of steady-state photosynthesis. Export during photosynthesis was calculated during the 90- to 120-min period of the $^{14}CO_2$ feed (as in Fig. 3, D–F). B, Values for $^{14}C$ efflux, which were derived from the data obtained by the GM detector during the initial 10 min of the chase period after correcting for respiratory exchange of $^{14}CO_2$. Data for 90 Pa $CO_2$ and 2 kPa $O_2$ and 180 Pa $CO_2$ were not available for calculation of $^{14}C$ efflux during a chase. C, Relationship between export rate and net photosynthesis, which are shown separately for data at leaf temperatures from 15 to 35°C and at 40°C, 40 Pa $CO_2$ (●, ○); 90 Pa $CO_2$ (▲, △); 180 Pa $CO_2$ (■, □). Closed symbols represent 21 kPa $O_2$ and open symbols represent 2 kPa $O_2$. Each point is an average of at least four replications.

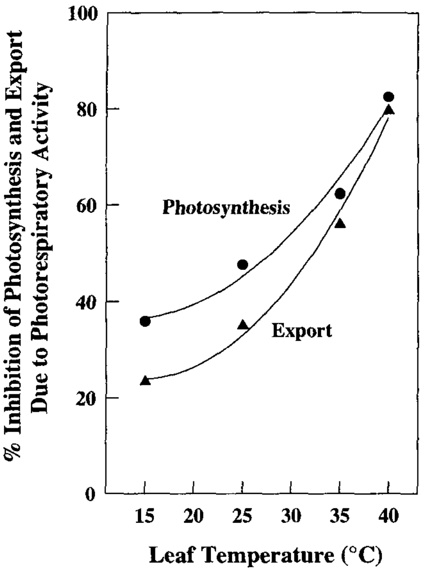

**Figure 5.** Effect of temperature on the percentage inhibition of photosynthesis (●) and export (▲) due to photorespiration. The percent inhibition due to photorespiration was estimated in a manner similar to that of Kozba and Edwards (1987). The rate (i.e. photosynthesis or export) at each temperature obtained under photorespiratory conditions was subtracted from the rate under nonphotorespiratory conditions, divided by the rate under nonphotorespiratory conditions, and multiplied by 100. The photorespiratory condition was 40 Pa $CO_2$ and 21 kPa $O_2$ at each temperature. However, the nonphotorespiratory conditions at each temperature were achieved with slightly different $CO_2$ levels. Photosynthetic and export rates at 15 and 25°C were those measured at 90 Pa $CO_2$ and 2 kPa $O_2$. At 35 and 40°C the nonphotorespiratory (i.e. $O_2$-insensitive) condition was established by supplying air containing 180 Pa $CO_2$.

except for Figure 4B, the terms "export" and "efflux" of C from the leaf refer to rates calculated from data obtained only during steady-state $^{14}CO_2$-labeling conditions.

It is noteworthy that over the temperature range of 15 to 35°C the export of C during photosynthesis was approximately 60 to 80% of the rate of C fixation (Fig. 4A). It is interesting that some of the lower values for concurrent export (i.e. 65% or less expressed as a percentage of photosynthesis) were obtained during $CO_2$ enrichment and at low $O_2$ (Fig. 4A). The possible significance of lower export rates at 15°C and 90 Pa $CO_2$, 21 kPa $O_2$, or at 35°C and 180 Pa $CO_2$, 21 kPa $O_2$ (Fig. 3, A and B) with respect to recycling of C or temporary storage of carbohydrates within the laminar tissues is currently not understood. Here it suffices to observe that, above 35°C, net export decreased as a percentage of photosynthesis regardless of the $O_2$ and $CO_2$ levels (Fig. 4A). When all export rates measured between 15 and 35°C were plotted against net photosynthesis rates (Fig. 4C), a linear relationship ($r^2$ = 0.88) was observed. However, the best line fit for the data collected at 40°C was a second-order regression ($r^2$ = 0.94), further indicating that export was inhibited in a manner other than that seen between 15 and 35°C by manipulating photorespiration.

