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Diurnal Trends in Net Photosynthetic Rate and Carbohydrate Levels of Soybean Leaves[1]

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ABSTRACT

A study was made of diurnal trends in net photosynthetic rate and carbohydrate levels of unifoliolate leaves of soybean (*Glycine max* L. Merrill) under constant environmental conditions (50,000-lux light intensity, 24.5 C air temperature, 60% relative humidity, and 300 microliters of CO_2 per liter of air).

Net photosynthetic rate remained relatively constant between 4 and 10 hours after the lights were turned on but then gradually declined to 85% of this rate by the end of the 16-hour photoperiod. The decline in net photosynthetic rate was due to increases in both stomatal diffusion resistance and residual resistance to CO_2.

The decline in net photosynthetic rate began when the rate of starch accumulation began to decline rapidly. At this time, there also appeared to be an increase in soluble carbohydrate level. The results suggest that when a high starch level was reached, further starch synthesis was impaired, leading to an increase in soluble carbohydrate level and, consequently, a reduction in net photosynthetic rate.

The subject of a recent review by Neales and Incoll (14) was the hypothesis that assimilate accumulation reduces leaf photosynthetic rate. It is notable that much of the evidence for the correlation between assimilate level and photosynthetic rate has been obtained in experiments employing treatments designed to increase the level of leaf assimilates above normal levels. In the study reported here, we evaluated the association between diurnal trends in net photosynthetic rate and carbohydrate level of young, unifoliolate leaves of soybean (*Glycine max* L. Merrill). The daily accumulation of leaf carbohydrates provided a range of normal assimilate levels and therefore eliminated some of the risks imposed by artificial treatments.

Tsuno and Fujise (17) performed similar experiments with sweet potato (*Ipomoea batatas*) and determined that there was no reduction in photosynthetic rate, in spite of an increase in leaf carbohydrates, up to 4:00 PM, at which time their measurements were terminated. We present evidence that later in the day there is a reduction in net CO_2 assimilation rate of soybean leaves, which may be associated with leaf carbohydrate level.

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EXPERIMENTAL PROCEDURE

Plant Culture. Beeson soybean seedlings for use in carbohydrate analyses and gas exchange measurements were grown in a controlled environment room with a 16-hr photoperiod (8:00 AM to 12:00 M). Light was supplied by a mixture of fluorescent and tungsten-filament lamps yielding approximately 40,000 lux at plant level. Approximately 90% of the total illumination was furnished by the fluorescent lamps. Air temperature was 24.0 ± 0.5 C in the light and 22.5 ± 0.5 C in the dark. Relative humidity was maintained at approximately 50%.

Seeds were planted in vermiculite saturated with deionized water. Seedlings were transplanted into nutrient solution 4 days later. Each seedling was placed in an aerated plastic pot containing 750 ml of nutrient solution (0.05 mM KH_2PO_4, 1.0 mM KNO_3, 1.0 mM $Ca[NO_3]_2$, 0.1 mM NH_4NO_3, and the micronutrient concentrations of Johnson *et al.* [6]). One milliliter of 2 mM $FeSO_4$ was added to each pot every 3 or 4 days.

Unifoliolate leaves emerged by the 7th day after planting and attained near-maximal area by the 13th day. The first trifoliolate leaf emerged by the 12th day.

Carbohydrates. Unifoliolate leaf laminae were cut from the plants, and lamina area was determined from paper tracings with a polar planimeter.

The freshly cut leaves were placed immediately into an oven at 100 C for 40 min. After continued drying to constant weight at 60 C, the leaves were weighed and ground through a 20-mesh screen in a small Wiley mill.

Approximately 100 mg of dried, ground plant material were placed into a 50-ml centrifuge tube with 20 ml of 80% (v/v) ethanol. The tubes were heated at 80 C in a water bath for 1 hr. After centrifuging at 1800*g* for 15 min the solute was decanted. A second washing was made, and the total extract was brought to volume. A sucrose equivalent of total carbohydrate in this extract was determined by the phenol-sulfuric acid procedure (3). This fraction shall be referred to as sugar.

