Influence of Leaf Starch Concentration on CO₂ Assimilation in Soybean

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ABSTRACT

Net photosynthetic rate, CO₂ compensation concentration, and starch and soluble sugar concentrations were measured in soybean (Glycine max [L.] Merrill) leaves in an attempt to evaluate the effect of carbohydrate concentration on rate of CO₂ assimilation.

Plants were grown in a controlled environment room at 23.5 °C, 50% relative humidity, 16-hour photoperiod, and quantum flux (400–700 nm) of 510 μeinsteins/m²-sec (30,090 lux) at plant level. On the 21st day after seeding, plants were subjected for 12.5 hours to one of three CO₂ concentrations (50, 300, or 2000 μl/l) in an attempt to alter leaf carbohydrate levels. Following the CO₂ treatment, gas exchange measurements were made at a CO₂ concentration of 300 μl/l in the lowermost trifoliolate leaf. Immediately after measurement, the leaf was removed and stored at −20 °C until carbohydrate analyses were performed.

Increasing the CO₂ concentration for 12.5 hours significantly increased leaf starch concentration but not soluble sugar concentration. There was a strong negative correlation between net photosynthetic rate and starch concentration. Net photosynthetic rate declined from approximately 38 to 22 mg CO₂/dm² leaf area-hr as starch concentration increased from 0.5 to 3 mg/cm² leaf area. Carbohydrate concentrations had no effect on compensation concentration.

The decrease in net photosynthetic rate as starch concentration increased resulted from an increase in mesophyll (liquid phase) CO₂ diffusion resistance. This suggests that starch accumulation may reduce net photosynthetic rate by impeding intracellular CO₂ transport.

Neales and Incoll (13) reviewed the research dealing with the relationship between leaf carbohydrate level and photosynthetic rate. Most of the evidence supporting a product inhibition hypothesis has come from experiments in which the source/sink balance was altered in an attempt to change leaf carbohydrate level. Such alteration may also have changed the hormonal balance in the plant (13); certain hormones have been shown to affect photosynthesis (18). The presence of such an effect makes the interpretation of results difficult. The review also pointed out that the mechanism of photosynthetic reduction under high carbohydrate level had yet to be satisfactorily explained.

Though much of the earlier work focused on the effect of soluble carbohydrates, some recent studies have examined the effect of leaf starch concentration on Pn. Chatterton et al. (6) discovered that nontillering pangolagrass plants accumulated leaf starch in the light while tillering plants did not. Following a cold night, nontillering plants, which retained starch in the leaves, had lower rates of photosynthesis than did tillering plants. The reduction in Pn was proportional to the amount of starch in the leaves.

Results of two recent studies with soybean (Glycine max [L.] Merrill) have also suggested a relationship between starch concentration and Pn. Upmeyer and Koller (19) found that Pn began to decline when starch reached a high level in the afternoon. Thorne and Koller (17) noted that Pn rose by 25% as starch concentration dropped from 23% to 2% in leaves under an induced high sink demand.

This paper reports an attempt to quantify the relationship between leaf carbohydrate level and Pn in soybean leaves. Because most previous research of this type has probably failed to distinguish carbohydrate effects from other possible effects such as hormonal control of Pn (13), we chose to alter carbohydrate levels by controlling the amount of CO₂ available to the plants during part of 1 day. Since this technique should produce little disruption of plant processes, evaluation of the independent effect of carbohydrate concentration on Pn should be possible.

Effects of carbohydrate concentration on the components of CO₂ diffusion resistance were observed in order to better understand the mechanism by which a carbohydrate buildup may affect Pn.

MATERIALS AND METHODS

Plant Culture. Seeds of ‘Amsoy 71’ soybean (Glycine max [L.] Merrill) were planted in 1-liter plastic pots containing a fertile greenhouse soil-vermiculite mix (5:2 v/v). Plants were thinned to one per pot 1 week after seeding and were watered daily. Plants were grown in a controlled environment room with a 16-hr photoperiod and a 23.5 ± 1.0°C temperature. A mixture of fluorescent and incandescent lamps supplied a quantum flux (400–700 nm) of 510 ± 50 μeinsteins/m²-sec (30,090 lux). Relative humidity was maintained at about 50%.

