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Studies on Genetic Male-Sterile Soybeans[1]

II. EFFECT OF NODULATION ON PHOTOSYNTHESIS AND CARBON PARTITIONING IN LEAVES

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ABSTRACT

Soybean (*Glycine max* L. Merr.) germplasm, essentially isogenic except for loci controlling male sterility (ms_1) and nodulation (rj_1), were developed to study the effects of reproductive development and nitrogen source on certain aspects of photosynthesis. Plants were sampled from flowering (77 days after transplanting) until maturity (150 days after transplanting). With all four genotypes, net carbon exchange rates were highest at flowering and declined thereafter. Photosynthetic rates of the sterile genotypes (nodulated and non-nodulated) declined more rapidly than the fertile genotypes, and after 105 days, both sterile genotypes maintained low but relatively constant carbon exchange rates (<3 milligrams CO_2/gram fresh weight per hour). Photosynthetic rates and starch accumulation (difference between afternoon and morning levels) declined with time. The sterile genotypes attained the highest morning starch levels, which reflected reduced starch mobilization. After 92 days, the proportion of photosynthetically fixed carbon that was partitioning into starch (relative leaf starch accumulation) in the sterile genotypes increased dramatically. In contrast, relative leaf starch accumulation in the fertile genotypes remained relatively constant with time. Throughout the test period, all four genotypes maintained leaf sucrose levels between 5 and 15 micromoles glucose equivalents per gram fresh weight.

The activities of sucrose phosphate synthase (SPS) in leaf extracts of the four genotypes declined from 77 to 147 days. Nodulated genotypes tended to maintain higher activities (leaf fresh weight basis) than did the non-nodulated genotypes. In general, relative leaf starch accumulation was correlated negatively with the activity of SPS (normalized with leaf net carbon exchange rate) in leaf extracts for all four genotypes during early reproductive development, and for the fertile genotypes at all sampling dates. In contrast, leaf sucrose content was correlated positively with SPS activity during early reproductive development. These results suggested that a direct relation existed between the activity of SPS and starch/sucrose levels in soybean leaves. However, the interaction between these processes also may be influenced by other factors, particularly when leaf photosynthetic rates and plant demand for assimilates is low, as in the sterile genotypes.

Several studies (2, 3, 14) describe the changes in photosynthesis and carbohydrate metabolism in soybean leaves during reproductive development. After flowering, leaf soluble sugars remain relatively constant although leaf photosynthetic rates decline. In contrast, leaf starch content (typically measured about mid-day) increases until mid-podfill, and thereafter decreases. With the removal of reproductive tissues, however, the demand for assimilates is apparently reduced as the levels of both leaf starch and soluble sugars increase (4, 16) and photosynthetic rates are depressed more severely than in control plants. The inhibition of photosynthesis by pod removal may be attributed partially to the accumulation of leaf ABA (17) which may cause stomatal closure (16). It is also possible that lower photosynthetic rates result from feedback inhibition by end products. Thus, the response of photosynthetic rate to altered reproductive tissue development should be evaluated with respect to photosynthetic starch formation and starch mobilization in the dark. In that regard, estimates of starch content at mid-day are inadequate and only reflect the balance between the two processes.

Because starch and sucrose are the principal end products of photosynthesis (6), factors which control the partitioning of carbon between the two products, and changes during reproductive development, are of fundamental importance. One factor which may influence carbon partitioning in leaves is the activity of SPS[2] (9). It has been postulated that SPS is one of the rate-determining steps in the sucrose formation pathway (7, 9, 10, 18). In addition, the rate of sucrose formation may indirectly regulate starch formation in the chloroplast by affecting metabolite transport across the chloroplast envelope (4, 8, 20).

In previous studies (10), carbon partitioning in four soybean cultivars was determined in relation to stage of development and plant N source (nodulated *versus* nitrate-dependent). Intraspecific variation was observed in leaf starch content, activities of SPS in leaf extracts, and the response to nodulation. In general, genotypic differences increased as plants progressed from vegetative to reproductive development, and with three of the four cultivars tested, nodulated plants exhibited increased sucrose formation as compared to nitrate-dependent plants.

