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Alterations in Leaf Carbohydrate Metabolism in Response to Nitrogen Stress[1]

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ABSTRACT

A series of experiments was conducted to characterize alterations in carbohydrate utilization in leaves of nitrogen stressed plants. Two-week-old, nonnodulated soybean plants (*Glycine max* [L.] Merrill, 'Ransom'), grown previously on complete nutrient solutions with 1.0 millimolar NO_3^-, were transferred to solutions without a nitrogen source at the beginning of a dark period. Daily changes in starch and sucrose levels of leaves were monitored over the following 5 to 8 days in three experiments. Starch accumulation increased relative to controls throughout the leaf canopy during the initial two light periods after plant exposure to N-free solutions, but not after that time as photosynthesis declined. The additional increments of carbon incorporated into starch appeared to be quantitatively similar to the amounts of carbon diverted from amino acid synthesis in the same tissues. Since additional accumulated starch was not degraded in darkness, starch levels at the beginning of light periods also were elevated. In contrast to the starch effects, leaf sucrose concentration was markedly higher than controls at the beginning of the first light period after the N-limitation was imposed. In the days which followed, diurnal turnover patterns were similar to controls. In source leaves, the activity of sucrose-P synthase did not decrease until after day 3 of the N-limitation treatment, whereas the concentration of fructose-2,6-bisphosphate was decreased on day 2. Restricted growth of sink leaves was evident with N-limited plants within 2 days, having been preceeded by a sharp decline in levels of fructose-2,6 bisphosphate on the first day of treatment. The results suggest that changes in photosynthate partitioning in source leaves of N-stressed plants resulted largely from a stable but limited capacity for sucrose formation, and that decreased sucrose utilization in sink leaves contributed to the whole-plant diversion of carbohydrate from the shoot to the root.

The activities of carbon and nitrogen assimilatory processes are closely related to rates of plant growth and development. The constancy of this association has led modelers of physiological responses to explain whole-plant growth and coordinated growth between the shoot and root predominantly in terms of carbon and nitrogen interactions (31, 33). It is not surprising, therefore, that disruption in the supply of either of the nutrients can result in marked changes in the assimilation of the other. In the case of nitrogen stress, for example, two distinct changes in carbon utilization are commonly observed: (a) starch accumulates in leaves (1, 19, 21, 34) and (b) a larger portion of available carbohydrate is translocated from leaves into the root system, resulting in a decline in the shoot to root weight ratio (3, 11). The responses imply a general decline in carbohydrate utilization efficiency within the leaf canopy; however, little is known about the regulatory processes involved.

Accumulation of starch in leaves of plants undergoing nitrogen stress could result from increased net synthesis in the light and/or decreased degradation in darkness. An increase in starch formation could reflect a decline in the rate of sucrose biosynthesis. The rate of sucrose synthesis in the cytosol of leaf mesophyll cells determines the availability of cytosolic Pi, which in turn regulates Pi/triose-P[2] exchange at the chloroplast membrane and the efficiency of carbon diversion from starch formation reactions in the chloroplast (30). Decreased activity of SPS and/or an increased level of F26BP could result in a decline in sucrose formation and the associated generation of cytosolic Pi. Adjustments in the two regulatory factors have been proposed to be important in the control of sucrose biosynthesis *in vivo* (8, 30).

Increased translocation of carbohydrate to roots with nitrogen stress probably is related, at least in part, to a decreased rate of leaf canopy development. The sensitivity of leaf area expansion to nitrogen limitation is well documented (24 and Refs. therein). A general decrease in meristematic activity would be the expected consequence of a decrease in the supply of reduced nitrogen to shoot growth centers. As long as the restriction of leaf canopy development exceeds the coincident decline in photosynthetic rate of existing leaves, then increased translocation of fixed carbon to the root system can occur. It is unknown to what extent alterations in carbohydrate utilization reactions of existing sink tissues also are involved in the whole-plant carbohydrate redirection.

