Short Communication

Maintenance of High Photosynthetic Rates during the Accumulation of High Leaf Starch Levels in Sunflower and Soybean

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ABSTRACT

Sunflower (cv. "Mammoth Greystripe") and soybean (Merr. cv. "Amsoy 71") leaves were exposed to continuous light for at least 52 hours in an attempt to determine the relationship between leaf starch levels and photosynthetic rates. Immature rapidly expanding and relatively mature slowly expanding sunflower leaves were studied. After 52 hours continuous light, the rapidly expanding leaves accumulated high starch levels (3.3 milligrams per square centimeter, 43% of dry weight) with only about a 10% decline from the initial photosynthetic rate of 42 milligrams CO₂ per square decimeter per hour. Under the same conditions, the slowly expanding leaves accumulated less starch, but the photosynthetic rate declined 30%. Soybean leaves, which were slowly expanding, accumulated less starch than sunflower leaves (2.1 milligrams per square centimeter, 34% of dry weight), and their photosynthetic rates declined only about 10% after 54 hours continuous light.

In sunflower and, to a lesser extent, in soybean, the accumulation of large amounts of leaf starch was not necessarily associated with an appreciable decline in photosynthetic rate. However, in sunflower, the stage of leaf maturity was a major determinant in the photosynthetic response to continuous, relatively high light with its associated starch accumulation.

A number of investigators have tested the hypothesis that high leaf starch inhibits photosynthesis (4-7, 10, 12, 13). Some have concluded that the accumulation of starch has no effect on photosynthetic rates (5-7), but the highest starch concentration presented in these reports was 23% on a dry weight basis (5) or 0.3 mg cm⁻² (6).

Several authors have reported that photosynthetic rates declined when leaf starch content increased (4, 10, 12, 13), although Mauney et al. (10) found that only one of four species tested gave a negative correlation between leaf starch content and photosynthetic rates. Naefziger and Koller (13) concluded that, at lower levels, starch does not significantly inhibit photosynthesis. They suggested that the failure of some investigators to find correlations between high leaf starch and decreased photosynthetic rates is due to the shape of the starch content versus photosynthetic rate curve.

Their data with soybean indicate that only a 10% decrease in photosynthetic rate was associated with a leaf starch content of 1.5 mg cm⁻² or about 28% starch (dry weight basis) and that, to observe large decreases in photosynthetic rates, leaves must contain over 2.0 mg starch cm⁻².

Any experiment drastic enough to alter substantially the starch content can potentially alter the photosynthetic rate independently of starch content (14). Also, any finding that photosynthetic rates were unaffected by high starch levels invites the criticism that the treatment was not drastic enough. This work is an attempt to achieve a balance between these two constraints.

MATERIALS AND METHODS

Plant Material. Sunflower (Helianthus annuus L. cv. "Mammoth Greystripe") and soybean (Glycine max [L.] Merr. cv. "Amsoy 71") plants were grown from seed in a controlled environment in 15-cm diameter pots containing 1.5 1 Jiffy® mix (a mixture of peat and Vermiculite). Plants were watered daily and received nutrient solution twice weekly.

Day/night temperatures were 27/24 C and a mixture of fluorescent and incandescent light was provided for 14 h daily at 850 ± 50 µE m⁻² s⁻¹ (400-700 nm) at the level of leaves to be measured. Sunflower leaves at the second or third node above the cotyledons (plants 14 to 19 days past emergence) and soybean leaves at the third trifoliolate (plants 18 to 25 days past emergence) were used for photosynthesis measurements. The areas of cut-out tracings of leaves were measured with an electronic area meter.

Photosynthesis and Leaf Diffusive Resistance. A single attached leaf was sealed in a semiclosed assimilation chamber (2) at 25 ± 0.1 C and 48 ± 2.0% RH with a quantum flux density of 1,100 µmol m⁻² s⁻¹ (400-700 nm). The CO₂ concentration in the chamber was 320 ± 15 µmol l⁻¹ and the photosynthetic rate was determined by measuring the time required for the leaf to reduce the CO₂ concentration from 335 to 305 µmol l⁻¹ while the system was closed. The leaf remained in the chamber during the entire exposure period, and photosynthetic and transpiration rates were measured sequentially about 1 min apart. Transpiration water was condensed and drained through a tube submerged under 5 mm H₂O.