The residue was allowed to dry at room temperature. Ten milliliters of water, 10 ml of acetate buffer (pH 4.5), and 10 ml of filtered 0.5% glucoamylase ("amyloglucosidase") were added. This enzyme preparation was allowed to incubate for 48 hr at 38 C. Owing to the presence of background carbohydrate in the enzyme preparation, an enzyme blank was included with the samples.

After incubation the samples were filtered with Whatman No. 42 paper, and an aliquot was diluted for analysis of total carbohydrate by the phenol-sulfuric acid method. A glucose equivalent of this fraction was multiplied by 0.9 to obtain starch equivalent weight. This fraction shall be referred to as starch.

Gas Exchange. CO_2 and water vapor exchange measurements were made with an apparatus similar in basic design to that

described by Gaastra (2). A Vap-Air model 84 dew point hygrometer was used for the measurement of water vapor concentration. The acrylic leaf chamber contained an effective air-circulating fan and was water-jacketed for temperature control. The chamber accepted both of the plant's unifoliolate leaves, which were held in place by nylon string. The emerging trifoliolate leaf tissue was removed from the plant just prior to placement into the chamber. The root system was placed in an external container of aerated nutrient solution. Leaf temperature was measured with thermocouples held against the lower side of the leaf. Air temperature was monitored with shaded thermocouples.

Plants remained sealed in the leaf chamber throughout the measurement period (up to 16 hr). An attempt was made to provide constant environmental conditions, similar to those of the controlled environment room. A light intensity at leaf level of approximately 50,000 lux was produced by four General Electric Cool Beam 150-w lamps, filtered through 6 cm of water. Air temperature was 24.5 ± 0.5 C, and leaf temperature was 25.0 ± 0.5 C. Relative humidity was maintained at approximately 60% and CO_2 concentration (in air) at approximately 300 μl/liter.

Calculations of net photosynthetic rate and CO_2 diffusive resistances were carried out using methods described by Gaastra (2), with the following exceptions. The CO_2 and water vapor concentrations of air exiting from the chamber were assumed to be those external to the leaf. Preliminary measurements indicated that photosynthetic response to CO_2 concentration was linear to beyond 300 μl/liter, with a CO_2 compensation point of 40 μl/liter. The latter value was taken as the CO_2 concentration at the chloroplast (1). We did not partition the vapor phase diffusion resistance into stomatal and external air resistances but refer to these collectively as vapor phase diffusion resistance. Our "residual" resistance is equivalent to Gaastra's "mesophyll" resistance.

RESULTS AND DISCUSSION

Although carbohydrate analyses and gas exchange measurements were made on both 11- and 12-day-old plants, only the results from 11-day-old plants will be discussed. Trends in carbohydrate levels and gas exchange were essentially identical in both groups.

Unifoliolate leaf starch and sugar levels and leafy density (dry weight per unit area) were measured in a 24-hr experiment involving 11-day-old plants (age at beginning of experiment). Results are shown in Figure 1. Carbohydrates are presented on a leaf area basis.

Differences in sugar level throughout the day were statistically significant ($P < 0.01$). The regression line (Fig. 1) suggests that sugar level increased slightly for 3 to 4 hr after lights were turned on, then remained relatively constant for an additional 4 to 6 hr, followed by a further increase beginning 4 to 6 hr before darkness. Owing to the variability in sugar data, it is uncertain that these trends are real. However, it is apparent that sugar level increased during the light period by approximately 6 mg × dm^{-2}. Average sugar level was 20.2 mg × dm^{-2} (7.0% of total leaf dry weight).

Starch level increased during the day by approximately 31 mg × dm^{-2} (Fig. 1). Starch accumulation was most rapid between 3 and 10 hr after lights on. After about 10 hr of light, rate of starch accumulation decreased rapidly. Most of the starch that accumulated during the 16-hr day was removed during the 8-hr night. Average starch level was 35.5 mg × dm^{-2} (11.9%).

Average leaf density was 2.2 mg × cm^{-2} (Fig. 1). Diurnal change in measured carbohydrates accounted for approximately one-half the change in leaf density.