In an attempt to elaborate upon the effects of nodulation and reproductive tissue development on carbon partitioning in leaves, four isogenic soybean lines were developed that differ only at loci governing male fertility and nodulating ability. Genetic control of fertility to alter reproductive development eliminates the need to remove pods manually and should avoid undesirable or unnatural physiological effects imposed by the time or method of pod removal. Male-sterile soybean genotypes have been studied previously, in terms of lipid metabolism (21) and carbon and nitrogen distribution (1, 22). Photosynthesis, however, has not been previously measured in that germplasm. Hence, specific objectives of the present study were to determine the effects of plant nodulation and reproductive load on (a) NCER, (b) leaf starch and sucrose metabolism, and (c) activities of sucrose-

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[2] Abbreviations: SPS, sucrose phosphate synthase; DAT, days after transplanting; Glc, glucose; NCER, net carbon exchange rate (mg CO_2/$dm^2 \cdot h^{-1}$).

metabolizing enzymes in leaves from flowering to maturity. In addition, a distinction was made between starch formation and mobilization, and for the first time, changes in the activities of certain sucrose-metabolizing enzymes during reproductive development have been documented.

MATERIALS AND METHODS

Plant Material. Soybeans (*Glycine max* L. Merr. cvs N69-2774 and N59-5259) were mated in 1978. The N69-2774 parent was homozygous recessive at the ms_1 locus governing male sterility and homozygous dominant at the Rj_1 locus for nodulation. The N59-5259 parent was homozygous dominant for the Ms_1 locus and homozygous recessive for the rj_1 locus. Hence, the genotype of the maternal parent was $ms_1ms_1Rj_1Rj_1$ (male-sterile, nodulating) and the paternal parent was $Ms_1Ms_1rj_1rj_1$ (male-fertile, non-nodulating). The F_2 progeny of that mating segregated in a ratio of: 9 (male-fertile, nodulated), 3 (male-fertile, non-nodulated), 3 (male-sterile, nodulated), 1 (male-sterile, non-nodulated). Seeds from male-fertile, nodulated plants were inbred through self-pollination to the F_5 generation. The seed advanced in each consecutive generation of inbreeding was selected only from male-fertile, nodulated ($Ms_1\_Rj_1\_$) plants to maintain heterozygosity at the ms_1 and rj_1 loci. Several lines were developed in that manner. In the F_5 generation, each of those lines was grown in progeny rows. At maturity, single fertile plants were harvested from within the respective rows that segregated for sterility and nodulation. Twenty seeds from each of those plants were grown in progeny rows to identify genotypes within each F_5 line which were $Ms_1ms_1Rj_1Rj_1$ or $Ms_1ms_1rj_1rj_1$. Pairs of those genotypes were selected from six F_5 lines, and were considered to be nearly isogenic with respect to all gene loci except the ms_1 and rj_1 loci. One of the pairs was chosen for use in this study. The respective genotypes were designated N80-96-1 ($Ms_1ms_1Rj_1Rj_1$) and N80-96-2 ($Ms_1ms_1rj_1rj_1$). All plants exhibited determinant growth habit.

