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Carbon Assimilation and Translocation in Soybean Leaves at Different Stages of Development

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JOHN E. SILVIUS, DIANE F. KREMER, AND DAVID R. LEE
Light and Plant Growth Laboratory, Plant Physiology Institute, SEA, Federal Research, United States Department of Agriculture, Beltsville, Maryland 20705

ABSTRACT

Carbon assimilation, translocation, and associated biochemical characteristics of the second trifoliolate leaf (numbered acropetally) of chamber-grown soybean, *Glycine max* (L.) Merr., plants were studied at selected stages of leaf development during the period from 10 to 25 days post-emergence. Leaves of uniform age were selected on the basis of leaf plastochron index (LPI).

The test leaf reached full expansion (A_{max}) and maximum CO_2 exchange rates on a leaf area basis at 17 days postemergence (LPI 4.1). Maximum carbon exchange rates per unit dry weight of lamina were attained several days earlier and declined as specific leaf weight increased. Chlorophyll and soluble protein continued to increase beyond the attainment of A_{max}, but were not accompanied by further increases in photosynthetic rates.

Much of the fixed carbon in leaves is partitioned between starch and sucrose. Starch content of leaves as a percentage of dry weight at the end of an 11-hour photoperiod was taken as an indication of the potential energy reserve accumulated by the leaf. Starch levels were the same regardless of leaf age during the period from 0.3 A_{max} to 7 days after attaining A_{max}. Respiratory and synthetic activity of leaves decreased considerably during the same period, suggesting that starch accumulation is not entirely controlled by the energy demands of the leaf.

Sucrose content increased steadily during leaf expansion and was accompanied by corresponding increases in sucrose phosphate synthetase (EC 2.4.1.14) activity and translocation rates. Sucrose phosphate synthetase may have an important regulatory role in photosynthate partitioning and translocation.

The growth of green plants depends not only upon photosynthesis, but also upon the translocation of photosynthates from sites of carbon fixation in differentiated photosynthetic tissue to sites of storage or utilization where growth and differentiation occur. Photosynthesis and translocation rates in expanding leaves reach maxima as leaf expansion ceases, and decline shortly thereafter (8, 13). The efficiency of the leaf as an assimilatory organ depends upon a large array of biochemical and physiological processes which exist in a dynamic relationship with leaf ontogeny.

The present study is part of an effort to identify physiological and biochemical processes which restrict the photosynthetic efficiency of leaves. Our approach was to determine the rates of carbon assimilation and translocation at precisely identified stages of leaf development. At each stage of development, specific biochemical components of the assimilatory process were characterized. A comparison of the changes in these components in relation to the changes in the over-all assimilatory activity during leaf development provides a basis for identifying those components which may restrict the efficiency of carbon assimilation in soybean leaves.

MATERIALS AND METHODS

Plant Material. Soybean, *Glycine max* (L.) Merr. cv. Amsoy 71, plants were grown from uninoculated seed in 15-cm plastic pots containing Vermiculite. Four days after emergence, seedlings were thinned to one plant/pot. Incandescent and cool-white fluorescent lamps supplied a photosynthetic photon flux density (400–700 nm) of 500 μE $m^{-2}sec^{-1}$ at pot height during a 14-hr photoperiod. Temperature and RH were maintained at constant levels of 27 ± 1 C and 60 ± 2%, respectively. Horizontal temperature variation within the chamber was less than 2 C. The plants received an excess of a nutrient solution developed by F. W. Snyder of this laboratory; it contains the following salts (mM concentration): $Ca(NO_3)_2$, 4; KCl, 2.5; KH_2PO_4, 1; KNO_3, 3; K_2SO_4, 1; $MgSO_4$, 3.5; $NH_4H_2PO_4$, 2.5; and the following micronutrients (μM concentration): H_3BO_3, 13.7; $CuSO_4$, 0.16; $MnSO_4$, 4.5; $(NH_4)_6Mo_7O_{24}$, 0.07; $ZnSO_4$, 0.34; metallic Fe, 107.4, as Sequestrene 330 Fe powder formulation (CIBA-GEIGY Corp., Greensboro, N.C.).[1]

