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Photosynthate Partitioning into Starch in Soybean Leaves

II. IRRADIANCE LEVEL AND DAILY PHOTOSYNTHETIC PERIOD DURATION EFFECTS

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ABSTRACT

Two photosynthetic periods and photosynthetic photon flux densities (PPFD) were used to study the relationship between the rate of photosynthesis and starch accumulation in vegetative soybean leaves (Merr. cv Amsoy 71). Plants grown in short daily photosynthetic periods (7 hours) had higher rates of CO_2 fixation per unit leaf dry weight and of leaf starch accumulation than plants grown in long daily photosynthetic periods (14 hours) irrespective of PPFD. CO_2 fixation rates per unit leaf area were similar in 7-hour and 14-hour plants grown at low PPFD but were highest in 14-hour plants at the high PPFD. When single leaves of 14-hour plants were given 7-hour photosynthetic periods, their rates of starch accumulation remained unchanged. The programming of starch accumulation rate and possibly of photosynthetic rate by the length of the daily photosynthetic period is apparently a whole-plant, not an individual leaf, phenomenon. Programming of chloroplast starch accumulation rate by length of the daily photosynthetic and/or dark periods was independent of PPFD within the ranges used in this experiment.

Future increases in crop yields will be highly dependent upon our ability to make changes in the biochemical and physiological processes of plant growth through genetic modifications. It is important to identify and characterize those metabolic processes which influence photosynthate partitioning. A major unanswered question is: "How is photosynthate partitioning regulated?" Because a significant amount of newly formed photosynthate is initially partitioned via sugar phosphates into chloroplast starch (4, 10, 11, 15, 21–23, 25), an understanding of the regulation of leaf starch synthesis is important. A knowledge of the control mechanisms of photosynthate partitioning will increase the potential for appropriate genetic modifications and perhaps lead to increased crop yields.

Previous work has shown that leaf starch accumulation rates are programmed by the length of the daily photosynthetic period (7) in a variety of species (8); an acclimation occurs in CER_W[2] (1, 9) and in the rate of leaf starch accumulation (9) when plants grown in one photosynthetic period are abruptly changed to one of another duration. Reviews of the literature (13, 17, 19, 25) point to a superficial understanding of the mechanisms involved in partitioning and also to the challenges of using the results of *in vitro* studies to understand the functioning of intact plant systems. Here, we obtained information from whole plants with the inherent complications of intact plant partitioning controls.

The study presented here was conducted to determine whether the influence on CER_W and starch accumulation rates by duration of the photosynthetic period was dependent on PPFD. In addition, we sought to determine whether a starch-accumulating response was induced when a single attached leaf was subjected to a shortened photosynthetic period, while the rest of the plant received a long photosynthetic period.

MATERIALS AND METHODS

Soybean plants (*Glycine max* (L.) Merr. cv. Amsoy 71) were planted, four seeds/pot, in black plastic pots (10 × 10 × 15 cm) containing vermiculite as previously described (7). Seedlings were thinned to one plant/pot about 4 days postemergence. Plants (non-nodulated) were grown in model M-2 controlled-environment chambers (Environmental Growth Chambers, Chagrin Falls, OH).[3] Air temperature and RH were maintained at constant 27 ± 1 C and 60 ± 2%, respectively. Plants were watered daily with sufficient nutrient solution to flush through the potting medium (21).

Light Treatments. Plants were grown in four light treatments. Two PPFDs (64 and 32 nE/s·cm^2 at pot height) were supplied by 10 60-w incandescent and cool-white fluorescent lamps located above a *Mylar* barrier. The 32 nE/s·cm^2 irradiance was obtained by positioning sufficient layers of cheesecloth on the *Mylar* barrier to reduce the normal 64 nE/s·cm^2 by one-half. The PPFD measurements were made with a quantum sensor (model LI-185, Lambda Instruments Corp., Lincoln, NE). Additionally, two photosynthetic period treatments, 7- and 14-h, were applied daily at both PPFD. The 7-h photosynthetic period treatments were followed by an additional 7-h of low incandescent light (about 1 nE/s·cm^2). Therefore, all four treatments received a daily 14-h photoperiod.

