Photosynthesize Partitioning into Starch in Soybean Leaves

I. EFFECTS OF PHOTOPERIOD VERSUS PHOTOSYNTHETIC PERIOD DURATION

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ABSTRACT

Photosynthesis, photosynthesize partitioning into foliar starch, and translocation were investigated in soybean plants (Glycine max (L.) Merr. cv. Amsoy 71), grown under different photoperiods and photosynthetic periods to determine the controls of leaf starch accumulation. Starch accumulation rates in soybean leaves were inversely related to the length of the daily photosynthetic period under which the plants were grown. Photosynthetic period and not photoperiod per se appears to be the important factor. Plants grown in a 14-hour photosynthetic period partitioned approximately 60% of the daily foliar accumulation into starch whereas 7-hour plants partitioned about 90% of their daily foliar accumulation into starch. The difference in starch accumulation resulted from a change in photosynthesize partitioning between starch and leaf residual dry weight. Residual dry weight is defined as leaf dry weight minus the weight of total nonstructural carbohydrates. Differences in photosynthesize partitioning into starch were also associated with changes in photosynthetic and translocation rates, as well as with leaf and whole plant morphology. It is concluded that leaf starch accumulation is a programmed process and not simply the result of a limitation in translocation.

Plant growth depends upon the net fixation and transport of carbon from the chloroplast to inter- and intracellular sites of photosynthesize demand. However, net photosynthesize efflux from the chloroplast during photosynthesis may be 30 to 50% less than the CO₂ fixation rate due to chloroplast starch formation from newly formed sugar phosphates within the chloroplast (3, 8, 12, 22). The result is a linear increase in foliar starch concentration during illumination that may represent 10 to 30% of the laminar dry weight by the end of each diurnal photosynthetic period (6, 8, 16, 20).

Foliar starch metabolism is the subject of an increasing number of physiological and biochemical studies (3, 5, 11, 16, 20) and reviews (13, 14, 22). However, the relationship between plant growth and this large energy reserve, which is unavailable for meristic growth during the light period, is uncertain. The observations that diurnal declines in foliar CO₂ exchange rates (CER)¹ have been correlated with elevated starch levels (4, 20) support the hypothesis that starch accumulation may be an inefficient process in plant growth.

An understanding of the mechanisms controlling starch synthe-

² Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
Table I. Irradiance Treatments Administered to Soybean Plants During Growth in Controlled Environment Chambers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Photoperiod (h)</th>
<th>Low Irradiance Period (h)</th>
<th>Photoperiod</th>
<th>Dark Period (h)</th>
<th>Cycle Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>0</td>
<td>17</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>D First 20 days</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Next 5 days</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>17</td>
<td>24</td>
</tr>
</tbody>
</table>

treatment D were grown for 20 days as in treatment A then transferred to conditions of treatment C for 5 days.

Carbohydrate Analyses. Carbohydrate content of soybean leaf laminae was determined on the third and fourth trifoliolate leaves (T3 and T4), numbered acropetally, harvested at intervals throughout the day. The two leaves were combined for analysis. Leaves were excised, leaf areas quickly determined (model LI-3000 portable area meter, LI-COR, Inc., Lincoln, Nebr.), and frozen in liquid N2. The samples were lyophilized and finely ground (60 mesh) in a Cyclotac tissue grinder (Tecator/udy, Boulder, Colo.). A 100-mg sample was suspended in 100 ml distilled H2O at room temperature for 30 min. The resultant water-soluble carbohydrate fraction was quantified (mg/100 mg) by reducing sugar analyses following hydrolysis with 0.6 N HCl. Phosphorylated sugars exhibit reducing power and are therefore measured along with hexoses, hydrolyzed sucrose, and water-soluble starch in the carbohydrate analyses. Another 100-mg sample of leaf tissue was treated with dianalyzed takadiastase (17). 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 Instr. Corp., Tarrytown, N.Y.). Per cent starch was calculated as the difference between TNC and water-soluble carbohydrates.