All observations were made on the second trifoliolate leaf (T_2) numbered acropetally at six stages of development from 11 to 25 days postemergence. In order to reduce ontogenetic variability, plants were selected for each stage on the basis of plastochron index (PI)[2] (7). The PI system was devised by Erickson and Michelini (7) to provide a precise numerical indication of the stage of development of a vegetative shoot and individual leaves thereon. Briefly, the PI of soybean was calculated as follows: the parameter chosen to indicate leaf size was the midvein length of the terminal leaflet. A reference length of 20 mm was arbitrarily selected. Then, the node number (n) having the youngest leaf with a reference length exceeding 20 mm was determined by counting acropetally from the cotyledonary node. The midvein length of this leaf (L_n), and that of the leaf at the adjacent node above (L_{n+1}) were measured and the PI of the plant calculated from the following relationship:

$$PI = n + \frac{\log L_n - 1}{\log L_n - \log L_{n+1}}$$

The leaf plastochron index (LPI) of a leaf at any given node, n, equals PI minus n.

Carbon Assimilation and Translocation. CO_2 exchange rate (CER) for the attached T_2 leaves was measured under growth

[1] 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.

[2] Abbreviations: F6P: fructose 6-phosphate; UDPG: uridine diphosphate glucose; ADPG: adenine diphosphate glucose; SPS: sucrose phosphate synthetase; SPP: sucrose phosphate phosphatase; CER_A: carbon dioxide exchange rate—leaf area basis; CER_W: carbon dioxide exchange rate—leaf dry weight basis; LPI: leaf plastochron index; PGA: 3-phosphoglyceric acid; PI: plastochron index; SLW: specific leaf weight.

conditions in acrylic plastic chambers representing an adaptation of the air-seal technique (28). Four leaf chambers were connected in parallel fashion into a flow-through IR gas analysis system. The CER for each of the four T_2 leaves was measured hourly for 10 hr following 1 hr of acclimation at the beginning of the light period. In order to express CER and translocation rates in the same units, mg of CO_2 was converted to mg of CH_2O since a large proportion of leaf organic matter is represented by this empirical formula; thus, $CER_A = CER \times 0.68$, where 0.68 represents the molar ratio of the two forms of carbon. The CER is expressed on a leaf area basis as mg of CH_2O $dm^{-2}hr^{-1}$ (CER_A) and on a leaf dry wt basis as mg of CH_2O $g^{-1}hr^{-1}$ (CER_W); $CER_W = CER_A \times 1/SLW$ (mg dm^{-2}). During the last two stages of development, neighboring leaves were reoriented to maintain leaf T_2 at the same photosynthetic photon flux density as in previous stages of development. Dark respiration rates were determined from CO_2 exchange rates during the 30 min of darkness before the start of the photoperiod.

Three leaf discs, each having an area of 0.52 cm^2, were removed from T_2 of each of seven plants at the beginning and at the end of the 10-hr period. The discs were placed in small envelopes, immediately frozen in liquid N_2, and lyophilized. Dry wt accumulation (mg $dm^{-2}hr^{-1}$) was determined from the dried discs. Mass carbon translocation rates were estimated from the difference between total carbon fixed and the dry wt accumulation according to the method of Terry and Mortimer (22). Validity of their method was checked by comparing resultant translocation rates with those determined from export of ^{14}C from $^{14}CO_2$-fed leaves at comparable stages of leaf development according to the method of Thorne and Koller (23).

Following the 10-hr test period, leaf areas of T_2 leaves were determined (LI-COR model LI-3000 portable area meter, Lambda Inst., Inc., Lincoln, Neb.), the leaf blades excised, frozen in liquid N_2, lyophilized, and stored at −20 C for chemical analyses and enzyme assays.

Chemical Analyses. Finely ground 200-mg samples of lyophilized leaves were extracted twice in boiling 80% ethanol, the extracts combined, and the ethanol removed by evaporation. Sucrose concentration of the aqueous extracts was determined according to the method of van Handel (27). Starch concentration was estimated by titration of reducing sugars following enzymic digestion of the remaining solid residue (21). Sucrose and starch content of the leaves were expressed as a percentage of dry wt. Chl content was determined according to the method of Schmid (18).

Extraction and Assay of Enzymes. Samples of lyophilized leaf blades weighing 100 mg were ground in a mortar with sand and 5 ml of extraction medium containing 0.1 M HEPES buffer (pH 7) and 20 mM mercaptoethanol. The extract was centrifuged at 35,000*g* for 15 min and the supernatant passed through a column (1 × 15 cm) of Sephadex G-25 (coarse) equilibrated with 5 mM HEPES buffer (pH 7) containing 1 mM mercaptoethanol. Aliquots (0.1 ml) were removed from the supernatant and from the Sephadex eluant for protein analysis (14). All steps were performed at 3 C.