CO₂ Treatments. Twenty-one days after seeding, eight plants were randomly selected for each treatment. At the beginning of the photoperiod, the plants were placed in a glass chamber (30 × 30 × 60 cm) equipped with a circulating fan. Plants were watered and a clear acrylic lid was placed over the chamber. The chamber was then placed into a growth cabinet, and the temperature inside the plant chamber was maintained at 26 ± 1°C. A mixture of fluorescent and incandescent lamps provided a quantum flux (400–700 nm) of 400 ± 30 μeinsteins/m²-sec (23,600 lux). Relative humidity in the plant chamber was 65 ± 10%.

Treatment consisted of maintaining CO₂ concentration in the chamber at low (50 ± 10 μl CO₂/l of air), normal (300 ± 30 μl/l), or high (2000 ± 100 μl/l) levels for 12.5 hr. An attempt was then made during the next 0.5 hr to equalize stomatal aperture among the three treatments by reducing the CO₂ concentration from the high treatment level to 50 μl/l and by increasing the low treatment level to about 300 μl/l. The normal treatment level

1 Contribution from the Purdue University Agricultural Experiment Station, West Lafayette, Ind. 47907. Journal Paper No. 5966.

2 Abbreviation: Pn: net photosynthetic rate; Γ: compensation concentration; rm: mesophyll resistance.
was unaltered. At the beginning of the 13th hour, the CO₂ concentration was returned to 300 µl/l in all treatments. Plants were removed singly for gas exchange measurements, which were made on the terminal leaflet of the lowermost trifoliate leaf.

Gas Exchange. Photosynthetic and transpiration rates were determined using a clamp-on assimilation chamber similar to that described by Čatský and Slavík (5). The chamber, formed by two closed-cell sponge rubber gaskets, was about 2.5 x 2.5 x 0.8 cm in size. A thermocouple pressed to the underside of the leaf measured leaf temperature, which was 25.6 ± 2.5 °C during the measurements.

A quantum flux (400-700 nm) of 1800 µmol/m² sec (70,200 lux) was supplied at leaf level by nine General Electric Cool Beam 150-w lamps filtered through 6 cm of water. Air of about 300 µl CO₂/l was supplied from a compressed-air cylinder. The air was humidified to a dew point of about 10 °C and entered the assimilation chamber at about 1.2 l/min. Dew point of the entering and exiting airstream was measured with a Vap-Air Model 84 dew point hygrometer. The difference between ingoing and outgoing CO₂ concentrations was measured using a Beckman Model 215A differential CO₂ analyzer.

Calculations of net CO₂ exchange rate and CO₂ diffusion resistances were made according to Gaastra (8) with the following modifications. Carbon dioxide compensation concentration (Γ'), determined in a separate experiment, was assumed to represent chloroplast CO₂ concentration (3). Boundary layer and stomatal diffusion resistances were calculated using the methods of Gale and Poljakoff-Mayber (9). Because of small differences in ambient CO₂ concentrations among measurements, calculated diffusion resistances were used to adjust Pn to an ambient CO₂ concentration of 300 µl/l.

Following measurement of gas exchange, the leaf was quickly removed, its area determined with a Hayashi AAM-5 area meter, and it was stored immediately at −20 °C until carbohydrate analysis.

CO₂ Compensation Concentration. A separate experiment was conducted in which the effect of carbohydrate concentration on Γ' was determined. Plants were treated with different CO₂ levels exactly as described above, then Γ' measurements were taken on the lowermost trifoliate leaf. A precision mixing valve was used to bleed 10% CO₂ into a humidified stream of CO₂-free air from a compressed-air cylinder. The mixing valve was adjusted until the differential CO₂ analyzer indicated zero net CO₂ exchange by the illuminated leaflet clamped into the assimilation chamber. At this point, Γ' was read directly from a Beckman Model 315 absolute CO₂ analyzer which measured the CO₂ concentration of the airstream exiting the differential analyzer.

Following the measurement the leaf was excised and stored as described above.