Carbon dioxide and water vapor exchange measurements were made during the lighted period on unifoliolate leaves of 11-day-old plants treated similarly to those used for carbohydrate analyses. Shown in Figure 2 are diurnal trends in net photosynthesis, CO_2 vapor phase diffusion resistance, and CO_2 residual resistance. Plant 2 was moved into the leaf chamber later in the day in order to detect any possible effects of the measurements on long term trends in gas exchange. The higher vapor phase diffusion resistance and consequent lower net photosynthetic rate early in the measurement period (Fig. 2) was apparently due to reduction in stomatal aperture, a result

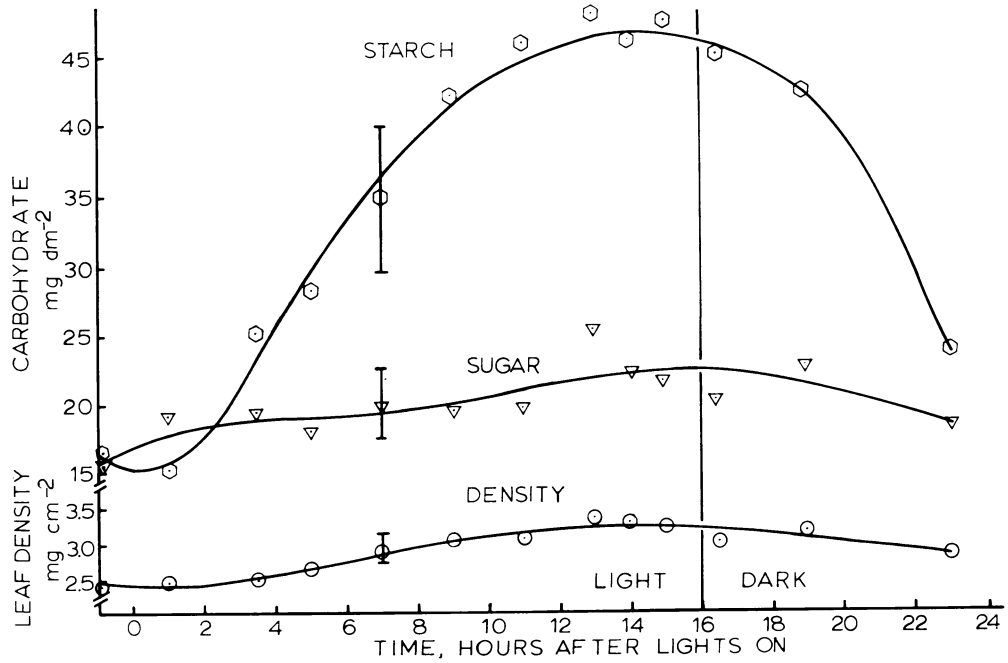

FIG. 1. Diurnal trends in unifoliolate leaf carbohydrate levels and leaf density of 11-day-old soybean plant. Plotted curves are fifth-order polynomial fits on means of four plants per sampling time. Vertical bars represent 95% confidence interval of means. Lights were turned on at time 0 (8:00 AM).