1 Oregon Agricultural Experiment Station Technical Paper No. 5276.

2 Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
Aliquots of CO₂ (as 5% CO₂ in air) were injected to restore the amount fixed by the leaf.

The transpiration rate and the rate of water loss by a wetted paper leaf model were determined by measuring the time required for the leaf or model to increase the water vapor content of the chamber air by 8.8 μl. The change in water vapor content was measured as a change in RH monitored with a LiCl element calibrated against a steady-state flow of water vapor through the assimilation chamber. Stomatal and boundary layer resistances were determined by the method of Gaasstra (8), modified so that the diffusion coefficient ratio for H₂O/CO₂ = 1.56 (9).

Starch and Sugar Analysis. At selected intervals, the assimilation chamber was opened, and a disc (4.0 cm²) was cut from the leaf, avoiding major veins. Half of each disc was dried at 70°C to determine the specific leaf weight, whereas the other half was ground with a mortar and pestle in 15 mL 80% ethanol. The homogenate was boiled for 2 min and centrifuged at 12,000×g for 5 min. The supernatant fraction was decanted, and the boiling ethanol extraction and centrifugation were repeated twice. The supernatant fractions were combined and the pellet was dried at 60°C.

The dried pellet was dispersed in 10 ml 100 mM acetate buffer (pH 4.8) and boiled for 10 min. Starch was digested with 20 mg amyloglucosidase (Grade II, Sigma) at 55°C for 90 min (3). The digest was centrifuged at 12,000×g for 5 min and a 30-μL aliquot of the supernatant fraction was analyzed for glucose using glucose oxidase (Statzyme, Worthington). Corn starch (Sigma) suspensions were used as standards, and the moisture content was accounted for.

The combined supernatant fractions, containing the soluble sugars, were dried at 100°C, dissolved in 1.0 mL boiling acetate buffer (100 mM; pH 4.8), and assayed for glucose as above. The sucrose content was also determined with glucose oxidase following digestion for 30 μL of the soluble sugar fraction for 90 min at 55°C with 10 μL of a suspension of invertase (Grade V, Sigma) containing 1.0 mg ml⁻¹ acetate buffer (100 mM; pH 4.8). The sucrose concentration of the digest was calculated after correcting for the presence of glucose before digestion. Glucose and sucrose were used as standards for their respective assays.

RESULTS

Rapidly expanding sunflower leaves maintained net photosynthetic rates within 10% of initial rates, while the leaf starch content increased 14-fold to 3.3 mg cm⁻² (43% dry weight) during 52 h continuous light (Table I). Photosynthetic rates, initially over 40 mg CO₂ dm⁻² h⁻¹, fluctuated between adjacent measurements, and there was a suggestion of a mid-day maximum (Fig. 1). The photosynthetic rates and stomatal resistances in Table I are means of hourly rates determined between 10:00 and 16:00 h. Figure 1 shows that the rate of starch accumulation per unit leaf area was constant for the first 44 h and then declined during the final 8 h.

The discontinuities in photosynthetic rate and stomatal resistance (Fig. 1) represent partial stomatal closure and an associated drop in photosynthetic rate caused by opening the chamber and cutting leaf discs. Photosynthetic rates returned to previous values within 1 h after the chamber was resealed.

Sunflower leaves could be separated into two groups based on their rate of expansion and their decline in photosynthesis. In one group (rapidly expanding), the leaf area increased over 20% in 52 h of continuous light, during which the photosynthetic rate declined by less than 10%. In the second group (slowly expanding), leaves expanded less than 10% and photosynthetic rates decreased by over 30% during the assimilation period (Table I). The mean leaf area of each group at the beginning of the 52-h light period was 1.5 dm². For the rapidly expanding leaves, stomatal resistance did not change during the 52-h light exposure, but it increased 40% under the same conditions for the slowly expanding leaves (Table I).

The sucrose content of sunflower leaves increased by as much as 3.5 times the starting value, but the final values were less than 0.2 mg cm⁻² (Table I). Glucose increased slightly in rapidly expanding leaves but remained essentially unchanged in slowly expanding leaves (Table I).