Resolving cause and effect relationships in plants subjected to a nitrogen limitation is difficult because nitrogen stress initiates many complex physiological responses which vary over time and with the degree of stress. Many of the effects could indirectly influence carbohydrate metabolism. Indirect effects, for example, would be expected to result from altered water relations (18) and net synthesis of protein (16). In addition, restrictions in photosynthetic rate (17) can mask separate regulatory adjustments within the carbohydrate metabolic pathways. In this series of experiments, we attempted to identify metabolic factors controlling accumulation and utilization of starch and sucrose in leaves

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[2] Abbreviations: triose-P, triose-phosphate; SPS, sucrose phosphate synthase; F26BP, fructose 2,6-bisphosphate; FBPase, fructose 1,6 bisphosphatase; PPi-PFK, pyrophosphate-dependent phosphofructokinase; STR, structural; PGA, 3-phosphoglycerate; Pi, inorganic phosphate; CER, net CO_2 exchange rate.

of nitrogen limited plants. Due to the possibility of confounding interactive effects, the experiments were designed to investigate rapid changes in carbohydrate relations following an abrupt nitrogen limitation, primarily changes occurring prior to decreases in photosynthetic rate. The intent was to identify sensitive metabolic processes which might contribute to longer term adjustments in carbohydrate utilization.

MATERIALS AND METHODS

Plant Culture. Seeds of soybean (*Glycine max* [L.] Merrill, 'Ransom') were germinated in paper towels at 25°C and 98% RH in a controlled environmental chamber. The germinating seeds were kept moist by capillary action from a 0.1 mM $CaSO_4$ solution. After 3 d, 64 seedlings were selected for radical lengths between 8 and 12 cm and placed into four 115-L continuous-flow, hydroponic culture systems with pH control. The culture systems were located in a growth room programmed for day/night (12 h/12 h) temperatures of 28/22°C. Irradiance, provided by high pressure sodium vapor and metal halide lamps, was 1000 ± 50 μE m^{-2} s^{-1} at canopy height. The culture solution temperature was 25 ± 1°C and pH was maintained at 5.8 ± 0.2 by additions of 0.2 N H_2SO_4. During the 2-week pretreatment period, plants were exposed to solutions with the following nutrient concentrations: 1.0 mM NO_3^-, 0.1 mM $H_2PO_4^-$, 1.1 mM K^+, 1.0 mM Ca^{2+}, 1.0 mM Mg^{2+}, 1.0 mM SO_4^{2-}, 17 μM B, 3 μM Mn, 0.3 μM Zn, 0.1 μM Cu, 0.04 μM Mo, and 18 μM Fe as Fe-DPTA (CIBA-Geigy Corp., Greensboro, NC).[3] The solutions were changed every 3 d to avoid depletion effects.

Experimental Conditions. The study consisted of three experiments which differed in length of time and sampling procedures. In each experiment, treatments were started at the beginning of the dark period (2000 h) on d 14, when the first trifoliolate leaf had reached full expansion. In control plants the second trifoliolate leaf was fully expanded by d 16, and the third on d 21. Plants in two of the culture systems were exposed to solutions identical with that in the pretreatment, which contained 1.0 mM NO_3^-. Plants in the other two systems were exposed to similar solutions, with the exceptions that NO_3^- was omitted and K_2SO_4 was added to provide a K^+ concentration of 1.1 mM. All other environmental conditions were the same as during the 2-week pretreatment. No nodules were found on plant roots during the experiment, thus NO_3^- supplied in solutions was the only nitrogen source available to the plants.

In experiment 1, plants were sampled at 0800 and 2000 h (the beginning and end of the light period) on the days which followed imposition of the nitrogen treatment. At each sampling, three plants were removed from each treatment, separated into leaves, stems, and roots, then weighed and frozen promptly at −20°C for later analysis. In experiment 2, the leaf canopy was separated into source and sink leaves at the time of sampling (0800 and 2000 h). Source leaves consisted of primary and first trifoliolate leaves, sink leaves were those on the main stem or lateral branches with a middle leaflet smaller than 3 cm in length. The groups of leaves were weighed and immediately frozen (−20°C). In experiment 3, a source leaf, trifoliolate no. 1, and a sink leaf, trifoliolate no. 3, from three plants were sampled individually and frozen rapidly in liquid nitrogen for later enzyme analyses. The samplings were done at 1100 and 1700 h to allow general characterization of SPS activity and the concentration of F26BP during the light period. The data presented in Figure 6 are means of all six replicates sampled at those times, which did not vary significantly. Specific sampling days for each experiment are specified in "Results."