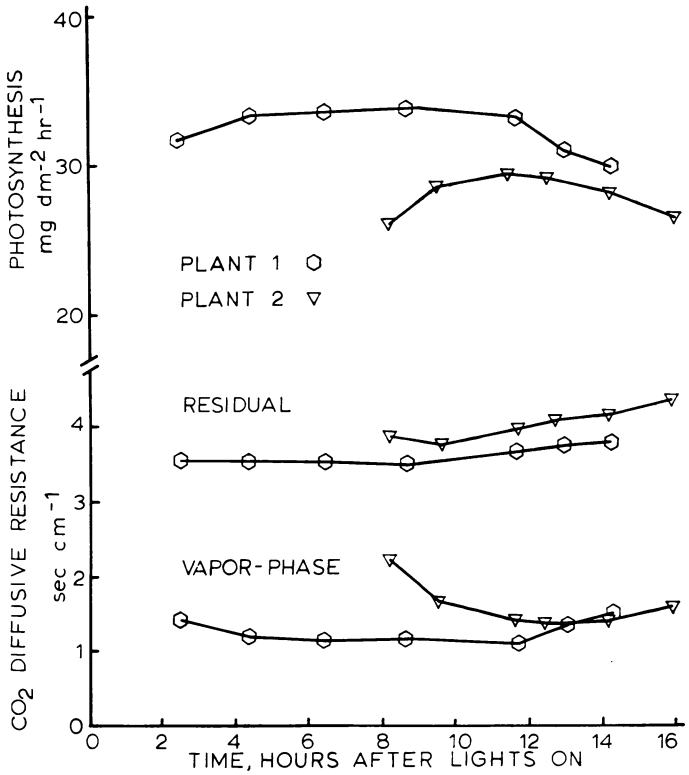

FIG. 2. Diurnal trends in unifoliolate leaf net photosynthetic rate and CO_2 diffusive resistances of 11-day-old soybean plants. Each point represents mean of three observations within 10-min period. Net photosynthetic rates are adjusted to 300 μl/1 ambient CO_2 concentration. Lights were turned on at time 0 (8:00 AM).

of plant manipulation during placement into the chamber. The data indicate, however, that duration of the measurement period had no significant long term influence on gas exchange. Net photosynthetic rates shown in Figure 2 were adjusted to 300 μl/liter ambient CO_2 concentration, using the calculated total diffusion resistance.

Diurnal change in net photosynthetic rate has been observed previously. In many instances, such change has been attributed to changing environmental conditions (12, 13, 15, 16). There are reports, however, of diurnal change in photosynthetic rate in constant environments (4, 5, 10). Diurnal change in photosynthetic rate under changing environmental conditions has also been attributed to factors other than the changing environment (7).

Our results indicate that net photosynthetic rate of young, unifoliolate soybean leaves may change during the normal photoperiod under constant environmental conditions (Fig. 2). The necessity to manipulate plants just prior to the initial measurements precluded establishment of the real trend in net photosynthetic rate during the early portion of the lighted period. From 4 until approximately 10 hr after lights on the rate remained relatively constant, but then it gradually declined until darkness to approximately 85% of the midday rate.

The conditions under which these measurements were made (saturating light, optimal temperature, and limiting CO_2) permitted an evaluation of the changes in photosynthetic rate on the basis of CO_2 diffusive resistances (2). The decline in net CO_2 assimilation during the evening was due to increases in both vapor phase and residual resistances to CO_2 (Fig. 2). Since our leaf chamber contained an effective fan operating at constant speed, we assume that any change in vapor phase diffusive resistance resulted from change in stomatal resistance. Residual resistance, as measured, may include chemical resistances as well as physical diffusive resistance (8, 11).

Since net photosynthetic rate declined in a constant environment, it is apparent that an internal factor or factors were responsible for the decline. The mechanism whereby this factor(s) depressed the rate of CO_2 uptake involved (*a*) a decrease in mean stomatal aperture and (*b*) an increase in the liquid phase resistance to CO_2 transport through the mesophyll and/or increased chemical resistances in the chloroplasts. The possibility exists, of course, that stomatal and residual resistances were influenced by different internal factors (or different levels of the same factor). There is some indication (Fig. 2) that the incline in residual resistance may have preceded that of stomatal resistance. It is conceivable that an increase in residual resistance could lead indirectly to increased stomatal resistance, *e.g.*, as a result of higher intercellular CO_2 concentration (9).

Although the results do not establish that carbohydrate buildup caused the reduction in photosynthetic rate, there are associations between carbohydrate and photosynthetic trends which suggest that the two are related. Although starch level increased substantially during the midday period while photosynthetic rate remained constant, photosynthetic rate began to decline when rate of starch accumulation began to decline rapidly (*cf.* Figs. 1 and 2). This is also the point at which there appeared to be a further increase in sugar level (Fig. 1). Calculations indicate that the reduction in total carbohydrate accumulation rate was approximately equivalent to the reduction in net photosynthetic rate in terms of carbohydrate production. These results suggest that, when a high starch level was reached, further starch synthesis was impaired, leading to an increase in soluble carbohydrate level and, consequently, a reduction in net photosynthetic rate.

Various hypotheses have been proposed regarding the mechanism by which a buildup of sugars or starch or both might depress photosynthetic rate (14). It was suggested that assimilate accumulation might lead to increased stomatal or mesophyll diffusion resistance (14). If it is assumed that in the present experiments carbohydrate level influenced net photosynthetic rate, any proposed mechanism should explain the basis of the increases in both stomatal and residual resistances to CO_2. It would appear necessary also to determine which component or components of residual resistance are affected by carbohydrate buildup.

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