Carbohydrate Analyses. Leaves were freeze-dried, weighed, and ground through a 1-mm screen. Approximately 100 mg of this tissue were weighed into a 50-ml centrifuge tube with 15 ml of 95% (v/v) ethanol. The tubes were fitted with gas-release stoppers, heated at 80 °C for 30 min, then centrifuged for 15 min at 1800g. After decanting the supernatant, two more extractions were made, each with 10 ml of ethanol, for 30 and 60 min, respectively. Supernatant fractions were combined and brought to 35 ml with ethanol.

Reducing sugar and sucrose concentrations of the extract were found using Nelson’s test (14) and a modification of the resorcinol procedure (1), in which free fructose was destroyed by 0.5 N NaOH prior to sucrose determination. Data from these two tests were combined and are referred to as soluble sugar.

The residue from the ethanol extraction was dried overnight at 60 °C. One ml of ethanol and 15 ml of H₂O were added, and the tubes were placed in a boiling water bath for 30 min. After cooling, 10 ml of acetate buffer (pH 4.5) and 10 ml of 0.5% glucoamylase (“amyloglucosidase”) were added, the tubes were shaken and then covered and incubated for 44 hr at 39 °C. Following incubation, the contents were filtered and glucose concentration of the filtrate determined (14). Starch equivalent was obtained by multiplying the result by 0.9.

Each group of three different CO₂ treatments (eight plants/treatment) was designated as one block of a randomized complete block design for purposes of analysis of variance of the carbohydrate data (16).

RESULTS

Carbohydrate Concentration and Pₐ. Table I gives the mean carbohydrate concentrations of the leaves used in the gas exchange measurements. Starch concentration was significantly lower in plants kept at low CO₂ and significantly higher in plants kept at high CO₂ when compared (P < 0.05) to plants kept at normal CO₂ levels. Starch concentrations among individual leaves ranged from 0.14 to 3.19 mg/cm² leaf area. Net photosynthetic rate was regressed on starch concentration of individual leaves; the results are shown in Figure 1. Both the linear and quadratic components of the trend were significant at P < 0.05.

Soluble sugar concentrations did not differ significantly (P > 0.05) due to CO₂ treatments (Table I). However, individual leaf sugar concentrations ranged from 0.05 to 0.23 mg/cm² and there was a significant positive correlation (r = 0.39, P < 0.01) between Pₐ and soluble sugar concentration on an individual plant basis. Mean soluble sugar concentration was 0.15 mg/cm².

Carbohydrate Concentration and Diffusion Resistances. Boundary layer resistance to CO₂ diffusion was assumed to vary only with air flow rate and was nearly constant, ranging from 0.41 to 0.49 sec/cm among measurements.

Stomatal resistance to CO₂ diffusion did not vary significantly due to CO₂ treatments (P > 0.05), but ranged from 0.53 to 1.13 sec/cm among individual plants. The mean value was 0.73 sec/cm. Correlation between stomatal diffusion resistance and starch concentration among individual leaves (r = 0.22) was significant at P < 0.05. Correlation was not significant (P > 0.05) between stomatal diffusion resistance and soluble sugar concentration.

Figure 2 shows the result of regressing mesophyll resistance to CO₂ diffusion on starch concentration of individual leaves. Mesophyll resistance ranged from 2.77 to 8 sec/cm. Both the linear and quadratic components of the trend were significant (P < 0.05).

CO₂ Compensation Concentration. Mean leaf starch concentrations were significantly different (P < 0.05) among the three CO₂ treatments in the CO₂ compensation experiment (Table I). Individual leaf starch concentrations ranged from 0.43 to 2.32 mg/cm². Soluble sugar concentrations (Table I) were not significantly different (P > 0.05) among the three treatments; individual leaf values ranged from 0.08 to 0.17 mg/cm² with a mean of 0.12 mg/cm².

Regression of Γ on starch concentration and on soluble sugar concentration showed that there was no significant association (P > 0.05) between Γ and leaf carbohydrate level in these plants. The average Γ was 57.4 µl/l; this value was taken as the chloroplast CO₂ concentration in the calculation of mesophyll resistance.