## Photorespiratory versus Nonphotorespiratory Conditions

Figure 5 provides an interesting qualitative assessment of the importance of photorespiration in regulating the net flux of C through the leaf. Clearly, these calculations, which compare data from ambient levels of $CO_2$ and $O_2$ with those under $O_2$-insensitive conditions, do not provide truly quantitative values for the involvement of the glycolate pathway in net C fixation (Fock et al., 1979; Sharkey, 1988) or export (Madore and Grodzinski, 1984). At 15°C, suppressing photorespiration increased photosynthesis by 35% and export by 25%. At 40°C, suppressing photorespiration increased photosynthesis and export by about 80%, consistent with the view that photorespiration is greater at high leaf temperatures, and that suppressing its activity increases the flux of C to sugar synthesis and export.

## Partitioning of $^{14}C$ Photoassimilates

As temperature increased from 25 to 40°C at ambient gas levels, less sugar was labeled and exported (Fig. 6A). Sup-

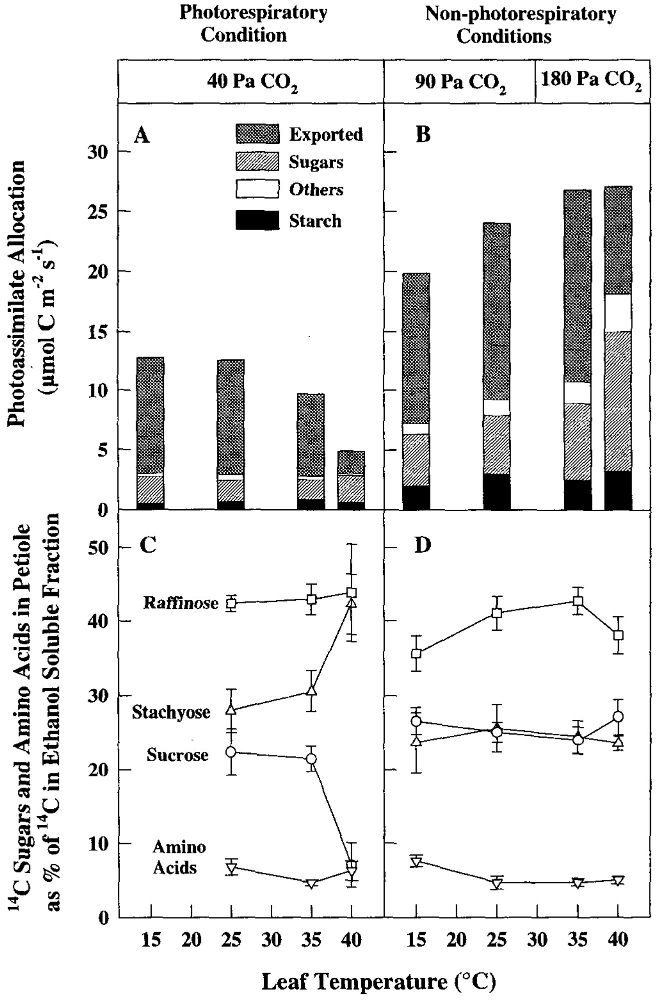

**Figure 6.** Effect of leaf temperature on $^{14}C$-photoassimilate allocation (A and B) and the proportion of $^{14}C$-sugars and $^{14}C$-amino acids (C and D) in the petiole of the feed leaf that was exposed to photorespiratory (ambient $CO_2$ and $O_2$) and nonphotorespiratory (i.e. $O_2$-insensitive) conditions (see Fig. 5 and "Materials and Methods").

pressing photorespiration by enrichment with $CO_2$ increased starch at all temperatures (Fig. 6B). As temperature was increased to 40°C, proportionally more of the newly fixed C was allocated to synthesis of sugars that constituted the major pool of newly fixed $^{14}C$ in both the export stream and in the leaf (Figs. 6B and 7). At 40°C export was inhibited (Figs. 3, 4, A and C, and 6A), even though a high rate of C fixation was maintained under nonphotorespiratory conditions (Figs. 3, C and F, and 6B). In one condition (ambient $CO_2$ and $O_2$, 40°C), labeled Suc in the petiole decreased while there was a concomitant increase in stachyose labeling (Fig. 6C). The decrease in labeling of Suc in the petiole under ambient conditions (Fig. 6C), but not under the nonphotorespiratory condition at 40°C (Fig. 6D), paralleled the lowest fixation (Fig. 3A) and export rates measured (Fig. 3D). Under nonphotorespiratory conditions, the rate of export at 40°C, although 50% lower than at 35°C (Fig. 6B), was still similar to export rates observed at 25 and 35°C at ambient $CO_2$ and $O_2$ (Fig. 6A). Raffinose labeling in the petiole was similar at all leaf temperatures (Fig. 6, C and D), even though the export rate was markedly inhibited at 40°C (Fig. 6, A and C). Taken together, the data show that when photorespiration was suppressed, more C was fixed and the relative proportion of label in Suc, raffinose, and stachyose remained unchanged in the petiole (Fig. 6D). Labeled Gly and Ser were exported at all leaf temperatures under the photorespiratory conditions (data not shown). As noted in *Cucumis melo* (Mitchell and Madore, 1992), the amino acid fraction contained a slightly higher percent of the label at 15°C; however, the total amount of label in the amino acid pool was less than 8% of the total in the petiole (Fig. 6, C and D).