Tissue and Enzyme Analyses. In experiments 1 and 2, the plant tissue was freeze-dried, weighed, and ground. A portion of the ground tissue was used for carbohydrate analyses. The tissue was extracted with hot 80% ethanol, and the supernatant enzymically analyzed for sucrose (12). The particulate fraction, containing starch, was suspended in 1.0 ml of 0.2 N KOH and placed in boiling water for 30 min. After cooling, the pH was adjusted to about 5.5 with 200 μL of 1.0 N acetic acid. To each sample, 1.0 mL of dialyzed amyloglucosidase solution (from *Aspergillus niger* [Sigma], 70 units/ml in 50 mM Na-acetate buffer, pH 4.5) was added and the tubes were incubated at 55°C for 15 min. After digestion, the tubes were placed in boiling water for 1 min, centrifuged, and the glucose in the supernatant was analyzed using hexokinase and glucose-6-P dehydrogenase (12).

Additional tissue was used to determine insoluble reduced nitrogen, which was used to estimate the concentration of protein and nucleic acids. The tissue was extracted with methanol:chloroform:water (13:4:3). Following separation of the chloroform from the methanol:water fraction, the chloroform was added back to the tissue residue, with this constituting the insoluble reduced-N fraction. Total nitrogen in the fraction was determined by Kjeldahl digestion (15) and colorimetric determination of NH_4^+ (4).

In experiment 3, frozen leaf tissue (about 250 mg fresh weight) was ground in a glass homogenizer (10 mL of medium per g fresh weight) in a medium containing 40 mM MOPS-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2% (w/v) polyethylene glycol-20,000, 0.5% (w/v) bovine serum albumin, and 2.5 mM dithiothreitol. After homogenization, the extracts were centrifuged (10,000*g*, 30 s) in a Beckman Microfuge, and the supernatants were used for enzyme assays. Preliminary experiments confirmed that desalting the extracts prior to assay had no effect on measured enzyme activity; consequently, the desalting procedure was not routinely performed.

The activity of sucrose-P synthase was assayed by measurement of fructose-6-P-dependent formation of sucrose (+ sucrose-P) from UDP-glucose. The assay mixture (70 μL) contained 7.5 mM UDP-glucose, 7.5 mM fructose-6-P, 15 mM $MgCl_2$, 50 mM Hepes-NAOH (pH 7.5), and an aliquot of leaf extract. Mixtures were incubated at 25°C, and reactions were terminated after 10 min by addition of 70 μL of 1 N NaOH. Unreacted fructose-6-P (or fructose) was destroyed by placing the tubes in boiling water for 10 min. After cooling, 0.25 ml of 0.1% (v/v) resorcinol in 95% ethanol and 0.75 ml of 30% HCl were added, and the tubes incubated at 80°C for 8 min (23). The tubes were allowed to cool, and the A_{520} was measured.

Levels of F26BP were determined following a separate extraction of frozen leaf tissue (approximately 20 mg fresh weight) in 10 mM NaOH (50 ml of base/g fresh weight of leaves) using a cold glass homogenizer. After extraction, a small amount (approximately 50 μL) of activated charcoal was added as a suspension in ethylene glycol (200 mg charcoal/3 ml ethylene glycol). After centrifugation at 10,000*g* for 30 s, the supernatant was used for measurement of F26BP. Detection of F26BP employed the bioassay based on stimulation of potato tuber pyrophosphate-dependent phosphofructokinase (32).

RESULTS

The N-Stress Response. Accumulation of reduced nitrogen during early growth of soybean plants in this culture system is shown in Figure 1. Past experiments have revealed that substantial amounts of NO_3^- taken up by plants in darkness are retained temporarily in the root system, and then assimilated into reduced-N molecules in the following light period (25). Thus, to restrict NO_3^- assimilation during a given light period, it was

[3] The use of trade names in this publication does not imply endorsement by the U.S. Department of Agriculture or the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

necessary to impose N-stress treatments at the beginning of the previous dark period. In the experiments presented herein, plants were first exposed to treatment solutions without a nitrogen source at the end of the light period on d 14, and tissue analyses confirmed that no additional nitrogen was accumulated after that time (data not shown). From the acquisition curve (Fig. 1), it can be calculated that control plants exposed continuously to 1.0 mM NO_3^- reduced approximately 9.8 mg of N as NO_3^- per plant on d 15, which is designated as d 1 in the following graphs.