DISCUSSION

Controlling the amount of CO₂ available to soybean plants was an effective means of altering leaf starch concentration but not soluble sugar concentration. These results are similar to those of Madsen (11), who found that starch concentration of tomato leaves rose with increasing levels of CO₂. He noted that soluble sugar concentration did not increase as CO₂ concentration was raised above 400 µl/l.
Gas exchange Starch per compensation CO2 plants

Means Carbohydrate resistance sugars block but increased relationship that after photosynthesis by accumulated soluble sugars. Data of Thorne and Koller (17), however, do not support this feedback hypothesis. The present study detected no feedback effect of accumulated soluble sugars. The small but significant positive correlation ($r = +0.39$) between $P_n$ and soluble sugar can probably be explained by the fact that soluble sugars are produced more rapidly in plants with higher $P_n$.

Figure 1 indicates that $P_n$ did not respond proportionately to an increase in starch concentration. The rate of decline in $P_n$ increased as the starch concentration increased. The data of Thorne and Koller (17) and of Upmeyer and Koller (19) show a greater sensitivity of $P_n$ to starch concentration than would be predicted by the present data. These differences may be due to the fact that leaves of different maturity were used in the two previous studies. The conclusion of Crookston et al. (7) that $P_n$ is not inhibited by accumulated starch in Phaseolus leaves may have been due to the fact that their maximum reported leaf starch level was about 0.2 mg/cm². This level of starch, according to our findings, would be too low to cause an appreciable decrease in $P_n$.

The data of Chatterton et al. (6) indicate a fairly severe depression in $P_n$ at relatively low starch concentrations in pango- lagrass, a C₄ plant. The increased sensitivity of $P_n$ to starch level in C₄ plants has been attributed to physiological and biochemical differences between C₃ and C₄ plants (7).

In the present study, changes in boundary layer and stomatal diffusion resistances played little part in the reduction of $P_n$. Boundary layer resistance was nearly constant among plants and accounted for about 9% of total resistance to CO₂ flux. The weak positive correlation between stomatal resistance and starch concentration probably indicates that the technique used to equalize stomatal aperture prior to gas exchange measurements was not entirely successful. High treatment CO₂, which produced high starch levels, probably also caused partial closure of the stomata (8). This effect may have partially carried over into the $P_n$ measurements and caused the small correlation between stomatal resistance and starch concentration.

The reduction of $P_n$ in leaves with high starch concentration was primarily due to increased $r_m$. This resistance is calculated as the difference between total and vapor phase diffusion resistances to CO₂ flux. It may contain components that are not purely diffusive in nature (10). One such component may be a "photochemical resistance" due to conditions under which light is not saturating. Wildman (21) has speculated that starch grain formation may cause disorientation of chloroplasts and result in less light interception. However, $P_n$ was measured, in the present study, at a light intensity about 3-fold higher than that at which the plants were grown. Since $P_n$ light saturates at about the intensity under which plants are grown (2), it was assumed that light was not limiting photosynthesis in the present study.

Another possible nondiffusive component of $r_m$ is the "biochemical resistance" associated with carboxylation (10). We attempted to eliminate this component from the measured $r_m$ by utilizing $\Gamma$ as the chloroplast CO₂ concentration (10). The con-
stancy of $\Gamma$ among treatments suggests that the biochemical resistance probably did not vary significantly due to starch concentration.

The increase in $r_m$ at high starch concentrations was apparently due to an increase in the diffusion resistance to CO$_2$ flux in the cell. Much of this increase may have resulted from an increase in the pathlength of diffusion. Rackham (15) concluded from microscopic investigation that starch accumulation may increase the diffusion pathlength considerably.

There may also be other mechanisms by which starch accumulation could increase observed $r_m$. Cytoplasmic streaming could be a means of facilitating CO$_2$ transfer to the chloroplasts (12). The enlargement of chloroplasts due to starch granule growth may cause the chloroplasts to protrude farther toward the center of the cell, thus reducing cytoplasmic streaming and resulting in less efficient transfer of CO$_2$.

Results of this study indicate that $P_n$ was negatively associated with the concentration of starch in soybean leaves. The decline in $P_n$ as starch concentration increased, was the result of increasing $r_m$. This suggests that starch accumulation may reduce $P_n$ by impeding intracellular CO$_2$ transport.

**LITERATURE CITED**


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