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Influence of Leaf Starch Concentration on CO_2 Assimilation in Soybean[1]

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ABSTRACT

Net photosynthetic rate, CO_2 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 CO_2 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/m^2·sec (30,090 lux) at plant level. On the 21st day after seeding, plants were subjected for 12.5 hours to one of three CO_2 concentrations (50, 300, or 2000 μl/l) in an attempt to alter leaf carbohydrate levels. Following the CO_2 treatment, gas exchange measurements were made at a CO_2 concentration of 300 μl/l on the lowermost trifoliolate leaf. Immediately after measurement, the leaf was removed and stored at −20 C until carbohydrate analyses were performed.

Increasing the CO_2 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 CO_2/dm^2 leaf area·hr as starch concentration increased from 0.5 to 3 mg/cm^2 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) CO_2 diffusion resistance. This suggests that starch accumulation may reduce net photosynthetic rate by impeding intracellular CO_2 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 P_n.[2] 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 P_n 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 P_n. Upmeyer and Koller (19) found that P_n began to decline when starch reached a high level in the afternoon. Thorne and Koller (17) noted that P_n 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 P_n 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 P_n (13), we chose to alter carbohydrate levels by controlling the amount of CO_2 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 P_n should be possible. Effects of carbohydrate concentration on the components of CO_2 diffusion resistance were observed in order to better understand the mechanism by which a carbohydrate buildup may affect P_n.

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/m^2·sec (30,090 lux). Relative humidity was maintained at about 50%.

CO_2 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/m^2·sec (23,600 lux). Relative humidity in the plant chamber was 65 ± 10%.

Treatment consisted of maintaining CO_2 concentration in the chamber at low (50 ± 10 μl CO_2/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 CO_2 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

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[2] Abbreviation: P_n: net photosynthetic rate; Γ: compensation concentration; r_m: mesophyll resistance.

was unaltered. At the beginning of the 13th hour, the CO_2 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 trifoliolate 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 × 2.5 × 0.8 cm in size. A thermocouple pressed to the underside of the leaf measured leaf temperature, which was 26.5 ± 2.5 C during the measurements.

A quantum flux (400–700 nm) of 1800 ± 100 μeinsteins/m^2·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 CO_2/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 CO_2 concentrations was measured using a Beckman Model 215A differential CO_2 analyzer.

Calculations of net CO_2 exchange rate and CO_2 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 CO_2 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 CO_2 concentrations among measurements, calculated diffusion resistances were used to adjust P_n to an ambient CO_2 concentration of 300 μl/l.

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

CO_2 Compensation Concentration. A separate experiment was conducted in which the effect of carbohydrate concentration on Γ was determined. Plants were treated with different CO_2 levels exactly as described above, then Γ measurements were taken on the lowermost trifoliolate leaf. A precision mixing valve was used to bleed 10% CO_2 into a humidified stream of CO_2-free air from a compressed-air cylinder. The mixing valve was adjusted until the differential CO_2 analyzer indicated zero net CO_2 exchange by the illuminated leaflet clamped into the assimilation chamber. At this point, Γ was read directly from a Beckman Model 315 absolute CO_2 analyzer which measured the CO_2 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 H_2O 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 were 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 CO_2 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 P_n. 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 CO_2 and significantly higher in plants kept at high CO_2 when compared ($P < 0.05$) to plants kept at normal CO_2 levels. Starch concentrations among individual leaves ranged from 0.14 to 3.19 mg/cm^2 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 CO_2 treatments (Table I). However, individual leaf sugar concentrations ranged from 0.05 to 0.23 mg/cm^2 and there was a significant positive correlation ($r = +0.39$, $P < 0.01$) between P_n and soluble sugar concentration on an individual plant basis. Mean soluble sugar concentration was 0.15 mg/cm^2.

Carbohydrate Concentration and Diffusion Resistances. Boundary layer resistance to CO_2 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 CO_2 diffusion did not vary significantly due to CO_2 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 CO_2 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 ($P < 0.05$).