The sucrose phosphate synthetase (EC 2.4.1.14) (SPS) assay was initiated by adding 0.1 ml of enzyme preparation, containing about 100 μg of protein and 0.1% BSA, to the reaction medium equilibrated in a 30 C water bath. The 0.1-ml reaction medium (pH 7) contained the following (in μmol): $MgCl_2$, 2; UDPG, 6; F6P, 6; and HEPES, 95. The reaction was stopped after 10 min by addition of 40 μl of 30% trichloroacetic acid. The resultant sucrose-P and sucrose were determined colorimetrically according to the method of van Handel (27). Reaction tubes acidified immediately upon addition of the enzyme were used as blanks. Conditions suitable for maximum catalytic activity were identified from SPS activity under various pH levels and compositions of extraction and reaction media. Substrate requirements for SPS were monitored by omission of UDPG or F6P, and by substitution of ADPG for UDPG, and D-fructose for F6P. Release of Pi from sucrose-P by sucrose phosphate phosphatase (EC 3.1.3.00) (SPP) was measured colorimetrically (2). Reported rates are means of three or more extractions of leaves from separate plants. The SPS activity was not significantly reduced by lyophilizing leaves before extraction.

RESULTS

The LPI provided an accurate means of identifying the morphological stage of development of soybean plants since a repeatable, linear relationship between LPI and time was obtained (Fig. 1). The six stages of development at which the second trifoliolate leaf (T_2) was studied are identified by plant age and LPI (Fig. 1). Data collected at each point are means representing 11 plants with the same LPI ± 3%.

At the earliest developmental stage selected for study (LPI 1.8), leaf T_2 had already reached 30% of its maximum area (A_{max}) (Table I). Leaves attained A_{max} by LPI 4.1. Specific leaf wt (SLW) did not increase during the early, rapid leaf expansion phase but then increased between LPI 2.6 and 6.6. Soluble protein/unit leaf area increased 2-fold, and total Chl 6-fold during the experimental period.

CO_2 exchange rates, expressed on a per unit area basis (CER_A), reached the maximum at LPI 3.6 (Fig. 2), as the leaf attained A_{max} (Table I). Maximum CER_W occurred 2 days earlier, at LPI 2.6 (Fig. 2). The decline in CER_W which followed the maximum rates was associated with an increase in SLW (Table I).

Significant differences ($P < .01$) among mean hourly CER_A were not observed for any of the five developmental stages during the first 11 hr of the 14-hr photoperiod. Hourly rates were not determined during the remainder of the photoperiod. Dark respiration rates decreased from 4.1 to 1.8 mg of CO_2 $dm^{-2}hr^{-1}$ as LPI progressed from 1.8 to 5.5.

Starch percentage in leaves excised after 11 hr in light were taken as an estimate of the starch accumulation for the current photoperiod, based on other reported studies (4, 26). The contribution of starch to the leaf dry wt did not change significantly (*P*

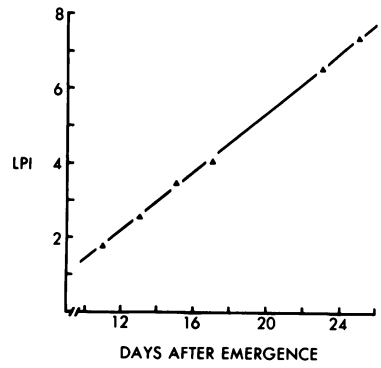

FIG. 1. LPI of second trifoliolate leaves (T_2) (numbered acropetally) of soybean plants as a function of time after seedling emergence.

Table I. Characteristics of the second trifoliolate leaf of soybean plants at different stages of development.

L P I	Leaf Area	Specific Leaf Weight	Chlorophyll (a + b)	Soluble Protein
	dm^2	mg dm^{-2}		
1.8	0.36 c[1]	307.9 cd	0.97 d	27.83 e
2.6	0.70 b	284.9 d	1.90 c	30.48 de
3.6	0.92 a	330.6 c	2.64 c	39.49 cd
4.1	1.03 a	363.9 b	3.97 b	42.54 bc
6.6	1.07 a	430.2 a	5.32 a	51.80 ab
7.4	1.10 a	461.0 a	5.89 a	57.95 a

[1]Values are means of at least three measurements from separate plants, and differ significantly at the 1% level if accompanied by a different letter.

