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Communication

Carbon Accumulation during Photosynthesis in Leaves of Nitrogen- and Phosphorus-Stressed Cotton

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ABSTRACT

Leaves of cotton (*Gossypium hirsutum* L.) accumulate considerable dry mass per unit area during photosynthesis. The percentage of C in that accumulated dry mass was estimated as the regression coefficient (slope) of a linear regression relating C per unit area to total dry mass per unit area. Plants were grown on full nutrients or on N- or P-deficient nutrient solutions. In the fully nourished controls, the mass that accumulated over a 9-hour interval beginning at dawn contained 38.6% C. N and P stress increased the C concentration of accumulated mass to 49.7% and 45.1%, respectively. Nutrient stress also increased the starch concentration of accumulated mass, but starch alone could not account for the differences in C concentration. P stress decreased the estimated rate of C export from source leaves, calculated as the difference between C assimilation and C accumulation. The effect of P stress on apparent export was very sensitive to the C concentration used in the calculation, and would not have been revealed with an assumption of unchanged C concentration in the accumulated mass.

In recent years much interest has developed in C balances of photosynthesizing leaves, primarily for the purpose of calculating rates of assimilate export (4, 8–12, 21, 22, 24). In a mature (nonexpanding) leaf, export can be estimated simply as the difference between C gained by photosynthesis and C accumulated in the leaf. The latter quantity is often estimated from increases in leaf mass, assuming that all the increased mass is carbohydrate of the formula CH_2O, *i.e.* 40% C (4, 8, 10–12, 21).

Although conceptually simple and theoretically applicable under all circumstances, such C balances must be interpreted with caution when applied to plants under different growth conditions (*e.g.* 4, 10–12, 22). Because environmental changes alter the mix of organic and inorganic compounds accumulating in leaves, the assumption of constant C concentration between environments needs verification. Of the studies listed above, only two (9, 22) reported direct C analyses of leaf tissue. Actual leaf C concentrations can vary greatly from 40% (*e.g.* 9). Errors in estimating C accumulation can assume great proportions because the export rate is sometimes much smaller than accumulation (8, 21, 22). Here we report large effects of inorganic nutrition (N and P status) on C concentration of accumulated dry mass in cotton leaves, and show how these effects alter calculations of assimilate export rates.

MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L. cv. Deltapine 70) plants, grown from seed in pots in a glasshouse as described earlier (15, 20), were watered three times per week with a modified half-strength Hoagland solution. N and P deficiencies were imposed by altering the solution composition to contain 20% of the normal amount of N or no added P, respectively. Normal concentrations of N (as NO_3^-) and P (as $H_2PO_4^-$) were 5 mM and 0.5 mM, respectively. All measurements were made on fully expanded leaves of vegetative plants in full sunlight on clear days.

Beginning before dawn and at 90-min intervals thereafter, leaves were sampled by removing 20 discs 15 mm in diameter. This size of sample provided sufficient material for analysis of starch and total C determination of mass per unit area. Discs were dried in a forced-draft oven at 70°C and weighed after at least 24 h, then ground in a mill and the powder stored in a freezer until analysis. Tissue dry mass, total C, and starch C contents are expressed in units of mg cm^{-2}. The concentrations of both total C and starch C were determined as the slopes of linear regressions relating those variables to change in leaf mass.

Total C was determined by oxidation of the tissue in a sample oxidizer (R. J. Harvey Instruments, Hillsdale, NJ).[1] For each sample, three replicates of 10 mg each were combusted to CO_2 and the CO_2 was trapped by bubbling the gas stream through a saturated solution of $Ba(OH)_2$. The precipitated $BaCO_3$ was washed, dried, and weighed. Recoveries of CO_2 were determined by similar analyses of 10-mg samples of soluble starch (assumed to have the formula $(C_6H_{10}O_5)_n$) and were typically greater than 95%. Recovered C contents of tissue were adjusted for losses during combustion.

Starch was extracted and purified by standard procedures. Briefly, lipids were extracted from samples with $CHCl_3$:ethanol (1:4, v:v), then with $CHCl_3$, and sugars were extracted with hot 70% ethanol. The residue was dried in an oven. Insoluble PVP and water were added to the sample, which was then gelatinized in a boiling water bath. Starch was hydrolyzed by addition of amyloglucosidase (Sigma Chemical Co.) in Na acetate buffer, pH 4.5, and glucose production determined with phenol-H_2SO_4 reagent (5). Starch content was quantitated by comparison to a standard curve obtained with soluble starch.