Starch levels at the beginning (0800 h) and end (2000 h) of each light period, with all leaves of a plant combined, are depicted in Figure 2. Daily accumulation and degradation of starch can be estimated from differences between the 0800 and 2000 h plots. In control plants, the starch content at 2000 h increased exponentially during the 8 d experiment, reflecting increases in total starch accumulation during light periods as the leaf canopy developed (*cf.* Fig. 3A). The starch content at 0800 h always approached zero, which indicates that accumulated starch always was degraded completely during dark periods. Exposure of plants to a nutrient solution without NO_3^- resulted in an immediate elevation of starch levels relative to controls. This was the result of an initial increase in starch accumulation on d 1 and, subsequently, incomplete degradation of starch which was apparent throughout the experiment. The tendency for starch contents at 0800 and 2000 h to level off and (after d 6) to decline generally coincided with inhibition of leaf canopy growth (*cf.* Fig. 3A) and, presumably, decreased photosynthetic activity.

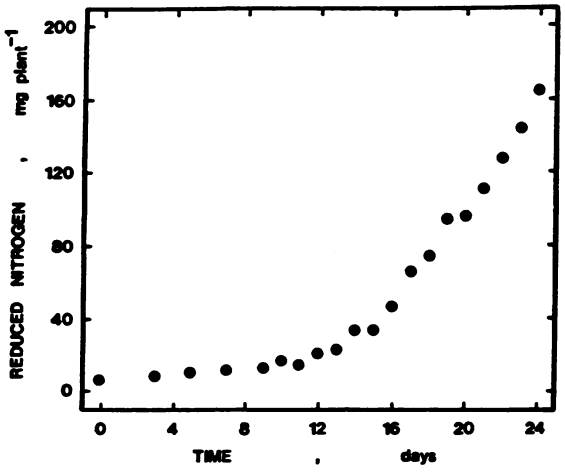

FIG. 1. Accumulation of reduced nitrogen by nonnodulated soybean plants exposed to complete nutrient solutions containing 1.0 mM NO_3^- for 24 d after germination. Nitrogen stress treatments began at the end of the light period on d 14. D 15 corresponds with d 1 in subsequent figures.

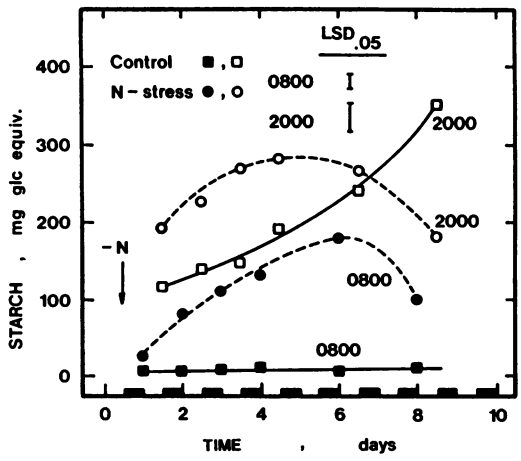

FIG. 2. Alterations in the starch content of leaves of plants exposed to solutions without NO_3^-. Leaves were sampled at the beginning (0800 h) and end (2000 h) of light periods (experiment 1).

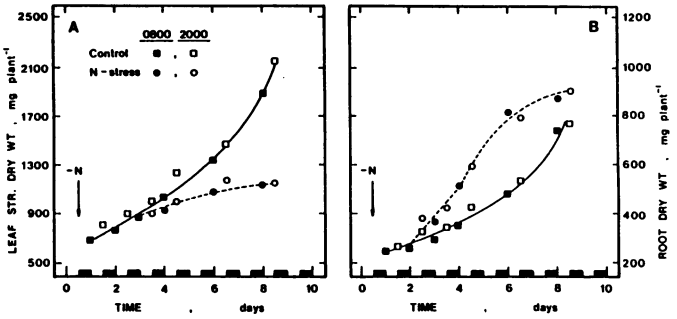

FIG. 3. Alterations in leaf and root structural dry weight in plants exposed to solutions without NO_3^-. Plants were sampled at the beginning (0800 h) and end (2000 h) of light periods. Individual points for N-limited plants are plotted only when statistically different (LSD 0.05) than controls (experiment 1).