The SLW (mg/dm²) of the experimental leaves was calculated from the lyophilized dry weight divided by leaf area at harvest. Conversion of per cent TNC (mg/100 mg) to mg/dm² was accomplished via the following relationship:

\[ \text{mg TNC/dm}^2 = \frac{\% \text{TNC} \times \text{SLW}}{100} \]

Residual dry weight of leaves, representing protein, cellulose, and other components of the residue following takadiastase treatment, was calculated by subtracting mg TNC/dm² from SLW. Diurnal rates of TNC and residual accumulation were obtained from mathematically determined slopes of regression lines. Relative growth rates (RGR, g/g-day) were calculated as follows:

\[ \text{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \]

where \( W_1 \) and \( W_2 \) are dry weights of plant parts harvested at two different times, \( t_1 \) and \( t_2 \). All reported values for SLW, TNC, and water-soluble carbohydrates are means of six replicate determinations, each from separate plants.

Carbon Assimilation and Translocation. The CER for attached leaf T3 was measured under growth conditions in acrylic plastic chambers using an adaptation of the air-seal technique (25). Four leaf chambers were connected in parallel to a flow-through IR gas analysis system. The CER for T3 leaves was measured during the photosynthetic period on each of four plants, and the mean diurnal CER determined. Leaf T3 was fully expanded as ascertained from periodic measurements of leaf area using comparable plants.

In order to express CER and rates of carbohydrate accumulation in the same units, mg of CO2 fixed was converted to mg of CH2O since the chemical composition of the leaf is approximated by this empirical formula. Thus, CER\( A \) (mg CH2O/dm²-h) equals CER (mg CO2/dm²-h) × 0.68, where 0.68 represents the molar ratio of the two forms of carbon. CER\( w \) (mg CH2O/g-h) equals CER\( A \) × 1/SLW (g/dm²). Translocation rates (mg/dm²-h) were calculated by subtracting foliar accumulation rates from CER\( A \). The foliar accumulation rate is the sum of residual and TNC accumulation rates.

RESULTS

The results presented are representative of those obtained from experiments repeated in time. The third trifoliolate leaf (T3) of soybean plants grown under a 14-h photosynthetic period (treatment A) had higher CER\( A \) (Table II) than plants grown under a 7-h photosynthetic period (treatment B). However, the CER\( w \) of 7-h plants was significantly higher than that of 14-h plants (Table II). Leaf area of the fully expanded leaves of 14-h plants was not significantly greater than that of 7-h plants.

Diurnal starch accumulation rates of leaves T3 and T4 were altered by the length of the photosynthetic period under which the soybean plants were grown (Fig. 1). This difference is apparent when rates are expressed either on a leaf dry weight basis (mg/100 mg-h) or on a leaf area basis (mg/dm²-h). Foliar starch accumulation rates in the 7-h photosynthetic period were much higher than those in the 14-h photosynthetic period in spite of the lower CER\( A \) of 7-h plants (Fig. 1 and Table II). Starch content of the leaves at the end of the 7-h photosynthetic period was about 15% of the laminar dry weight compared to about 10% in the 14-h treatment (Fig. 1). In all treatments starch was depleted during the dark period to about 1 to 3% of laminar dry weight (2-6 mg/dm²) by the beginning of the subsequent photoperiod (Fig. 1). Water-soluble carbohydrates, primarily sucrose and monosaccharides, attained higher concentrations in leaves T3 and T4 of 7-h plants than in corresponding leaves of 14-h plants grown under the same PPFD (Fig. 2).

Plants grown in a 7-h photosynthetic period followed by a 17-h dark period (Table I, treatment C) had the same starch accumulation rates as plants grown in a 7-h photosynthetic period followed by 7 h of low irradiance incandescent light and 10-h dark (treatment B). Foliar starch percentages of treatment C at hours 1 and 6 are indicated by the stars in Figure 1.