Photosynthesis rates were measured with an ADC portable photosynthesis system (Analytical Development Co., Hoddesdon, Herts, England). This open, steady-state gas exchange system utilized a clip-on cuvette of 6.25 cm^2 area and an IR CO_2 analyzer which was operated in the differential mode. Flow rate of air was 300 ml min^{-1}. At each time of day, photosynthetic

[1] Mention of a trademark or proprietary product 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 that may also be suitable.

rates were determined on four separate plants using the youngest fully expanded leaf. Cumulative assimilation of C during the day was estimated by integration of the photosynthetic rate with time, assuming a constant rate during the time interval surrounding each measurement.

RESULTS AND DISCUSSION

Figure 1 shows linear regression relating leaf C content to mass. The regression coefficients (slopes) indicate the gain of C per unit gain of dry mass. In the examples shown, C represents about 39, 50, and 46% of the accumulated mass in fully nourished control, N-stressed, and P-stressed plants, respectively. The regressions are noteworthy in several respects. First, the correlation coefficients were normally high and significantly different from zero ($P < 0.01$). Those few regressions for which $P > 0.05$ were discarded. Second, both N and P stress noticeably increased the slope of the relationship; and third, they also decreased the daily fluctuation in mass (Fig. 1). However, the increased C concentration tended to compensate for the decreased range of mass, so that daily change in leaf C content was similar for all treatments. Fourth, P stress increased the predawn C content (Δ mass = 0 in Fig. 1). This increase was associated with a greater predawn mass, but not all of the increase was from structural mass. Some of the difference was from a higher predawn starch level in P-stressed leaves (see subsequent discussion). It seems unlikely that P stress would increase leaf thickness (13).

It should be noted that the C concentration of accumulated mass is not equivalent to the C concentration of the entire leaf. For example, the predawn mass of the N-stressed leaves shown in Figure 1 was 2.29 mg cm^{-2}. The C concentration of the leaves at that time was 0.91/2.29 = 40%, but the C concentration of accumulated mass was nearly 50% (Fig. 1). Presumably this discrepancy arises from the different compositions of fluctuating and nonfluctuating dry mass. An advantage of regression analysis is that it separates these two components of the leaf: treatment effects on nonfluctuating mass appear an an altered Y-intercept, whereas effects on accumulated mass appear as an altered slope.

Numerous data sets were collected for N- and P-stressed plants and for well-nourished controls. Day-to-day variation within treatments was greater than the uncertainty associated with each day's regression, so each day of measurement was treated as a replicate for purposes of statistical analysis. N and P stress strongly affected the mean regression coefficient (C concentration of accumulated mass), increasing it from 38.6% to 49.7% and 45.1%, respectively (Table I). This variation in C concentration of accumulated mass can be partially explained by its starch content. Starch constituted 55% of the accumulated mass in controls but 75% and 87% of accumulated mass in N- and P-stressed plants, respectively (Table I). Because starch has a higher percentage of C (44.4%) than the accumulated mass in fully nourished control plants, this shift must have contributed to the increased C concentration of that mass with nutrient stress. However, the 'nonstarch' fraction must also have been enriched in C compared to the controls, because with either nutrient stress the actual C concentration to the accumulated mass exceeded that of starch itself. Changes in the C concentration imputed to the 'nonstarch' fraction of accumulated mass remain unexplained, but nutrient stress could have decreased 'C-poor' constituents (inorganic solutes, or highly oxidized compounds such as organic acids) or increased 'C-rich' constituents (reduced compounds such as lipids, phenolics, amino acids, or even proteins). N stress eliminates diurnal osmotic cycling in cotton leaves (18, 19), indicating that the stress greatly decreases the accumulation of low mol wt solutes. All of these changes show that changes in leaf mass are inadequate by themselves to calculate accumulation of C in plants grown in different environments.

The mean predawn starch content of well-nourished, N-stressed, and P-stressed leaves was 0.22 ± 0.03, 0.24 ± 0.08, and 0.49 ± 0.04 mg cm^{-2}, respectively. The failure of P-stressed leaves to degrade all starch during the night indicates some

Table I. *Components of Accumulating Dry Mass in Leaves*

Both starch and total C concentrations were determined as the slopes of linear regressions relating content of that component per unit area to mass per unit area. Regressions were based upon samples collected from dawn to midafternoon. Values shown as means ± SE, treating each day's regression as a replicate. Numbers in parentheses indicate starch mass as a percentage of total mass.