Single Leaf Treatment. The third trifoliolate leaves of 24 soybean plants grown for 21 days (64 nE/s·cm^2) in a 14-h photosynthetic period were shifted to a 7-h treatment by enclosure in black zip-lock plastic bags (opaque to far-red light) for 4 days during the second 7 h of the normal 14-h photosynthetic period. Therefore, a 7-h photosynthetic period treatment was applied to leaves on plants that were receiving 14-h daily photosynthetic periods. Air was circulated through one-half of the leaf bags to minimize alterations in the ambient air surrounding the shaded leaves. During illumination (64 nE/s·cm^2), the air temperatures were about 27 and 29 C in the bags with and without positive air

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[2] Abbreviations: CER_W, mg CH_2O/g dry weight·h; CER, carbon dioxide exchange rate; CER_A, mg CH_2O/dm^2·h; PPFD, photosynthetic photon flux density; SLW, specific leaf weight (mg dry weight/dm^2); TNC, total nonstructural carbohydrates.

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

circulation, respectively. Twelve leaves were harvested and measured to determine starch accumulation rates from 1- to 6-h illumination. Because no differences in starch acucmulation rates were observed, the data from leaves with and without positive air circulation were combined.

Carbohydrate Analyses. The nonstructural carbohydrate concentrations were determined from lyophilized tissue obtained from two leaves from each of six plants harvested at intervals throughout the day. The two leaves were combined for analysis. A 100-mg sample was suspended in 100 ml distilled H_2O at room temperature for 30 min. The resultant water-soluble carbohydrate fraction was quantified (mg/100 mg) by reducing sugar analyses following hydrolysis in 0.6 N HCl. Another 100-mg sample of dry leaf tissue was treated with dialyzed takadiastase (Clarase 900, Miles Laboratories) for 44 h (7). Per cent TNC (mg/100 mg) was determined colorimetrically by potassium ferricyanide analysis for reducing sugars in the enzyme digest following 0.6 N HCl hydrolysis in an Autoanalyzer II (Technicon Instruments Corp., Tarrytown, NY). Per cent starch was calculated as the difference between TNC and water-soluble carbohydrates.

Specific Leaf Weight. The SLW (mg/dm^2) was determined from the lyophilized leaf dry weight (minus petiole) and leaf area at the time of harvest. Two leaves were harvested from each of six plants to determine mean SLW at each sampling time. The values for mg TNC/dm^2 were obtained from the relationship: mg TNC/dm^2 = (%TNC × SLW)/100. Similarily, mg starch/dm^2 was also calculated from per cent starch and SLW.

Carbon Assimilation. The CERs of attached leaves (third trifoliolate) were measured under growth conditions using a flow-through IR gas analysis system described earlier (7). Measurements were made during the photosynthetic period on each of four plants, and the diurnal CER was determined. CER, mg of CO_2 fixed, was converted to mg of CH_2O to express CER in the same units as those used for the carbohydrate accumulation rates (7).

RESULTS

Whole Plant Treatments. The CER_A of the third trifoliolate leaf of soybean plants grown under 14-h photosynthetic periods at 64 nE/s·cm^2 was about 20% higher than that of plants grown in 7-h photosynthetic periods at the same PPFD (Fig. 1) and is inversely correlated with mean SLW (Table I). However, CER_A of plants grown in 32 nE/s·cm^2 was the same in 14- and 7-h grown plants. Given such CER_A relationships among the four treatments and the considerably lower SLWs in plants grown in 7-h than in 14-h photosynthetic periods at a given PPFD (Fig. 2), CER_W was much higher in plants grown at 7-h than in those at 14-h at both PPFDs (Fig. 1; Table I). Surprisingly, the CER_W of 7-h plants at 32 nE/s·cm^2 was comparable to that of 14-h plants receiving 64 nE/s·cm^2 (Table I).

Much of the change in SLW over time (Fig. 2) resulted from an accumulation of laminar starch (Fig. 3). The rates of starch and TNC accumulation, as evident from the slope of the lines (Figs. 3 and 4), were much faster in plants grown at 7-h than in those grown at 14-h. At both PPFDs, the percentage of total CH_2O synthesized that accumulated as starch was at least 2 times greater in plants grown at 7-h than in those grown at 14-h, whether expressed on an area or a dry weight basis (Table I).

Single-leaf Treatment. Starch accumulation rates were 3.26 mg/dm^2·h or 9.20 mg/g dry weight·h (1 to 6 h illumination) in leaves shaded during the second 7 h of a 14-h photosynthetic period while the remainder of the plant was illuminated for 14-h (64 nE/s·cm^2). The rates of starch accumulation in the shaded single leaves did not differ from those of the 14-h control leaves (compare with Table I). Water-soluble carbohydrate content remained about 2.00 to 2.50% from 1 to 6 h illumination in control and shade-treated plants.