Figure 7 shows the relationships among leaf temperature, photosynthesis, and total pools of each of the major transport sugars. Between 15 and 35°C *Salvia* leaves exported about 70% of the newly fixed C (Fig. 4A). Over the same temperature range, Suc accumulated in the leaf when the photosynthesis rate increased (Fig. 7A). When export was inhibited by warming the leaf to 40°C (Fig. 4A), both Suc (Fig. 7D) and raffinose (Fig. 7E) accumulated. Stachyose did not accumulate in the leaf lamina, even at 40°C (Fig. 7F).

### Reversing the Thermal Inhibition of Photosynthesis and Export

Table I shows the results of an experiment in which the same leaf was heated to 40°C and subsequently "cooled" to 35°C under both photorespiratory and reduced photorespiratory conditions. Export recovered more slowly than did photosynthesis, further indicating that the processes involved in export of C were more sensitive to leaf warming than the processes involved in $CO_2$ fixation per se.

## DISCUSSION

Taken together, the data indicate that temperature changes between 15 and 40°C had two effects on photosynthesis and concurrent export in *Salvia* (Figs. 3–6). Over the entire temperature range of 15 to 40°C, suppressing photorespiration increased both photosynthesis and export at each temperature (e.g. Fig. 5). The relationships between photosynthesis, photorespiration, and Suc synthesis were probably under the same type of control as they are in other $C_3$ species (Kozba and Edwards, 1987; Stitt and Grosse, 1988; Sage et al., 1990). However, above 35°C export in *Salvia* appeared to be inhibited directly by temperature under both photorespiratory and nonphotorespiratory conditions (Fig. 3, D–F). This fact was most clearly demonstrated when rapid rates of C fixation were maintained by adding 180 Pa $CO_2$ to suppress photorespiration (Fig. 3C). Since photosynthesis was high under nonphotorespiratory conditions at 40°C (Fig. 3C), it appears that the operation of the photosystems (Terzaghi et al., 1989; Havaux et al., 1991), photophosphorylation mechanisms (Berry and Björkman, 1980; Stidham et al., 1982; Farrar, 1988), carboxylation (Ku and Edwards, 1977; Jordan and Orgen,