< .01) until LPI 7.4 at which time a decrease was observed (Fig. 3). Sucrose constituted an increasing percentage of leaf dry wt as leaf age increased (Fig. 3).

Sucrose (Fig. 3), translocation rates (Fig. 4), and SPS activity (Fig. 4) were all low in leaves in LPI 1.8, suggesting that the leaves possessed only minimal assimilate export activity. Maximum *in vitro* SPS activity, approximately 4 μmol of sucrose synthesized mg of protein^{-1}hr^{-1}, occurred in leaves at LPI 4.1 (Fig. 4). This activity was totally dependent upon UDPG and F6P. Minor synthetic activity (<0.3 μmol of sucrose mg of protein^{-1}hr^{-1}) was measured when D-fructose was substituted for F6P, and this was attributed to sucrose synthetase (EC 2.4.1.13) (25). No measurable sucrose synthesis was observed when ADPG was substituted for UDPG.

Throughout the expansion of leaf T_2, SPS activity and translocation rates closely paralleled one another (Fig. 4). Both SPS activity and translocation rates, as well as leaf area (Table I) and CER_A (Fig. 2) peaked at LPI 4.1. The UDPG-dependent release of Pi from F6P during sucrose synthesis (4–7 μmol of Pi mg of protein^{-1}hr^{-1}) exceeded SPS rates (≃4 μmol of sucrose mg of protein^{-1}hr^{-1}), suggesting that sucrose-P conversion to sucrose was not limiting.

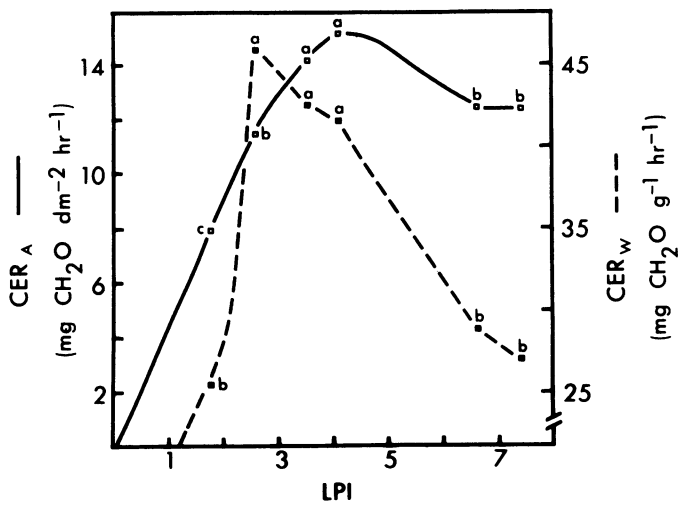

FIG. 2. CO_2 exchange rates in developing soybean leaves (T_2) expressed on a leaf area basis (CER_A) and on a leaf wt basis (CER_W); CER_A = CER × 0.68; CER_W = CER_A × 1/SLW (g dm^{-2}). Means accompanied by a different letter differ significantly at the 1% level.

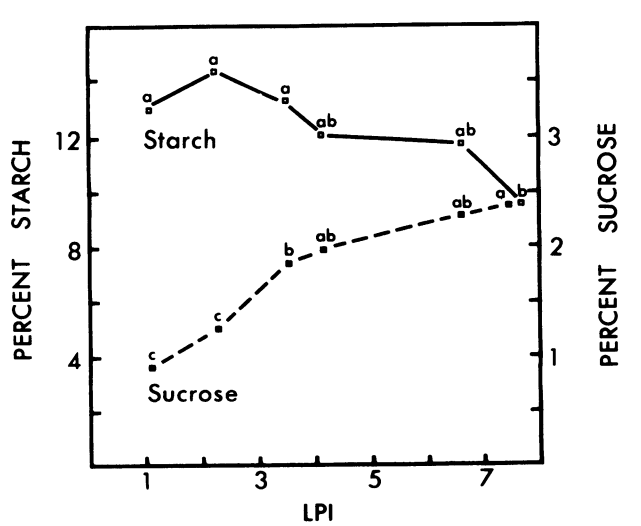

FIG. 3. Starch and sucrose contents of developing soybean leaves (T_2), measured after an 11-hr photoperiod. Means accompanied by a different letter differ significantly at the 1% level.