Alterations in whole-plant partitioning of assimilates can be estimated from relative changes in the growth of leaves and roots (Fig. 3). Leaf data are presented as structural dry weight (total dry weight − starch) to eliminate the confounding effects of unequal starch contents in the two treatment conditions (*cf.* Fig. 2). Leaves of control plants accumulated structural dry weight exponentially over the 8 d experimental period (Fig. 3A). When plants were deprived of external NO_3^-, however, a restriction of leaf growth became evident within 3 d, and by the end of the experiment growth had virtually ceased. In contrast, the absence of external NO_3^- resulted in a marked stimulation of root growth relative to the control within 2 d, and the stimulation increased up to d 6 before starting to decline (Fig. 3B). Associated with the differential growth responses, the leaf to root dry weight ratio of N-limited plants and, presumably, the proportion of available photosynthate utilized for growth in the leaf canopy, were appreciably decreased from 3 to 8 d after plants were deprived of an external nitrogen supply. The leaf to root weight ratio decreased from 2.9 to 1.2 during that time, while the ratio remained relatively constant in control plants.

Source and Sink Leaf Effects. A second experiment was conducted to examine alterations in leaf carbohydrate metabolism during the initial days of N-stress in greater detail. Effects on source and sink leaves were examined separately. The photosynthetic rate of source leaves (primary and first trifoliolate) of control plants was relatively stable over the 5-d experimental period (Fig. 4A). Exposure to N-free solution resulted in a reduction of photosynthetic rate which was detectable by d 3 and became more pronounced with time. A decrease in the concentration of insoluble reduced-N in the source leaves also was evident, indicating that net synthesis of protein was being restricted significantly by the third day after the nitrogen treatment was imposed.

The starch levels in source leaves responded in a similar fashion to that observed for the whole leaf canopy (*cf.* Figs. 2 and 5A). The starch concentration in leaves of control plants at 0800 and 2000 h remained stable, indicating uniformity in daily turnover, whereas the concentration of starch in N-stressed plants increased appreciably (Fig. 5A). Higher starch was due to (a) enhanced accumulation in the first 2 d of treatment and (b) incomplete degradation. The tendency for the 0800 and 2000 h data of stressed plants to merge with time indicates that, even though accumulation and degradation were restricted relative to controls after d 2, degradation was restricted to a greater extent.

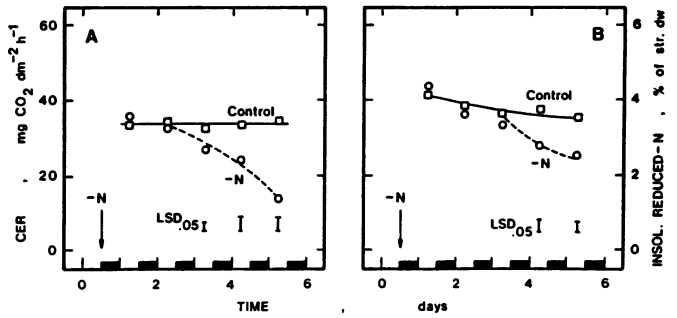

FIG. 4. Alterations in CER (A) and the concentration of insoluble reduced-N (B) of source leaves of plants exposed to solutions without NO_3^-. Data for CER represents the mean value from 6 measurements during each light period, and for insoluble reduced-N the mean of all source leaves sampled at the beginning and end of the light period (experiment 2).

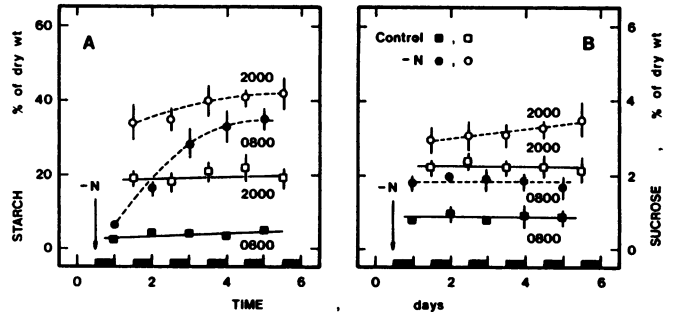

FIG. 5. Alterations in the concentration of starch (A) and sucrose (B) in source leaves of plants exposed to solutions without NO_3^-. Leaves were sampled at the beginning (0800 h) and end (2000 h) of light periods (experiment 2).

The concentration of sucrose in source leaves also increased in response to N deprivation, but the response was unlike that of starch (Fig. 5B). The concentration was elevated at 0800 h on d 1 of the experiment, but changed little thereafter, as daily turnover (the difference between 0800 and 2000 h plots) generally resembled that of the control. The higher concentration thus reflected an immediate adjustment in carbohydrate utilization during the first dark period in which plants were exposed to N-free solutions.