CO_2 Compensation Concentration. Mean leaf starch concentrations were significantly different ($P < 0.05$) among the three CO_2 treatments in the CO_2 compensation experiment (Table I). Individual leaf starch concentrations ranged from 0.43 to 2.32 mg/cm^2. 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/cm^2 with a mean of 0.12 mg/cm^2.

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 CO_2 concentration in the calculation of mesophyll resistance.

DISCUSSION

Controlling the amount of CO_2 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 CO_2. He noted that soluble sugar concentration did not increase as CO_2 concentration was raised above 400 μl/l.

Table I. *Mean Starch and Soluble Sugar Concentrations of Lowermost Trifoliolate Leaves of 21-day-old Soybean Plants after 13 Hr in Light*

Experiment	Carbohydrate	Treatment CO_2 Concn (μl/l)					
		50		300		2000	
		mg/cm²	% dry wt	mg/cm²	% dry wt	mg/cm²	% dry wt
Gas exchange	Starch	0.636 (0.098)[1]	14.02	1.294 (0.138)	24.28	1.808 (0.165)	30.48
	Soluble sugar	0.133 (0.006)	2.98	0.143 (0.010)	2.70	0.168 (0.009)	2.87
CO_2 compensation concn	Starch	0.670 (0.043)	14.87	1.289 (0.055)	24.65	2.055 (0.086)	34.91
	Soluble sugar	0.115 (0.009)	2.59	0.127 (0.010)	2.42	0.132 (0.008)	2.25

[1] Means of the gas exchange experiment are of four blocks and means of the CO_2 compensation experiment are of two blocks. There were eight plants per block per treatment. Numbers in parentheses are the standard error of the mean.

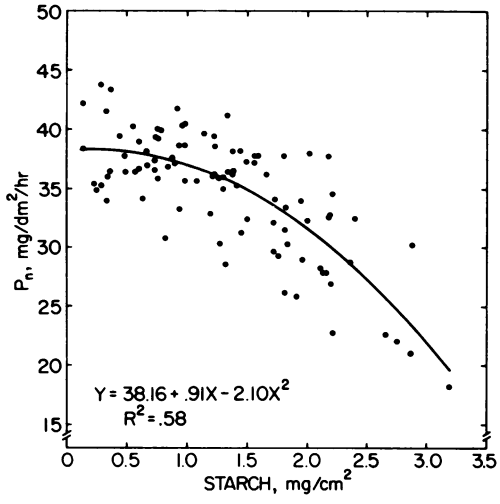

FIG. 1. Relationship between leaf starch concentration and net photosynthetic rate (P_n) of the lowermost trifoliolate leaves of 21-day-old soybean plants after 13 hr in the light.

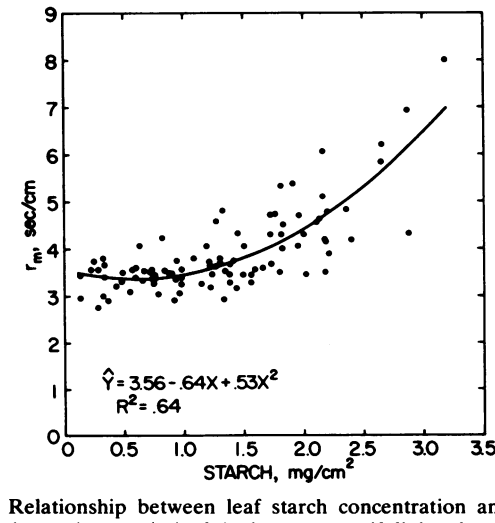

FIG. 2. Relationship between leaf starch concentration and mesophyll diffusion resistance (r_m) of the lowermost trifoliolate leaves of 21-day-old soybean plants after 13 hr in the light.

The fact that raising the CO_2 level had no effect on soluble sugar concentration but increased starch concentration indicates that soluble sugars were rapidly converted to starch. Warren-Wilson (20), Burt (4), and others (13) have proposed a feedback control of 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 pangolagrass, a C_4 plant. The increased sensitivity of P_n to starch level in C_4 plants has been attributed to physiological and biochemical differences between C_3 and C_4 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 CO_2 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 CO_2, 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 CO_2 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 Γ as the chloroplast CO_2 concentration (10). The con-

stancy of Γ 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.

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