Plant Culture. Approximately 400 seeds of N80-96-1 and N80-96-2, respectively, were germinated (72 h at 30°C and 95% RH in darkness) and transplanted (2 seedlings/pot) to 30.5-cm diameter plastic pots filled with Perlite.[3] N80-96-1 plants were inoculated with *Rhizobium japonicum* cells (strain USDA 110) as described previously (12). The plants were grown under greenhouse conditions at Raleigh, NC during the period June 10 to November 4, 1981. The seedlings were thinned to one plant per pot at 10 DAT. N80-96-1 plants received N-free nutrient solution plus 20 mM K_2SO_4. N80-96-2 plants received nutrient solution containing 20 mM KNO_3. Each plant was supplied nutrients and deionized H_2O every morning and afternoon throughout the duration of the experiment according to the following schedule: 0 to 10 DAT, 0.25 L nutrient solution twice daily; 11 to 60 DAT, 1 L water (morning), 1 L water plus 0.4 L nutrient solution (afternoon); 61 to 147 DAT, 1 L water plus 0.4 L nutrient solution twice daily. All plants were randomly distributed throughout the greenhouse. At 77 DAT (flowering) pollen analysis of each plant was conducted to identify male-sterile and male-fertile genotypes. At that time, the size of the experiment was reduced to 120 plants and was divided into 30 sub-blocks containing one plant of each genotype. Each sub-block was assigned at random to one of six sampling dates (77, 92, 105, 119, 133, or 147 DAT). Hence, each sample date was replicated five times in a randomized complete block design. All plants were treated with alternate applications of Plictran 50WP (1 g L^{-1}) and Cygon-2E (1 g L^{-1}) on a 2-week cycle to eliminate insect populations.

All photosynthetic measurements reported herein were made at the leaf position that was most recently fully expanded at 77 DAT. Three or four replicate plants were sampled, and the values reported are mean values ± SE.

Starch Analysis. Samples for starch analysis (six discs corresponding to 3.6 cm^2) were taken at 0800 and 1700 h. Preparation of the ethanol-insoluble fraction and analysis of starch by enzymic digestion were as described previously (10).

Enzyme Extraction and Assays. Leaves (about 3 g fresh weight) were harvested after the 1700 h sampling for starch. Samples were weighed, sliced, and frozen at −80°C prior to extraction and assay of SPS, sucrose synthase, and uridine diphosphatase activities (10, 11). Immediately after extraction, an aliquot was heat-denatured and stored at −80°C prior to enzymic analysis of sucrose and hexoses (13).

RESULTS

Photosynthesis and Carbon Partitioning. Net carbon exchange rates were monitored at the same leaf position in the top of the canopy from 77 to 147 DAT (Fig. 1). An overall decline in NCER was observed but the genotypes differed in the rate of change. In general, the fertile genotypes (nodulated and non-nodulated) maintained higher NCERs than did the corresponding sterile genotypes. The NCERs for both sterile genotypes declined to about 3 mg CO_2/g fresh weight h^{-1} by 105 DAT and thereafter remained relatively constant. In contrast, NCER of the fertile genotypes exhibited a continual decline. The fertile nodulated genotype, however, maintained higher activities than did the fertile non-nodulated genotype.

Leaf starch content was measured at early morning and late afternoon (Fig. 2) at the same leaf position used for the NCER measurements. In general, the level of starch in leaves at the morning sampling increased until mid-podfill (119 DAT). At that sampling, the sterile genotypes contained greater starch levels (up to 80 mg Glc/g fresh weight) than the fertile counterparts. However, photosynthetic starch accumulation (the difference

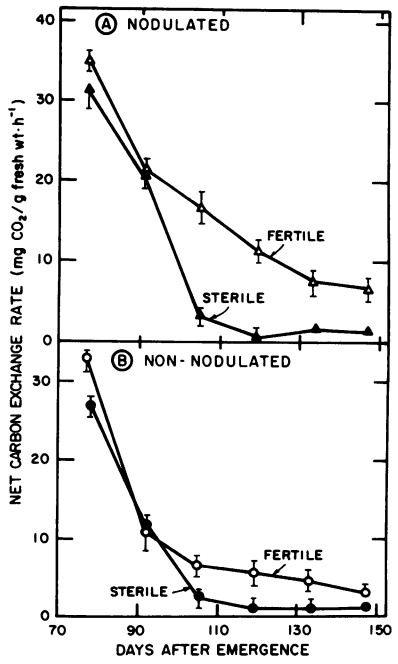

FIG. 1. Leaf photosynthetic rates of (A) nodulated and (B) non-nodulated male-sterile and fertile soybean plants during development. Each point is the mean of three determinations ± SE.