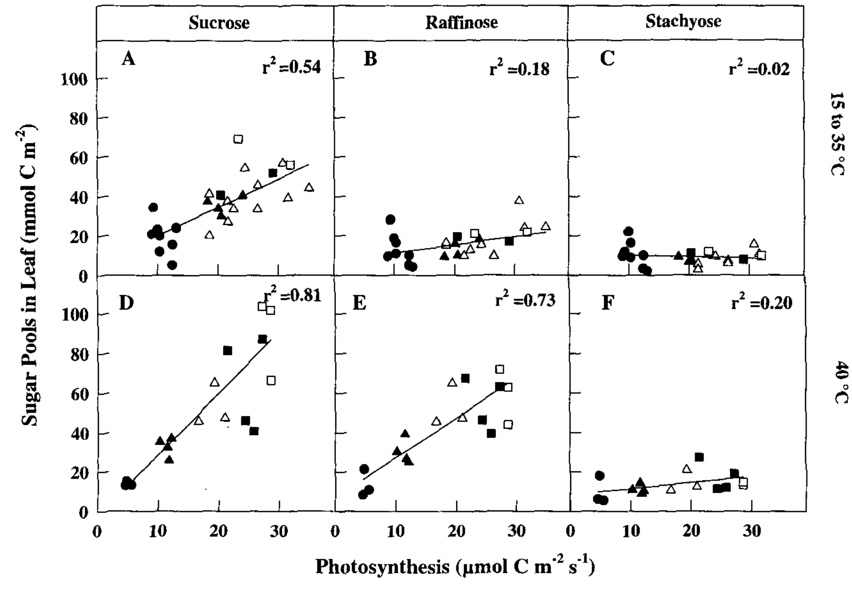

**Figure 7.** Photosynthetic rates and accumulation of Suc (A and D), raffinose (B and E), and stachyose (C and F) in leaves exposed to photorespiratory and nonphotorespiratory conditions at varying temperatures between 15 and 35° (A–C) and 40°C (D–F). The data and symbols used are for leaves exposed to conditions described previously in Figure 3. Each point is the average of at least four replications.

**Table I.** *Recovery of heat stress-induced inhibition of photosynthesis and export*

Leaves were assayed at 35 and 40°C and exposed to both ambient $CO_2$ (40 Pa) and $O_2$ (21 kPa), and high $CO_2$ (180 Pa) and low $O_2$ (2 kPa) as outlined in Figure 3. In addition, leaves that were preincubated at 40°C for 40 min and subsequently cooled to 35°C for 2 h were assayed at 40 Pa $CO_2$ or for 30 min at 180 Pa $CO_2$ prior to measurements of photosynthesis and export during a 2-h, steady-state $^{14}CO_2$ feeding (i.e. 40/35°C values).

| Temperature | Ci | Photosynthesis | Inhibition | Export | Inhibition |
|---|---|---|---|---|---|
| *°C* | *Pa* | *$\mu mol\ C\ m^{-2}\ s^{-1}$* | *%* | *$\mu mol\ C\ m^{-2}\ s^{-1}$* | *%* |
| Ambient $CO_2$ and ambient $O_2$ | | | | | |
| 35 | 21 ± 1 | 9.7 ± 1.1 | 0 | 6.8 ± 0.8 | 0 |
| 40 | 30 ± 1 | 4.8 ± 0.6 | 50 | 1.8 ± 0.7 | 73 |
| 40/35 | 19 ± 2 | 8.4 ± 0.8 | 13 | 4.1 ± 0.6 | 39 |
| High $CO_2$ and low $O_2$ | | | | | |
| 35 | 95 ± 6 | 24.8 ± 2.3 | 0 | 16.4 ± 1.8 | 0 |
| 40 | 121 ± 3 | 24.3 ± 1.2 | 2 | 8.7 ± 2.6 | 46 |
| 40/35 | 101 ± 8 | 25.1 ± 1.9 | −1 | 11.9 ± 1.5 | 27 |

1984; Brooks and Farquhar, 1985; Hubbs and Roy, 1993; Ghosh et al., 1994), triose phosphate/Pi exchange, and the capacity of the leaf to synthesize Suc (Kozba and Edwards, 1987; Stitt and Grosse, 1988; Sage et al., 1990) were not severely inhibited by simply warming the leaf from 35 to 40°C. In fact, Suc and raffinose both accumulated at 40°C (Figs. 6B and 7, D and E) and were present in the phloem stream (Fig. 6D). At temperatures higher than 40°C, chloroplast structure and function may limit photosynthetic C fixation, since they may demonstrate temperature sensitivity (Berry and Björkman, 1980; Farrar, 1988; Hubbs and Roy, 1993; Ghosh et al., 1994). However, direct inhibition of any of the processes responsible for light trapping or conversion of reduced C to sugars does not explain the reduced rates of export observed under all gassing conditions when the leaf temperature was raised from 35 to 40°C (Fig. 3, C and F).

Regardless of the explanation for the inhibition of export above 35°C, the data show that in *Salvia* suppressing photorespiration between 15 and 40°C resulted in (a) increased photosynthesis, (b) increased concurrent export of Suc, raffinose, and stachyose, and (c) increased storage of carbohydrates in the leaf, primarily in the form of starch, Suc, and raffinose (Fig. 6). Based on the observation that label in starch was not reduced during photosynthesis during a chase period (Fig. 1B), it appears that in *Salvia* leaves starch turnover in the light under steady-state photosynthetic conditions was very slow. The increase in starch reserves, especially at high $CO_2$ (Fig. 6B), supports enhanced export at night (data not shown), as noted by other authors (Madore and Grodzinski, 1985; Farrar, 1988; Geiger et al., 1988). At elevated leaf temperatures (e.g. 35–40°C) proportionally more of the newly fixed C was converted to sugars and less was partitioned to starch (Fig. 6B). These data are consistent with the sensitivity of starch synthesis to elevated temperatures (Stitt and Steup, 1985; Preiss, 1988).