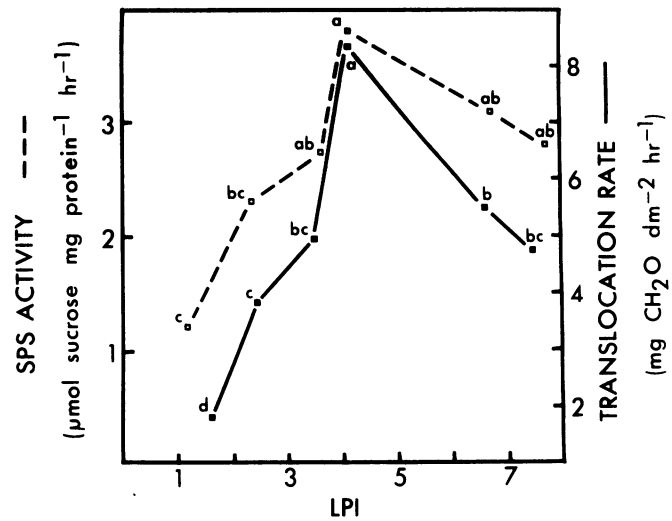

FIG. 4. Sucrose phosphate synthetase (SPS) activity and translocation rate of developing soybean leaves (T_2). The 0.1-ml SPS assay medium (pH 7) contained the following (in μmol): $MgCl_2$, 2; UDPG, 6; F6P, 6; HEPES, 95. Following a 10-min incubation at 30 C, the resultant sucrose was determined colorimetrically (27). Means accompanied by a different letter differ significantly at the 1% level.

Although major attention was given to leaf T_2, other developmental events occurring throughout the plant were noted. An axillary branch was initiated at the T_2 node beginning at LPI 3.6. Plants were flowering at the T_3 node at LPI 7.4.

DISCUSSION

The results show that a number of constituents and processes change during the development of soybean leaf T_2. From these data, inferences can be made regarding the significance of CO_2 exchange rates, the ontogenetic development of photosynthetic capacity, and the metabolic control of photosynthate partitioning and translocation in the test leaf.

Significance of CER. The total organic matter produced by a given leaf depends upon its CER integrated over the duration of its functional existence. The CER of leaves and whole plants may be based upon one or more of several photosynthetic components including leaf area, leaf dry wt, Chl content, and soluble protein content. CER_A provides an accurate, nondestructive measure of photosynthesis by a fixed leaf area exposed to a given quantity of light energy; differences in SLW are not considered. CER_W estimates photosynthesis/unit wt of photosynthetic, structural, and storage components; the area of light-absorbing surface is not considered. In addition, the evaluation of relative leaf photosynthetic capacity at different developmental stages may differ greatly depending upon which component is considered in the expression of CER (Fig. 2). Thus, in order to characterize the carbon economy of a single leaf in relation to whole plant growth, no one photosynthetic component, or point in time is sufficient.

Besides ontogenetic factors, both genotypic and environmental effects on CER are more clearly identified when both CER_A and CER_W are known. Ogren (17) has shown that the differential in CER_A among soybean cultivars is greatly reduced when expressed on a leaf wt basis (CER_W). He suggests that leaves having higher CER_A may contain more photosynthetic enzymes/unit leaf area than leaves having lower CER_A. Environmental conditions such as light regime (4), temperature (12), CO_2 concentration (12), and time of day (26) may differentially influence CER_A and CER_W, and these effects should be distinguished to accurately evaluate the plant response.

Ontogenetic Development of Photosynthetic Capacity. Chl and protein levels continue to increase in leaves beyond the ontogenetic stages in which maximum CER_W and CER_A were measured

(Table I and Fig. 2). The additional input of energy and resources to synthesize more of these two photosynthetic components, however, did not increase the assimilatory capacity of the leaves. The continued increase in leaf protein may reflect the storage of protein reserves which can be mobilized later, during pod filling and leaf senescence (20). However, the prolonged synthesis of Chl and protein in maturing leaves, which can no longer compete successfully with newly developed leaves for light and nutrients (10, 13), suggests that the plant may be somewhat inefficient in its partitioning of energy and resources to the various growing points.