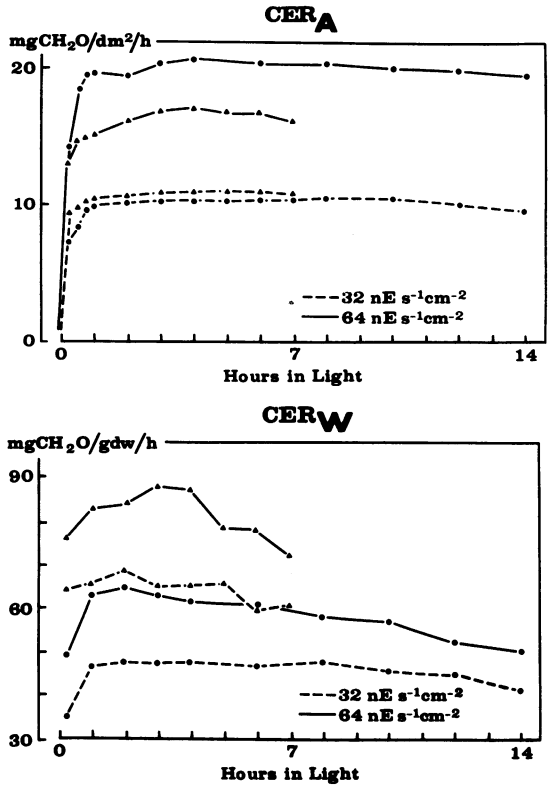

FIG. 1. Rates of carbohydrate (CH_2O) synthesis (mg/dm^2·h [*upper*]; mg/g dry weight·h [*lower*]) calculated from the CER of soybean leaves grown at two PPFDs (32 and 64 nE/s·cm^2) and at two daily photosynthetic periods (▲, 7 h; ●, 14 h).

DISCUSSION

We previously observed higher CO_2 fixation rates (mg/g dry weight·h) and starch accumulation rates in plants grown in or shifted to 7-h photosynthetic periods than in plants grown in 14-h photosynthetic periods at 64 nE/s·cm^2 PPFD (7, 8). Similarly, the length of the daily photosynthetic period influenced starch accumulation rates at the lower PPFD used in this experiment (32 nE/s·cm^2). More starch accumulated, both on a leaf area and a dry weight basis, in plants grown in a 7-h photosynthetic period than in those grown in a 14-h photosynthetic period, even though the rates of CO_2 fixation were considerably less at low than at high irradiance levels. Therefore, the rate of leaf-starch accumulation was not necessarily correlated with the rate of CO_2 fixation. The length of the daily photosynthetic period definitely influenced the rate of leaf-starch accumulation as well as the rate of CO_2 fixation. A change in CER may be the result of an alteration in the size of the photosynthetic unit (16, 24). Alternatively, Robinson *et al.* (20) have recently reported a difference in the specific activity of ribulose-1,5-bisphosphate carboxylase in intact plastids isolated from spinach (*Spinacia oleracea* L.) leaves of plants adapted to 7- and 12-h daily photosynthetic periods. The differences in CO_2 fixation rates and of starch accumulation rates in contrasting daylengths may be the result of a regulation of enzyme activities by length of the daily photosynthetic period.

A comparison of data from 7- and 14-h plants grown at two PPFDs indicated that the rate of leaf starch accumulation did not result simply from translocation not keeping pace with carbohydrate synthesis, as some data have suggested (19, 26). Further, the high rates of photosynthesis per unit leaf dry weight of leaves containing large quantities of starch contrasted sharply with other results that have suggested a feedback effect of starch on photosynthesis (5, 6, 14), but they did agree with the data of Little and

Table I. *Rates of Carbohydrate Synthesis (CER_A, CER_W) and Starch Accumulation in Leaves of Plants Grown in 14- and 7-h Photosynthetic Periods at Two PPFDs*

The ratios are also shown for the rates of starch accumulation over the rates of total carbohydrates synthesized. All values were calculated from measurements made at 2, 4 and 6 h illumination and at 2, 4, 6, 8, 10, and 12 h illumination for 7- and 14-h daily photosynthetic period treatments, respectively.

PPFD	Rates							
	CH_2O synthesis		Starch accumulation		Total starch accumulation		Starch/CH_2O synthesis[a]	$\bar{x}$ SLW
	mg/dm²·h	*mg/g dry wt·h*	*mg/dm²·h*	*mg/g dry wt·h*	*mg/dm²·day*	*mg/g dry wt·day*	*ratio*	*mg/dm²*
64 nE/s·cm²								
14 h	20.05a[b]	58.97b	2.90	8.53	40.63	119.50	0.14	340a
7 h	16.66b	82.06a	5.02	24.72	35.14	173.04	0.30	203b
32 nE/s·cm²								
14 h	10.34c	48.77c	1.59	7.50	22.26	105.00	0.15	212b
7 h	10.83c	64.08b	4.51	26.68	31.57	186.76	0.42	169c

[a] Measured in (mg starch/dm²) ÷ (mg CH_2O/dm²) or (mg starch/g dry weight) ÷ (mg CH_2O/g dry weight).
[b] Means within vertical rows followed by the same letter are not significantly different at $P \leq 5\%$ (Duncan's Multiple Range Test).