Treatment	Fraction of Accumulating Dry Mass	
	Total C	Starch C
	% of total mass	
Full nutrients	38.6 ± 1.3	24.5 ± 2.7 (55)
N stress	49.7 ± 3.0	33.2 ± 4.0 (75)
P stress	45.1 ± 4.2	38.5 ± 5.8 (87)

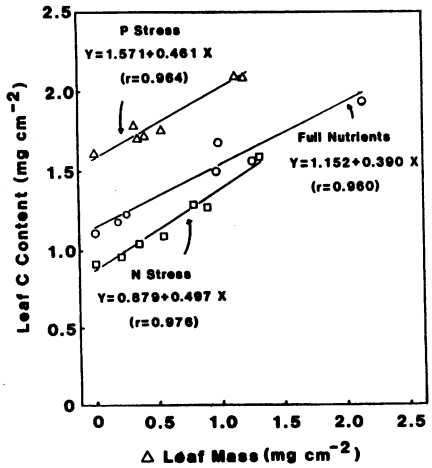

FIG. 1. Regression of leaf C content against the increase in leaf mass. Each regression consists of 7 data pairs from samples collected at 90-min intervals beginning at dawn (Δ leaf mass = 0). The masses of control, N-stressed, and P-stressed leaves at dawn were 2.59, 2.29, and 3.69 mg cm^{-2}, respectively.

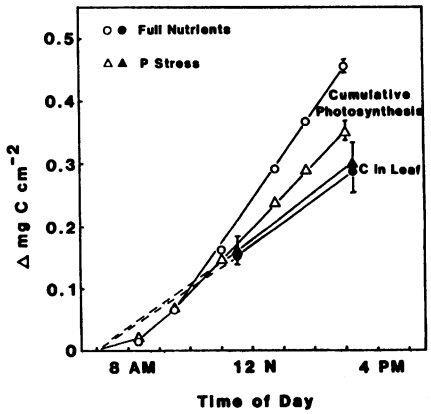

FIG. 2. Assimilation and accumulation of C in leaf blades during a day. Measurements were begun at 7 AM (dawn). Standard errors for cumulative photosynthesis are shown only at the last measurement, when differences between plants were greatest. The coefficient of variation was approximately constant during the day. The masses of control and P-stressed leaves at dawn were 3.59 ± 0.14 and 3.88 ± 0.09 mg cm^{-2}, respectively.

important metabolic effects specific to this nutrient deficiency. Ackerson (1, 2) reported similar findings for 'water stress-acclimated' cotton and also showed that increasing the P supply 10-fold decreased the predawn starch level to normal.

The coefficients shown in Table I were used to construct C balances for source leaves of fully nourished and P-stressed plants. Photosynthetic gas exchange was followed over several hours, and C acquisition was compared to actual C accumulation in the leaf. In control plants, C accumulation was 74% of assimilation in the morning (7:00 AM–11:30 AM), whereas in the afternoon (11:30 AM–3:15 PM) accumulation was only 49% of assimilation (Fig. 2). Export of C, calculated by difference, thus represented 26% and 51% of C assimilation during the two time periods. In P-stressed plants, C export was decreased to 9% and 30% of assimilation during the morning and afternoon, respectively (Fig. 2). Hendrix and Huber (8), working with unstressed cotton plants, also found a greater rate of C export from leaves in the afternoon. However, neither their study nor ours takes into account the possible changes in photosynthetic products (and their C content) between morning and afternoon (23). Although out correlation coefficients are very high (Fig. 1), indicating minimal error, small changes over short intervals would be extremely difficult to isolate. This uncertainty implies that only large differences in calculated export rates can be considered reliable.

Increases in starch (Table I) or other non-structural carbohydrates with nutrient stress are commonly reported (3, 6, 25, 26). Frequently this observation has been interpreted as evidence that nutrient stress decreases sink activities, such as leaf expansion, more than it decreases photosynthesis (14, 16, 17, 25, 26). Direct evidence for such a specific effect of N and P stress on leaf expansion has been reported and a mechanism proposed (16, 17). An alternative hypothesis is that nutrient stress may specifically limit sucrose synthesis and subsequent C export from source leaves in favor of starch accumulation (10, 11). Although the C balance shows decreased C export from nutrient-stressed leaves (Fig. 2), these data cannot by themselves distinguish between an effect at the source or an effect at the sink. Geiger et al. (7) described a similar dilemma with regard to the effect of daylength on starch accumulation in sugarbeet.

In conclusion, the C concentration of diurnally accumulating mass in leaves of well-nourished cotton plants approximates the C concentration in CH_2O. This finding provides support for C balances calculated under such growth conditions (*e.g.* 8). However, N and P stresses increase the C concentration of accumulated mass. Using C concentrations specific to each treatment, we show that P stress decreases the rate of export of C from source leaves. This effect of P stress would have been undetected without knowledge of the altered C concentration of accumulated mass.

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