Activity of the enzyme SPS in the first trifoliolate source leaf of plants exposed to 1.0 mM NO_3^- tended to decline somewhat over the 5-d experimental period (Fig. 6A). SPS activity was similar in the same leaf of N-limited plants during the initial 3 d of treatment, but activity was lower than the control on d 5 when insoluble reduced-N had declined (Fig. 4B). The concentration of F26BP increased with time in the source leaf of plants receiving NO_3^- (Fig. 6B). In the source leaf of plants without NO_3^-, the F26BP level was similar to the control on d 1, but then was noticeably lower through d 5. The tendency for F26BP to increase with ageing of the leaf still was evident.

Exposure of plants to nutrient solution without NO_3^- also increased starch and sucrose concentrations in sink leaves (Fig. 7). The concentration of starch in leaves of control plants at 0800 and at 2000 h did not change greatly over the 5-d experimental period, but in sink leaves of nitrogen limited plants, starch at both sample times was elevated on d 1 and increased considerably over time (Fig. 7A). The concentration of sucrose in sink leaves also was higher than controls, due primarily to increased 0800 h levels (restricted degradation) in the first 2 d of treatment (Fig. 7B). This response resembled that in source leaves

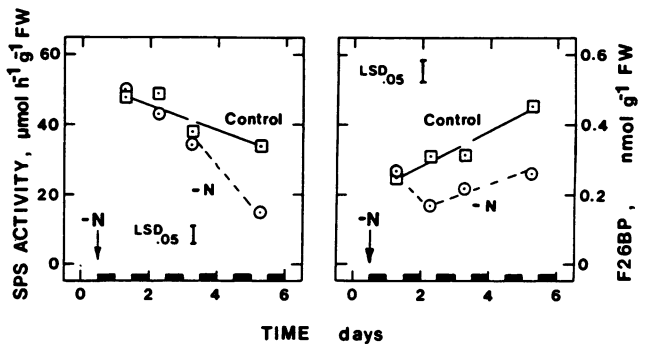

FIG. 6. Alterations in the activity of SPS (A) and the concentration of F26BP (B) in the first trifoliolate source leaf of plants exposed to solutions without NO_3^-. Data represent means of six plants sampled during each light period (experiment 3).

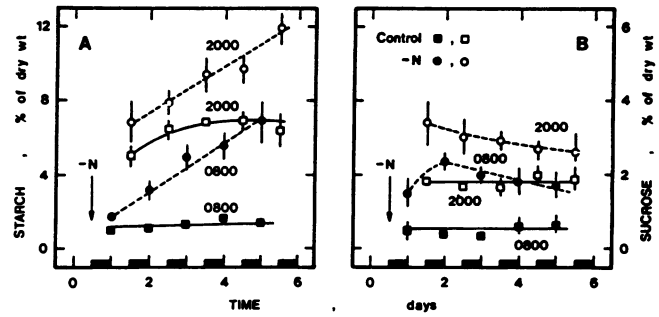

FIG. 7. Alterations in the concentration of starch (A) and sucrose (B) of sink leaves of plants exposed to solutions without NO_3^-. Leaves were sampled at the beginning (0800 h) and end (2000 h) of light periods (experiment 2).

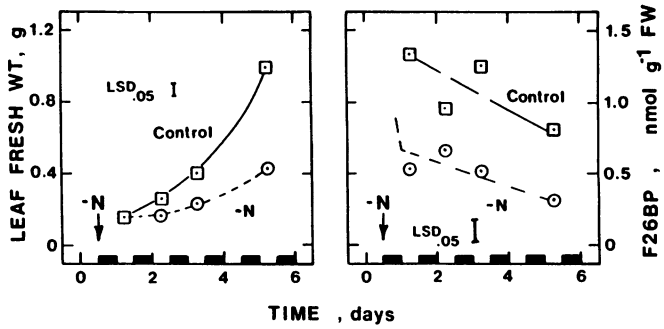

FIG. 8. Alterations in the accumulation of fresh weight (A) and the concentration of F26BP (B) in the third trifoliolate sink leaf of plants exposed to solution without NO_3^-. Data represent means of six plants sampled during each light period (experiment 3).

(*cf.* Fig. 5B) but was not identical because in sink leaves sucrose concentrations at 0800 and 2000 h tended to decline after d 2.