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

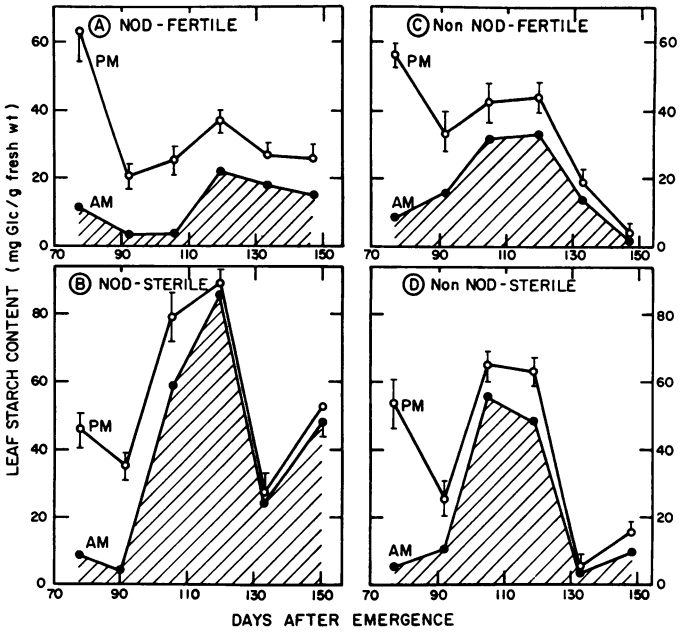

FIG. 2. Starch contents of leaves of (A) nodulated (NOD)-fertile, (B) nod-sterile, (C) non-nod-fertile, and (D) non-nod-sterile soybean plants during development. Starch level was measured at 0800 and 1700 h (AM and PM, respectively). Each point is the mean of three determinations (for simplicity, SE bars are shown for PM levels only).

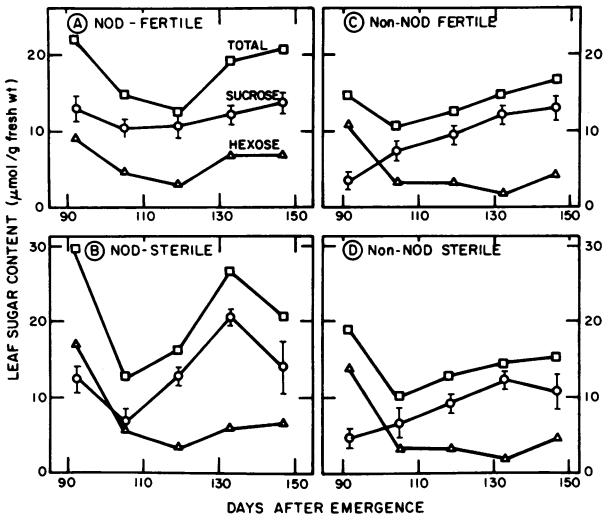

FIG. 3. Concentrations of soluble carbohydrates as mg glucose eq/g fresh weight of leaves of (A) nod-fertile, (B) nod-sterile, (C) non-nod-fertile, and (D) non-nod-sterile soybean plants during development. (○), sucrose; (△), hexose; (□), total. Each point is the mean of three determinations (for simplicity, SE bars are shown only for sucrose).

between PM and AM contents) declined from 77 to 147 DAT.

In contrast to the relatively large changes observed in NCER and starch content, leaf sugar content remained relatively constant (Fig. 3). With all four genotypes, sucrose was the principal sugar found in the leaf extracts.