The CER\( A \) and translocation rates of leaf T3 differed in plants grown under 14-h and 7-h photosynthetic periods (Table III). Nevertheless, the differences in rates of starch accumulation (Fig. 1) and in soluble carbohydrate levels (Fig. 2) resulted from differences in photosynthetic partitioning between TNC (starch plus soluble carbohydrates) and residual components within the leaf (Table III). Leaves T3 and T4 of 14-h photosynthetic period

Table II. Carbon Assimilation Rates (Leaf T3), Specific Leaf Weights, and Leaf Areas (Leaves T3 and T4) as a Function of Length of Photosynthetic Period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>14 h</th>
<th>7 h (7 h low)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td>Treatment B</td>
<td></td>
</tr>
<tr>
<td>CH2O synthesis</td>
<td>CER( A ) (mg/dm²-h)</td>
<td>19.89a</td>
</tr>
<tr>
<td>CER( w ) (mg/g-h)</td>
<td>59.20b</td>
<td>81.95a</td>
</tr>
<tr>
<td>Leaf area (dm²)</td>
<td>2.80a</td>
<td>2.41a</td>
</tr>
<tr>
<td>Specific leaf weight (mg/dm²)</td>
<td>336a</td>
<td>200b</td>
</tr>
</tbody>
</table>

1 Means within horizontal rows followed by the same letter are not significantly different at \( P \leq 5\% \) (F-test).
plants partitioned approximately 60% of the total foliar accumulation into TNC and the remainder into residual components. However, corresponding leaves of 7-h photosynthetic period plants partitioned about 90% of the total foliar accumulation into TNC (Table III) in spite of their lower CER.<br><br>Lowering the irradiance under which plants were grown from 64 to 32 nE/s/cm² did not obscure the effects of photosynthetic period duration on photosynthate partitioning and translocation (unpublished data). This was true in spite of an approximately 30% reduction in CER.<br><br>To determine the time course of plant adaptation to a shortened photosynthetic period, soybean plants were grown under a 14-h photosynthetic period until 21 days after planting and then transferred to a 7-h photosynthetic period (Table I, treatment D). Leaves T₂ and T₃ (fully expanded) were harvested only at hours 1 and 6 following the beginning of the photosynthetic period; therefore, the calculation of starch and residual accumulation rates by regression analysis was not possible. However, starch percentages in leaves at the 6th h on 4 consecutive days after the transfer clearly indicate that metabolic adjustments were triggered by the change in photosynthetic period (Fig. 1, O). On the 4th day, the starch accumulation rate was the same as that of leaves that had developed under a 7-h photosynthetic period. Lower rates of photosynthate partitioning into residual components accompanied the increase in foliar starch accumulation in plants transferred to the 7-h photosynthetic period.<br><br>DISCUSSION<br><br>CO₂ exchange rates and photosynthate partitioning into starch, residual dry weight, and translocation were altered when plants were grown under a short compared to a long photosynthetic period even though photoperiod was held constant [7]. Our results permit certain deductions regarding the relationship of CER to leaf morphology and carbohydrate accumulation and the relationships among starch synthesis, translocation, and plant growth strategies.<br><br>CER and Leaf Morphology. Leaves of plants grown under a 7-h photosynthetic period were thinner, but photosynthetically more efficient per unit of dry weight than those of plants grown in the 14-h photosynthetic period. Reduced SLW of 7-h leaves resulted from a sharp reduction in photosynthate allocation to residual components of leaves suggesting that, under a short photosynthetic period, the soybean plant curtails photosynthate allocations to leaf components that do not contribute to CER.<br><br>Previous ontogenetic studies of soybean leaves revealed an inverse relationship between SLW and CERw during leaf develop-
**Table IV. Comparison of Dry Weight Accumulation and Partitioning Patterns of Control Plants (14-h Photosynthetic Period) with Plants Shifted to a 7-h Photosynthetic Period**

Beginning at 21 days after planting, half of the plants were given a 7-h daily photosynthetic period, the others remained under a 14-h photosynthetic period. Harvests were made 1 h after the beginning of the photosynthetic period.