Sink leaf development was markedly restricted by limited external NO_3^- availability (Fig. 8A). Decreased accumulation of fresh weight by trifoliolate leaf 3 was evident by d 2 and became more pronounced with time. Also, the level of F26BP in leaf 3 was depressed relative to that in the control on the first day of treatment, and levels remained lower throughout the 5-d experimental period (Fig. 8B). The concentration of F26BP declined as the leaves expanded in both treatment conditions.

DISCUSSION

The nitrogen limitation imposed in these experiments resulted in rapid adjustments in carbohydrate formation and utilization

in leaves of vegetative soybean plants. Substantial accumulation of starch and sucrose occurred in both source and sink leaves on the first day of treatment, and increased translocation of available carbohydrate to the root system was evident by d 3, as indicated by reciprocal root and shoot growth alterations. The responses thus correspond with those observed in other experiments in which crop plants characteristically were exposed to less severe degrees of nitrogen stress over longer time periods (1, 19, 33).

Starch Accumulation. Higher starch concentrations in leaves of nitrogen limited plants were a consequence of increased accumulation in the light and decreased degradation in darkness. Increased accumulation of starch relative to controls was apparent only in the first 2 d of N-stress (Figs. 2, 5A, and 7A), preceding declines in photosynthetic rate (Fig. 4A).

In considering controls governing the starch accumulation effect, it is important to quantify how closely nitrogen and carbon assimilatory processes were coupled. This question can be approached using the leaf canopy data for the light period on d 1 (Fig. 2). Leaves of N-stressed plants accumulated 61 mg of starch in excess of that accumulated by controls in the light on d 1, which is equivalent to 339 μmol of glucose or 2034 μmol of carbon. Estimating the amount of carbon diverted from assimilation of NO_3^- into amino acids in the same tissues during the same time interval requires certain assumptions. Regression analysis indicates that about 9.8 mg or 700 μmol of reduced-N was accumulated by control plants on d 1 (designated d 15, Fig. 1). From previous experiments (25), it is known that (a) about 85% or 595 μmol of the reduced N (0.85 × 700) was derived from the reduction of NO_3^- taken up in the previous dark period and in the sample period in the light, both of which were eliminated in the N-stressed plants, (b) about 70% or 417 μmol of the reduced N (0.70 × 595) was associated with NO_3^- reduction in the light, and (c) about 80% or 333 μmol of the reduced N (0.80 × 417) resulted from NO_3^- reduction in leaves. If it is assumed that nitrogen molecules are assimilated primarily into amino acids with a C/N ratio of 5/2, then 832 μmol of carbon were required to provide carbon skeletons for amino acids synthesized during the light period on d 1. Organic acids are synthesized stoichiometrically with the reduction of NO_3^- (14, 20) to maintain pH and charge balance in the tissue, requiring an additional 1332 μmol (333 × 4) of carbon if malate is assumed to be the predominant organic acid involved. An additional 28 μmol of carbon (0.083 × 333 mol of NO_3^-, *cf.* Ref. 27) would have been required to provide energy for NO_3^- reduction, with nitrite reduction in leaves being energetically free (22). Thus it can be estimated that about 2192 μmol (832 + 1332 + 28) of carbon were diverted from NO_3^- assimilation events during the light period on the first day of the nitrogen limitation, which closely equates with the additional 2034 μmol of carbon incorporated into starch. The validity of these estimates is, of course, dependent on the accuracy of the assumptions.

At this stage of soybean development, source leaves accounted for 80% of the dry weight of the total leaf canopy and 85% of the total leaf area (data not shown). Also, relative increases in starch concentration were much larger in source than in sink leaves (*cf.* Figs. 5A and 7A). As a consequence, greater than 95% of the starch accumulation in the first day of nitrogen limitation occurred in source leaves. The nearly quantitative diversion of carbon from reactions associated with NO_3^- reduction and amino acid synthesis into starch suggests that the rate of sucrose formation in source leaves was unchanged relative to that of the controls. Adding support to this contention, there was no indication of metabolic restrictions within the sucrose biosynthesis system, as the activity of SPS and the concentration of F26BP were unchanged during this time. The activity of SPS did decrease, but not significantly until after d 3. The concentration of F26BP was decreased relative to the control on d 2, but restriction of sucrose formation by F26BP has been associated with increased levels of F26BP and the related inhibition of cytoplasmic FBPase (8). Furthermore, a somewhat analogous result has been noted in other studies, in which photosynthetic rates increased suddenly after raising of the light intensity or CO_2 concentration, and the additional increments of fixed carbon accumulated as starch (6, 7, 28). In our experiment, it seems likely that the capacity for sucrose formation was saturated, being acclimated to growth conditions existing prior to the withdrawal of NO_3^- which increased carbon availability. A limited capacity for sucrose formation, and the associated limited generation of cytosolic Pi required for transport of triose-P out of chloroplasts (30), would account for the diversion of photosynthetically fixed carbon from nitrogen assimilatory reactions into starch.