Differences in leaf starch accumulation (Fig. 2) among genotypes were normalized with respect to NCER (Fig. 1). Hence, the ratio of leaf starch accumulation to NCER (relative leaf starch accumulation) reflected the proportion of photosynthetically fixed carbon that was partitioned into starch. With the fertile genotypes, relative leaf starch accumulation remained rather constant from 77 to 147 DAT, although the nodulated genotype tended to partition less carbon into starch than did the non-nodulated genotype (Fig. 4A). In contrast, the sterile genotypes exhibited a substantial increase in relative leaf starch accumulation at the later sampling dates (Fig. 4B).

Changes in Sucrose-Metabolizing Enzymes. The activity of SPS in leaf extracts of the four genotypes declined from 77 to 147 DAT (Fig. 5). Of the fertile genotypes, the nodulated line maintained higher activities of SPS than did the non-nodulated line (Fig. 5A). With the sterile genotypes, however, the effect of nodulation was not apparent after the first two sampling dates (Fig. 5B). At 147 DAT, however, both sterile genotypes had higher activities of SPS in leaf extracts than did the fertile

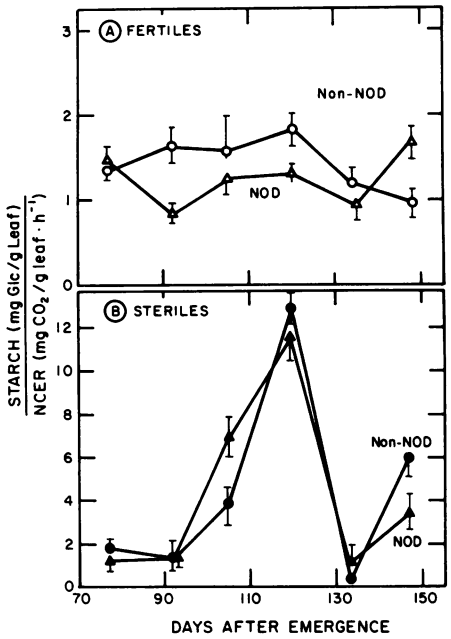

FIG. 4. Changes in relative leaf starch accumulation during development of (A) fertile and (B) sterile soybean plants. Each point is the mean of three determinations ± SE.

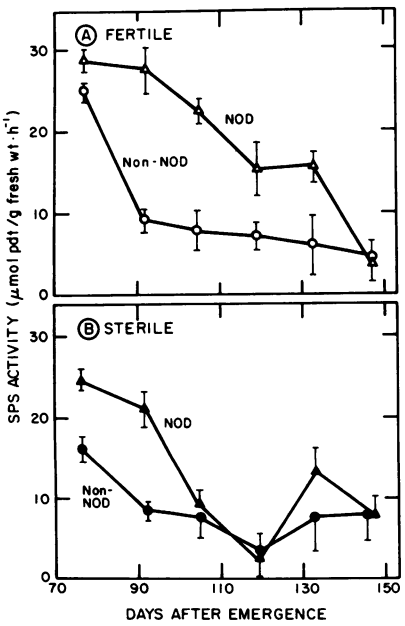

FIG. 5. Changes in activities of SPS in leaf extracts during development of (A) fertile and (B) sterile soybean plants. Each point is the mean of three determinations ± SE.

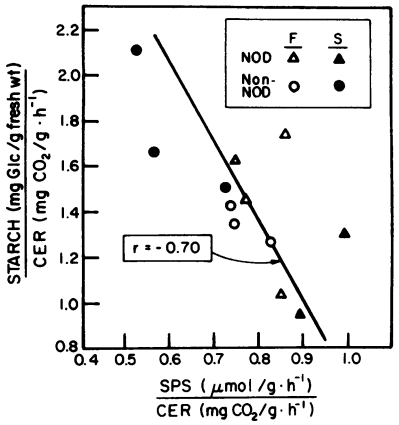

FIG. 6. Relation between relative leaf starch accumulation and relative SPS activity in leaves of the four soybean genotypes at flowering (77 DAT). Significant at the 0.05 level. CER, carbon exchange rate.