<table>
<thead>
<tr>
<th>Dry Weight Distribution</th>
<th>Photosynthetic Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 h (control)</td>
</tr>
<tr>
<td><strong>Day 22</strong></td>
<td></td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>2.21a</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>0.69a</td>
</tr>
<tr>
<td>Shoot/root ratio</td>
<td>3.20a</td>
</tr>
<tr>
<td><strong>Day 25</strong></td>
<td></td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>3.54a</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>1.26a</td>
</tr>
<tr>
<td>Shoot/root ratio</td>
<td>2.81b</td>
</tr>
<tr>
<td>RGR(^2) (g/g-day):</td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>0.16</td>
</tr>
<tr>
<td>Root</td>
<td>0.20</td>
</tr>
</tbody>
</table>

1 Means within horizontal rows followed by the same letter are not significantly different at P ≤ 5\% (F-test). Those followed by different letters are significantly different at P ≤ 1\%.

2 Rate of change in dry weight from day 22 to day 25.

**opment (16). The SLW continued to increase beyond \( A_{\text{max}} \) but the additional dry matter input, which included additional Chl and soluble protein, was not accompanied by increases in photosynthetic output.**

**CER and Foliar Carbohydrate Accumulation.** Diurnal declines in CER have been correlated with plastid starch accumulation in soybean (20) and other species (3–5, 14). This correlation has been interpreted as evidence for the existence of a feedback inhibition of photosynthesis. Although diurnal mean CER\(_A\) was less, CER\(_W\) was greater in leaves of plants grown under the 7-h photosynthetic period in spite of their higher starch percentages than in leaves of 14-h plants. Therefore, our CER\(_W\) data do not support the hypothesis that starch accumulation causes a feedback inhibition of CER.

**Control of Starch Synthesis.** Carbohydrate accumulation in leaves has been ascribed to a large photosynthate supply and limited photosynthetic demand (3, 14, 18, 24). For example, inhibition of corn leaf expansion by low temperatures resulted in carbohydrate accumulations in both source and sink leaves because leaf expansion was inhibited to a greater extent than CER (1). Conversely, increased photosynthetic demand favors induction of tillering in pansy leaves (Digitaria decumbens Stent.) associated with reduced chloroplast starch accumulation (5).

Although it is apparent from the above reports that starch accumulation is influenced by photosynthetic demand, little is known about the mechanism that controls the proportion of photosynthate retained within the chloroplast under steady-state growth conditions. Challa (3) recently reported higher rates of starch accumulation in cucumber (Cucumis sativus L.) leaves when plants received an 8-h versus a 14-h photosynthetic period. However, he (3) did not attempt to separate the effects of different irradiance levels from those of different photosynthetic periods.

Our results provide some insights into the mechanisms for control of foliar starch synthesis in the light. First, we have shown that the rate of starch accumulation in fully expanded soybean leaves is a function of the duration of the daily photosynthetic period but is unaffected by a classical photoperiod treatment. Although the phenomenon is not a classical photoperiod response, it may be a high irradiance reaction (9, 21). However, the length of the daily period when net photosynthesis does not occur may also affect the starch accumulation rate.

Second, rates of foliar TNC accumulation, representing primarily starch accumulation, were not necessarily proportional to CER\(_A\). If starch accumulation simply results from the retention within the chloroplast of a relatively constant proportion of the total carbon fixed, then shortening the photosynthetic period and reducing CER\(_A\) should decrease starch accumulation rates. However, our results indicate that while CER\(_A\) was reduced by shortening the photosynthetic period, the TNC accumulation rates increased 65\% (Fig. 1 and Table III). This suggests that starch accumulation is controlled independently of \( CO_2 \) fixation rate per se. Furthermore, starch accumulation is not simply a result of translocation potential being insufficient to keep pace with CER.