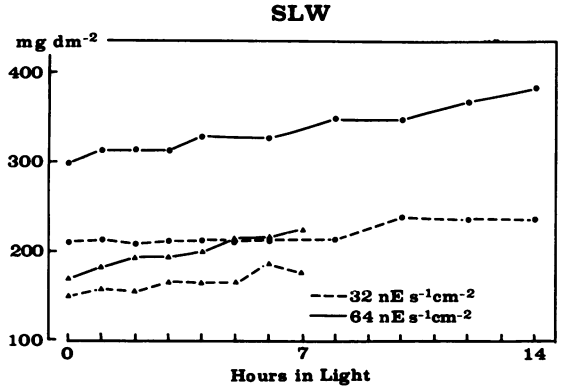

FIG. 2. Mean specific leaf weight (SLW, mg/dm²) of the third and fourth trifoliolate leaves of soybean plants measured at intervals over 7- and 14-h photosynthetic periods and at two PPFDs (32 and 64 nE/s·cm²). Values are means of six replicate determinations, each from separate plants.

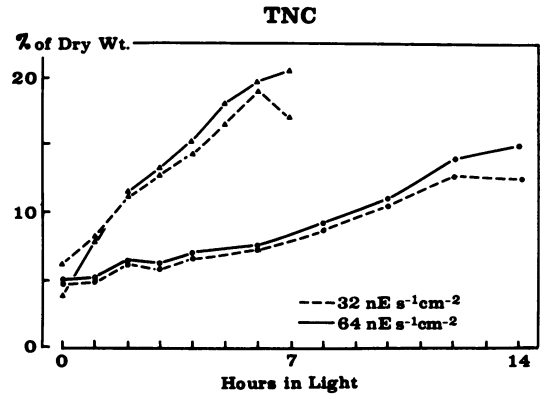

FIG. 4. Total nonstructural carbohydrate content (TNC, mg/100 mg dry weight) of soybean leaves of plants grown at 32 and 64 nE/s·cm² and given either 7 or 14-h daily photosynthetic periods.

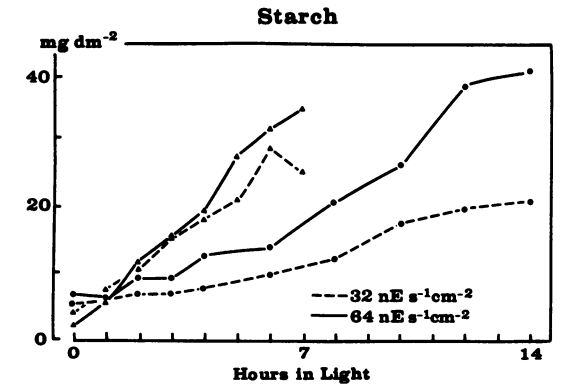

FIG. 3. Laminar starch content (mg/dm²) of soybean leaves from plants grown in two PPFDs (32 and 64 nE/s·cm²) and in two daily photosynthetic periods (7 and 14 h).

Loach (18), Carmi and Shomer (3), and Forde *et al.* (12). Therefore, further doubt is cast on the hypothesis that starch accumulation is a feedback regulator of leaf photosynthetic rates.

The absence of a significant change in the rate of starch accumulation in the single leaf treatment suggested either reprogramming did not occur when a single leaf was shifted to a short photosynthetic period while remaining attached to a plant receiving a long photosynthetic period or that the reprogramming effects of acclimation were over-ridden by whole plant effects. We found that the shoot/root ratio increased with a 14- to 7-h shift in length of the daily photosynthetic period (7). That increase suggested significant changes in the relative sink strength of the plant parts. Therefore, many alterations in the whole plant response may occur as a result of a shift in the length of the daily photosynthetic period that would be absent when a single leaf is shifted. However, because an acclimation in the rate of various metabolic processes following a shift in length of the daily photosynthetic period can be very rapid (9), we conclude that the responses observed in this experiment are not a function of the large differences in leaf and plant size that were present following long term growth in the contrasting environments. The endogenous control of photosynthesis and of starch synthesis may depend upon various plant hormones as suggested by Carmi and Koller (2). In any case, the programming of starch accumulation rate by length of the daily photosynthetic period is apparently a whole plant, not an individual leaf phenomenon, and is independent of PPFD, at least within the ranges used in this experiment.

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