The other factor responsible for the elevated starch levels in nitrogen limited plants was the lack of complete degradation of starch in darkness. Degradation in source leaves remained quantitatively similar to the controls in the first 2 d of treatment, even though starch accumulation in light phases of the diurnal cycle was greatly enhanced. There were indications that degradation was restricted in source leaves after d 2 (Fig. 5A), as the 2000 and 800 h plots began to merge. Little evidence is available concerning the regulation of starch degradation rates, thus the biochemical factors responsible for the effects observed here remain obscure.

The types of responses identified in these experiments may contribute to elevated starch levels in source leaves of plants exposed for extended periods of time to moderate degrees of nitrogen stress. The activity of SPS and assimilate export rates were decreased considerably in soybean plants growing at suboptimal levels of exogenous NO_3^- (10), indicating that the capacity for sucrose formation was limited, and that the tendency for excess starch accumulation was sustained even when the photosynthetic rate and growth were depressed. In another experiment with soybean plants exposed to growth limiting levels of NO_3^-, increased partitioning of photosynthate into starch was accompanied by an increased PGA/Pi ratio and decreased concentration of triose-P in source leaf tissue (21); biochemical adjustments again compatible with a limitation in sucrose synthesis and cytosolic generation Pi. The capacity of the sucrose biosynthetic system ultimately may be governed by the demand for assimilates (5, 26) which, in turn, would be determined by the rate of supply of reduced N to growth centers and associated physiological constraints which accompany a nitrogen limitation (*e.g.* altered water relations; 18). It also is notable that source leaf starch levels were found to be elevated at the start of light periods in soybean plants acquiring nitrogen at growth limiting rates (13), which suggests a long-term restriction of starch mobilization.

Sucrose Accumulation. The pattern of adjustment in leaf sucrose levels in nitrogen limited plants was very different from that of starch. In both source and sink leaves, sucrose levels were already elevated at the start of the light period on d 1, and levels remained higher than controls as subsequent diurnal fluctuations in the two treatment conditions were similar. Higher sucrose levels therefore were a consequence of decreased utilization during the initial dark period after the nitrogen limitation was imposed. It seems unlikely that sucrose transport processes in source leaves would be inhibited within several hours after removal of the supply of exogenous NO_3^-. A more plausible explanation is that additional amounts of sucrose were required to serve an osmotic function. An abrupt limitation in exogenous NO_3^- could rapidly influence the internal level of NO_3^- and organic acids generated during NO_3^- reduction, both of which may be involved in turgor maintenance in leaf cells (29).

Whole-Plant Partitioning. The restriction of leaf canopy development in nitrogen-stressed plants (*cf.* Fig. 3A) consists of

two general effects. One is a decrease in the rate of leaf initiation, described previously (24), and the other is a decrease in the development of existing sink leaves, which was evident in this experiment by the second day of treatment (Fig. 8A). Both effects contribute to decreased utilization of carbohydrate in the shoot.

Carbohydrate metabolism of sink leaves was altered in nitrogen-limited plants, as starch and sucrose levels were elevated relative to controls (Fig. 7), even as growth was being restricted (Fig. 8A). This observation implies that (a) growth was not limited by assimilate availability, and (b) sucrose degradation and, possibly, glycolysis were reduced. A large portion of the assimilate utilized in growth reactions in sink tissues is derived from sucrose imported from source leaves. It has been recently proposed (2, 9) that an important pathway for sucrose utilization in sink tissues is catalyzed by PPi-PFK, which is activated by F26BP. The sharp decline in the concentration of F26BP in sink leaves following the imposition of nitrogen stress (Fig. 8B), therefore, may have caused the accompanying decrease in carbohydrate metabolism. Further experimentation will be necessary to elucidate the exact role of F26BP in sink leaf response. Nonetheless, our results clearly suggest that decreased sucrose utilization contributed to decreased demand for assimilates within the shoot, and thus, to the related diversion of carbohydrate to the root system.

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