Because of the differences shown in Table I between rapidly and slowly expanding sunflower leaves, the time course of leaf area expansion and photosynthetic rates was followed for plants exposed to normal diurnal light-dark cycles. Plants remained in the growth chamber (14-h photoperiod) except for 90 min each day when photosynthesis measurements were made. The photoperiod measurements were made 1.5 to 9 h after the beginning of the photoperiod. The photosynthetic rate of sunflower leaves reached a maximum of about 42 mg CO₂ dm⁻² h⁻¹, which was maintained for only 1 or 2 days before the rate declined sharply (Fig. 2). The photosynthetic rates decreased before the leaf area reached final size.

As with the rapidly expanding sunflower leaves, the photosynthetic rate of the soybean leaves declined only 10% during 54 h of continuous light, while leaf starch increased 14-fold to 2.10 mg cm⁻² (34% dry weight) (Table I). The decrease in photosynthetic rate was accompanied by a 25% increase in stomatal resistance (Table I). The soybean leaves were enlarging very slowly, less than 5% in 54 h, and had initial photosynthetic rates about two-thirds those of sunflower. The sucrose content in the soybean leaves increased over 3-fold during the first 31 h and then rose more slowly, whereas glucose remained essentially constant.

DISCUSSION

The accumulation of high concentrations of starch was not associated with large decreases in the photosynthetic rates of either sunflower or soybean leaves (Table I). The differences in photosynthetic rates were associated with the rate of leaf expansion, and these differences were maintained throughout the measurement period.
The two populations of sunflower leaves which we sampled may have differed in more than just their expansion rate and photosynthetic response to constant light. Since both groups had similar areas at the start of the 52-h light period, those expanding slowly were likely more mature and presumably would have attained a smaller final size than the rapidly expanding leaves. Although the study was conducted over a period of several months, no clear relationship existed between the leaf type and the period when it was grown.

Others have reported declining photosynthetic rates under constant environmental conditions and they attributed at least some of the decline to factors other than starch or sugar accumulation (15, 16, 18). Here, at least part of the decrease in photosynthetic rate of soybean and slowly expanding sunflower leaves was due to increased stomatal resistance. The stomatal resistance of the rapidly expanding sunflower leaves was nearly constant during the 52 h light. Mesophyll resistances were not calculated because the plants were not light-saturated during photosynthesis measurements.

The disagreement among published accounts of the relationship between leaf starch and photosynthetic rates could result from variations in leaf age or other associated factors. Nafziger and Koller (13) suggested that the stage of leaf maturity is a determinant in the sensitivity of the photosynthetic system to starch concentration. One interpretation of the data presented here is that the photosynthesis apparatus of the relatively mature slowly expanding sunflower leaves is more sensitive to starch accumulation than that of the rapidly expanding leaves. However, the authors feel that the decrease in photosynthetic rates with time is more likely due to normal ontogenetic changes.

A decline in photosynthetic rates in sunflower leaves before full expansion was also reported by McWilliam et al. (11). Silvius et al. (17) found a similar, but less dramatic, pattern with soybean. The sunflower leaves used to generate the data in Table 1 were near the peak of photosynthetic activity described in Figure 2 because all the initial photosynthetic rates were about the same. However, in selecting leaves according to a given size, it would be easy to miss the peak by 1 day. If the slowly expanding sunflower leaves were at or beyond the photosynthetic peak, their photosynthetic rate would decline rapidly in the subsequent days, regardless of starch accumulation. Alternatively, measurements of photosynthetic rates of soybean and rapidly expanding sunflower leaves were probably initiated before the photosynthetic decline associated with leaf ontogeny.

Sucrose did not accumulate to a high concentration in either soybean or sunflower leaves; the values were always less than 3% of dry weight. Although soluble sugars have been suggested as possibly inhibiting photosynthesis by some feedback mechanism, Austin (1) concluded that the photosynthetic rate in sugar beet leaves was probably not inhibited until the sugar concentration reached over 30%, a 10-fold higher concentration than that reached in the present study.

Experiments designed to test the effect of starch on photosynthetic rates are inherently difficult to interpret because any manipulation that causes starch to accumulate can potentially alter the photosynthetic apparatus (14). Conclusions that starch interferes with photosynthetic rates should be made only if evidence can be provided that the treatment responsible for starch accumulation did not concurrently alter the photosynthetic apparatus. The extremely small decline in photosynthetic rates in the presence of massive accumulations of starch in the soybean and sunflower leaves reported here make it unlikely that mechanisms of starch inhibition of photosynthesis based on interference with CO₂ diffusion (13) or light absorption (19) were operating to an appreciable extent.

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LITERATURE CITED