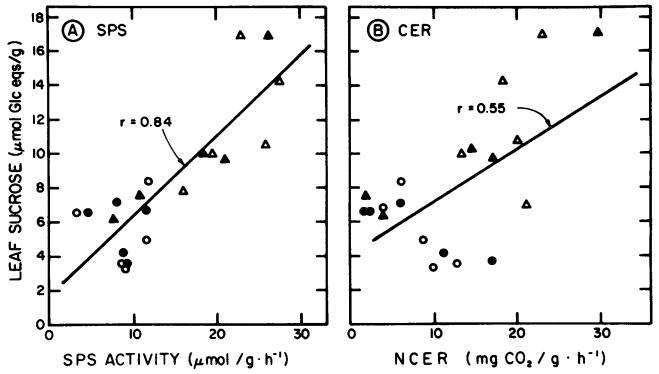

FIG. 8. Relation between leaf sucrose content and (A) SPS activity in leaf extracts and (B) NCERs of leaves of fertile (open symbols) and sterile (closed symbols) soybean plants at 92 and 99 DAT. Individual determinations are shown. (Δ, ▲), Nodulated; (O, ●), non-nodulated. Relations were significant at the 0.01 and 0.05 levels, respectively.

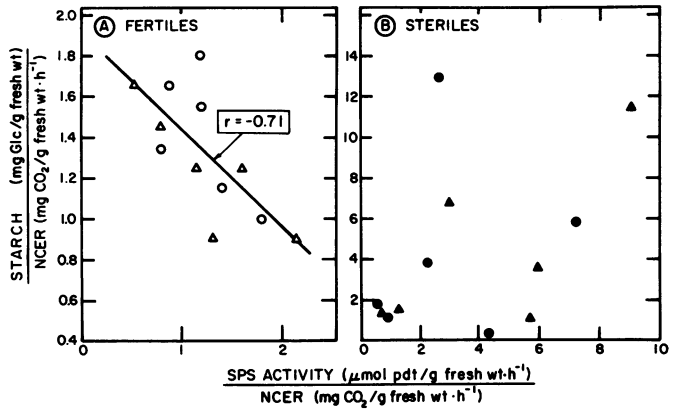

FIG. 7. Relation between relative leaf starch accumulation and relative SPS activity in leaves of (A) fertile and (B) sterile genotypes during development. (Δ, ▲), Nodulated; (O, ●), non-nodulated. Each point is the mean of three determinations of a given sampling date from 77 to 147 DAT. Correlation in A was significant at the 0.01 level.

genotypes.

Changes in the activities of sucrose synthase and uridine diphosphatase (11) in leaf extracts were also measured. In general, genotypic differences in changes in activities from 77 to 147 DAT were not observed (data not shown). However, uridine diphosphatase activity declined during the period (from about 700 to 70 μmol UDP hydrolyzed/g fresh weight h^{-1}) and sucrose synthase increased slightly (from about 8 to 12 μmol sucrose formed/g fresh weight h^{-1}).

At a given sample date, genotypic differences were observed in leaf starch accumulation, NCER, and activities of SPS in leaf extracts. The relation of relative leaf starch accumulation to activity of SPS in leaf extracts (normalized with NCER) is shown in Figure 6 for all genotypes at 77 DAT. As shown, a negative correlation ($r = -0.70$) between relative starch accumulation and the relative activity of SPS in leaf extracts was representative of all four genotypes.

The negative relation between starch accumulation and SPS activity was maintained from 77 to 147 DAT for the fertile genotypes (Fig. 7A), but not the sterile genotypes (Fig. 7B). After 92 DAT, the sterile genotypes accumulated high levels of starch but also had high activities of SPS in leaf extracts (in relation to NCER), and no correlation was apparent.