*Salvia* is a symplastic loader, but the possibility that apoplastic loading of Suc was reduced by high temperature cannot be eliminated (Farrar, 1988; Lucas and Madore, 1988; van Bel, 1993). As noted above, Suc and raffinose synthesis continued and both accumulated at 40°C (Fig. 7). Therefore, the synthesis of these precursors was not limiting stachyose production under nonphotorespiratory conditions. There are many possible sites of storage of Suc and raffinose within the symplasm, mesophyll, parenchyma, intermediary cells, companion cells, and phloem sieve tubes (Robards and Lucas, 1990; Holthaus and Schmitz, 1991; Beebe and Turgeon, 1992). Stachyose did not accumulate in the leaf tissue even at relatively high photosynthesis rates (Fig. 7F). In earlier studies with mesophyll cells isolated from *Salvia,* we did not detect labeling of stachyose from $^{14}CO_2$, but we were able to detect label in both Suc and raffinose (Grodzinski et al., 1987). More recently, Beebe and Turgeon (1992) showed that the intermediary cells in *Cucurbita pepo* leaves were sites of synthesis of the raffinose sugars. The labeling of the stachyose pool noted during the feed period (Fig. 2F) was consistent with the view that stachyose was found mainly in the phloem stream in *Salvia* (Madore and Grodzinski, 1984; Grodzinski et al., 1987).

The inhibition of export at temperatures above 35°C may be due to a negative effect of temperature on several different sensitive steps in the pathway of sugars to the phloem. For example, reduced enzyme activity leading to stachyose synthesis, or impairment of the physical transport pathway, or reduced vein loading, as suggested earlier (Webb, 1970; Geiger and Sovonick, 1975), could each account for the reduced rate of export observed at 40°C. A block in the symplastic pathway resulting from callose synthesis at high temperature (McNairn and Currier, 1968; McNairn, 1972) or occlusion of plasmodesmata due to formation of heat-shock proteins or altered phloem protein character are all consistent with our observations. Recovery of photosynthesis and partial recovery of export on return to 35°C (Table I) may have occurred because translocated photoassimilates bypassed the temperature-induced block via alternative transport paths (see Robards and Lucas, 1990, and refs. therein). The effect of high temperatures on the mechanisms of phloem loading are unknown (see van Bel, 1993), but we have observed a similar effect of high temperature on export in peas, an apoplastic loading species (data not shown). Clearly, we need to know whether, in *Salvia* and other species that transport raffinose-series sugars, there is a heat-sensitive biochemical step in the pathway to stachyose synthesis, or whether there is a physical impairment in the translocation pathway.

## SUMMARY

The data show that at ambient $CO_2$ and $O_2$ levels, photosynthesis and C export during photosynthesis of *Salvia* leaves were maximal at 25°C. Suppressing photorespiration between 15 and 40°C by $CO_2$ enrichment increased photosynthesis, sugar synthesis, and concurrent C export during photosynthesis. Maximum rates of export during photosynthesis were measured at 35°C during $CO_2$ enrichment. Export was inhibited at 40°C, even when the rates of net $CO_2$ fixation were maintained by $CO_2$ enrichment to 180 kPa, an $O_2$-insensitive condition. It is therefore postulated that independent of the photorespiratory conditions, biochemical steps in stachyose synthesis, vein loading processes, and/or phloem translocation processes were heat sensitive above 35°C. When the leaf was cooled from 40 to 35°C, the ability to export was recovered more slowly than was the ability to fix C. These results indicate the sensitivity of export processes, as well as photosynthetic mechanisms, to changes in leaf temperature. The accumulation of intermediates such as Suc and raffinose are dependent on both the rate of their synthesis and the rate of export during photosynthesis. In contrast, stachyose, another major transport sugar in *Salvia,* did not accumulate when export was inhibited, and its synthesis more closely paralleled export of newly fixed C under a variety of photorespiratory and nonphotorespiratory conditions. The extent to which the accumulation of sugars such as Suc and raffinose, or amino acids such as Gly and Ser, which "normally" are in efflux from the leaf during photosynthesis, might regulate the amount of C and N recycling within the leaf are subjects of ongoing research.

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