Third, rates of photosynthetic translocation from fully expanded soybean leaves increased with an increase in the length of the photosynthetic period. Our results do not rule out the possibility that translocation from the leaves of 7-h photosynthetic period plants is rate-limiting, and therefore at least partly responsible for starch accumulation. Greater starch accumulation under a 7-h photosynthetic period was associated with decreased synthesis of residual components but did not affect total foliar accumulation when compared with plants grown in a longer photosynthetic period (Table III). Therefore, a major difference in photosynthetic partitioning existed in the amount of photosynthesize allocated to starch versus residual components in soybean leaves grown under 7- and 14-h photosynthetic periods in controlled environments.

**Influences of Photosynthetic Period on Leaf and Whole-Plant Development.** Additional data are necessary for further characterization of the mechanisms of photosynthetic partitioning within the fully expanded soybean leaf; however, the adaptive significance of the phenomenon is evident. When an environmental factor such as light or water becomes limiting, detrimental effects of the less favorable condition may be minimized by alterations in photosynthetic partitioning. For example, soybean root growth is favored over shoot growth when vegetative plants are exposed to low soil moisture (15). The resultant growth favors acquisition of water during unfavorable soil conditions and reduces evaporative losses. In contrast, a shortened photosynthetic period favored shoot growth over root growth (Table IV). The soybean plants responded to a short photosynthetic period by diverting relatively more photosynthesize into the light-capturing shoot. Within the shoot itself more efficient energy utilization (increased CER\(_W\)) resulted from decreased photosynthesize partitioning into residual components of leaves. An increased proportion of the carbon fixed by plants grown in a short photosynthetic period was retained within the chloroplast during the day as starch, and translocated out of the leaf during darkness. The fact that shoot growth was favored over root growth under the shortened photosynthetic period suggests that photosynthesize were translocated preferentially to growth centers of the shoot.

Various other plant responses have been associated with the length of the photosynthetic period. Garner et al. (10) reported an increase in foliar soluble sugars of short day Cosmos bipinnatus and long day radish under short day and long day conditions, respectively. Similarly, Tsybul’ko (19) noted a foliar accumulation of assimilates in short day Petilla and long day Brassica under inductive photoperiods. He concluded that long day plants translocated most of their assimilates during the day, whereas short day plants translocated more during the night (19). In a review article, Wardlaw (23) concluded that the dominant effect of daylength is the transformation from vegetative to floral development and that the greater translocation of assimilates in long day plants under long days and short day plants under short days results from these developmental changes. Bodson et al. (2) concluded that although photosynthesize partitioning and translocation influenced floral induction in the long day plant Sinapis alba, additional determining factors may operate. In the present experiments, floral induc-
tion occurred under all treatments regardless of photoperiod or photosynthetic period; however, photosynthetic partitioning responses differed. We conclude that the change in partitioning with a change in photosynthetic period is independent of the vegetative to floral transformation.

Our results demonstrate that daily partitioning of photosynthates in soybean leaves among starch synthesis, residual dry weight accumulation, and translocation to sites outside the leaf are modified by the duration of the daily photosynthetic period. However, partitioning remained unchanged when photoperiod was varied with photosynthetic period held constant. Leaves receiving a short daily photosynthetic period accumulated much more foliar starch and conversely less residual dry weight during photosynthesis than leaves receiving a long photosynthetic period. Results of the present study suggest that: (a) a potentially inefficient partitioning of carbon occurs in a fully expanded soybean leaf grown in a 14-h photosynthetic period; (b) length of the daily photosynthetic period influences the partitioning of carbon within soybean leaves. Indeed starch synthesis seems to be a programmed process and possibly regulated by the same photomorphogenetic controls that determine leaf thickness and whole plant morphology. Any model designed to account for the photosynthetic period-dependent shift in photosynthetic partitioning should consider: starch synthesis within the chloroplast; photosynthate efflux from the chloroplast into the cytoplasm; synthesis of cellulose, protein, and other residual components of leaf cells; and the synthesis and extracellular translocation of sucrose.

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LITERATURE CITED

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