Within a sampling date, genotypic differences in leaf sucrose content were related to the activity of SPS in leaf extracts and the photosynthetic rate. At the first two sampling dates, leaf sucrose content was correlated positively with the activity of SPS in leaf extracts of the four genotypes ($r = 0.84$, Fig. 8A). The relation between sucrose content and NCER was also positive, but not as strong ($r = 0.56$, Fig. 8B). No correlation, however, was observed between sucrose content and the activities of sucrose synthase or uridine diphosphophatase (data not shown).

DISCUSSION

The results of the present study described the effects of plant N source and altered source-sink relations (fertile *versus* sterile) on photosynthesis and carbon partitioning in leaves. In general, the effects of reduced reproductive development were greater than the effect of plant N source. Because the genotypes used in this study exhibited determinant growth habit, after flowering the male-sterile genotypes would be expected to have a greatly reduced demand for assimilates. Indeed, the sterile genotypes exhibited the most rapid declines in NCER, and with time accumulated the highest levels of morning starch, which reflected reduced mobilization of stored carbon in the dark. Also, after 92 DAT, the sterile genotypes partitioned a greater proportion of photosynthetically fixed carbon into starch as compared to the fertile genotypes. These responses were consistent with previous studies (14, 16, 17) that showed inhibition of photosynthesis and accumulation of starch as a result of pod removal.

Of the fertile genotypes, the nodulated line maintained higher activities of SPS in leaf extracts and tended to partition less carbon into starch than did the non-nodulated line. The results are consistent with an earlier report (10) that nodulation of some, but not all, soybean cultivars increased photosynthetic sucrose formation at the expense of starch accumulation. Hence, it was postulated that increased sucrose formation accommodated the greater demand for assimilates in roots of nodulated plants (10).

During the early part of reproductive development, the activity of SPS in leaf extracts was correlated negatively with leaf starch accumulation, but positively with leaf sucrose content. These data support the postulate that the activity of SPS may effect coarse control of carbon partitioning between starch and sucrose. The activity of SPS in leaf extracts was apparently sufficient to account for photosynthetic sucrose formation. For example, with a NCER of 35 mg CO_2/g fresh weight h^{-1} and assuming that 40 to 60% of the total carbon fixed was partitioned into sucrose (5, 6), an activity of SPS of 26 to 40 μmol sucrose/g fresh weight h^{-1} would be required. Extractable activities of SPS were within that range when photosynthetic rates were high (*e.g.* at flowering). The correlations observed were generally consistent with the postulate originally proposed by Silvius *et al.* (18) that sucrose

synthesis regulates the partitioning of carbon between starch and sucrose. Apparently, the activity of SPS was associated with the rate of sucrose formation. Under steady state conditions, leaf sucrose content was in equilibrium with the rate of sucrose formation (15), which may explain the positive correlation between leaf sucrose and SPS activity.

The relationships observed during early reproductive development between SPS activity and starch/sucrose levels for the four genotypes were not maintained consistently through maturity. With the fertile genotypes, the negative relation between starch accumulation and SPS activity was maintained from flowering to maturity. However, the relation between leaf sucrose content and SPS activity varied with sampling date (data for other sampling dates not shown). With the sterile genotypes, after 92 DAT, no relation existed between SPS activity and starch accumulation or leaf sucrose content (data not shown). During that time, the sterile genotypes partitioned proportionally more carbon into starch as compared to the fertile genotypes. Thus, factors other than SPS activity were apparently regulating carbon partitioning in leaves when photosynthetic rates, and demand for assimilates, was low (*i.e.* sterile genotypes after 92 DAT, and the fertile genotypes near maturity).

Overall, the results obtained in the present study are consistent with previous reports which showed inhibition of photosynthesis, and accumulation of starch and sucrose in leaves as a result of decreased demand for assimilates (2, 14, 16, 17, 19, 22). The impact of reduced reproductive load on photosynthesis in the sterile genotypes was independent of plant N source. However, the relations observed between SPS activity and starch/sucrose levels in leaves when demand for assimilates was present support the suggestion that SPS activity is associated with the control of carbon partitioning in leaves.

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