Metabolic Control of Photosynthate Partitioning and Translocation. The question of what controls partitioning of photosynthate into starch has been prompted by reports that diurnal declines in CER were associated with the diurnal accumulation of starch in leaves (26). While diurnal declines in CER_A were not observed under the conditions of the present study, a better understanding of starch synthesis and its relationship to photosynthesis would permit a more thorough evaluation of leaf assimilatory efficiency. Numerous studies have demonstrated that diurnal patterns of starch synthesis and mobilization are coupled to energy and organic carbon demand during the night as influenced by temperature regime (4, 5), length of night (4, 15), light intensity (4, 15), CO_2 level (16), and sink demand (5, 15).

In the present study, leaf starch levels after 11 hr in light had attained the same percentage of total dry wt in each stage of development from LPI 1.8 to 6.6 (Fig. 3). However, the respiratory and synthetic activity of the leaf declined considerably during this period, suggesting that starch accumulation in soybean leaf T_2 is not controlled solely by the energy and organic carbon demands of the individual leaf.

The control of starch accumulation by photosynthate demands beyond the test leaf may be mediated through the synthesis and translocation of sucrose. If this hypothesis is correct, then SPS, the allosteric enzyme catalyzing cytoplasmic sucrose synthesis (1), may play a key regulatory role in the partitioning of photosynthate between starch and sucrose pools. Beck (3) has recently proposed that increased SPS and SPP activity may reduce carbon flow into starch synthesis by reducing levels of PGA, and increasing levels of Pi. Starch synthesis by isolated chloroplasts is inhibited under these conditions (11).

Recent studies by Thorne and Koller (23) show that increased sucrose synthesis and translocation rates, and decreased starch synthesis are associated with increased photosynthate demand. They altered photosynthate demand by darkening soybean plants in the pod-filling stage, allowing only one leaf to receive light. The response occurred gradually over a period of several days and must be interpreted in the light of possible changes in hormonal relationships and protein turnover. We observed a 25% increase in *in vitro* SPS activity in source leaves 48 hr after a similar darkening treatment of vegetative soybean plants (unpublished data), suggesting that SPS activity is influenced by assimilate demand.

During leaf expansion, corresponding changes in translocation rates, sucrose levels, and SPS activity suggest that the development and extent of photosynthate export capacity of the test leaf may be coupled to the activity of SPS. Fondy and Geiger (9) compared sucrose flux rates in the minor veins of sugarbeet leaves after supplying either ^{14}C-fructose of ^{14}C-sucrose. The lower flux rate for sucrose derived from fructose compared to sucrose supplied directly as attributed to a limitation in the rate of sucrose synthesis. Recent studies have demonstrated the dependence of translocation rate upon sucrose concentration in leaves (24).

The elevated sucrose content/unit leaf area (per cent sucrose (Fig. 3) adjusted for the increased SLW) following LPI 4.1 may have resulted from the decline in translocation rate in the absence of any apparent decline in SPS activity. Our results are in agreement with those of Shiroya *et al.* (19) who observed decreasing translocation rates and an increased sucrose pool size in older tobacco leaves. Evidence exists for a storage pool of sucrose in leaves that is not directly accessible to translocation (6). The enlargement of such a pool may accompany the maturation of the test leaf. A decreased translocation capacity with increasing leaf age has been associated with an apparent decline in levels of phosphorylated sugars (19) and Pi levels (13). In view of the apparent regulatory role of Pi in starch metabolism, declining levels of Pi following A_{max} (10, 13) may account for the sustained high levels of starch and decreased translocation rates following LPI 4.1.

The present study had addressed three aspects of carbon metabolism in developing soybean leaves which may represent limits to the rate and efficiency of the photosynthetic organ. The progressive decline in CER_W during soybean leaf development indicates that less CO_2 is fixed/unit of organic matter invested as the leaf develops. The prolonged synthesis of protein and Chl beyond the developmental stage representing A_{max} and maximum CER_A is a specific example of potentially inefficient partitioning of resources during leaf development. The efficiency of a leaf is also judged by its capacity to export assimilate from the chloroplast to centers of assimilate demand such as the root or shoot apex. The close association among sucrose levels, translocation rates, and SPS activity suggests that sucrose synthesis is a potential limit to the export process and may influence both the rate of photosynthate export and the amount of starch accumulated. All of these processes are coupled to the dynamics of leaf and whole-plant ontogeny. Therefore, photosynthetic efficiency may be limited by different metabolic processes at different developmental stages. Efforts to identify processes which limit plant growth and productivity should consider the metabolism of expanding as well as mature leaves.

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