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Starch and Sucrose Synthesis in *Phaseolus vulgaris* as Affected by Light, CO_2, and Abscisic Acid[1]

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ABSTRACT

Phaseolus vulgaris **L. leaves were subjected to various light, CO_2, and O_2 levels and abscisic acid, then given a 10 minute pulse of $^{14}CO_2$ followed by a 5 minute chase with unlabeled CO_2. After the chase period, very little label remained in the ionic fractions (presumed to be mostly carbon reduction and carbon oxidation cycle intermediates and amino acids) except at low CO_2 partial pressure. Most label was found in the neutral, alcohol soluble fraction (presumed sucrose) or in the insoluble fraction digestable by amyloglucosidase. Sucrose formation was linearly related to assimilation rate (slope = 0.35). Starch formation increased linearly with assimilation rate (slope = 0.56) but did not occur if the assimilation rate was below 4 micromoles per square meter per second. Neither abscisic acid, nor high CO_2 in combination with low O_2 (thought to disrupt control of carbon metabolism) caused significant perturbations of the sucrose/starch formation ratio. These studies indicate that the pathways for starch and sucrose synthesis both are controlled by the rate of net CO_2 assimilation, with sucrose the preferred product at very low assimilation rates.**

One of the first branch points for the use of newly fixed carbon is between starch and sucrose synthesis (or other transported sugars or sugar alcohols). Both starch and sucrose synthesis are stimulated by low Pi and high PGA[2] in photosynthesizing cells. In starch metabolism the enzyme sensitive to Pi and PGA is ADPglucose pyrophosphorylase (13) while fructose-6-phosphate 2-kinase, which produces the regulatory molecule fructose-2,6-bisphosphate is responsible, in part, for the sensitivity of sucrose synthesis to Pi and PGA (4). Additional Pi and PGA control occurs at sucrose phosphate synthase (1, 5). We reasoned that studies of the partitioning of starch and sucrose might provide a good probe for environmental perturbations of the carbon and phosphate metabolism of photosynthetic tissues.

There have been many investigations of carbon partitioning between starch and sucrose as affected by nutrition and photoperiod (12) between species (8–10), and between genotypes (9), but these studies have not examined environmental control of sucrose and starch synthesis.

We have investigated partitioning of recent photosynthate between alcohol-insoluble material (starch) and a water soluble, neutral fraction (sucrose) in leaves of *Phaseolus vulgaris* under steady state conditions while the rate of photosynthetic CO_2 assimilation was affected by variations in light and CO_2. In addition, we assessed partitioning between sucrose and starch under conditions which we expected to disrupt normal control of photosynthetic metabolism, namely after feeding detached leaves ABA and at high CO_2 when photosynthesis was inhibited by low O_2. We found that the starch/sucrose ratio remained constant except that at very low rates of photosynthesis, starch formation was reduced more than sucrose formation.

MATERIALS AND METHODS

Plant Material. *Phaseolus vulgaris* L. var Tendergreen (seeds from Northrup King) was grown in 4-L plastic pots in potting soil (compost/sand/perlite; 2/1/1 by volume) in a greenhouse. The temperature was controlled at 27°C day, 15°C night, RH was 50%, and the local photoperiod was extended to 18 h with 1 μmol photons m^{-2} s^{-1} light from fluorescent tubes (because daylength-sensitive plants were being grown in the same greenhouse). Plants were used when they were between 4 and 6 weeks old. Middle leaflets from the second and third trifoliolate leaf were used, but within any experiment only identical material was used.

Gas Exchange. Air was mixed from N_2, O_2, and 3% CO_2 in air using mass flow controllers (FC260, Tylan, Carson, CA). Some of this synthetic air passed through an aluminum chamber which had two fans for mixing the air, a glass window to admit light, and was temperature controlled by Peltier heating and cooling. The air flow was controlled by a mass flow controller. Some of the synthetic air and air from the leaf chamber was compared for water content and CO_2 content with a Binos IR gas analyzer (Leybold-Heraeus, Koln, W. Germany). Cross sensitivity of the water-analyzing section to CO_2 was reduced by optical filters while cross-sensitivity of the CO_2 measuring section to H_2O was reduced by passing the measurement air stream through a copper coil in an ice bath. Additional 3% CO_2 in air was injected through another flow meter directly into the chamber to compensate photosynthetic depletion of CO_2 from the air stream. In this way the CO_2 analyzer was used primarily as a null point detector.

Leaf temperature was measured with a copper-constantan thermocouple probe (SCPSS-020G-6, Omega Engineering Inc., Stamford, CT). 'Light' as used in this paper refers to photosynthetic photon flux (areal) density and was measured with a 190 SB quantum sensor from LiCor (Lincoln, NE).

Calculations of evaporation rate, conductance to gas exchange, photosynthesis, and intercellular CO_2 pressure were done according to von Caemmerer and Farquhar (19).

Leaves were fed $^{14}CO_2$ by switching the compensation CO_2

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[2] Abbreviations: PGA, glycerate-3-P; A, photosynthetic CO_2 assimilation rate; C_i, partial pressure of CO_2 in the intercellular spaces of a leaf.

flow from the unlabled 3% CO_2 tank to a supply of $^{14}CO_2$ labeled 3% CO_2 in N_2. The specific activity of the $^{14}CO_2$ was 0.1 Ci/mol and after dilution was approximately 0.05 Ci/mol, though the final dilution varied from experiment to experiment. The leaves were fed under steady state conditions. In most experiments the feedings were for 10 min, except when the assimilation rate was less than 5 μmol m^{-2} s^{-1} when the feeding was extended to 20 min. A 5-min chase with unlabeled CO_2 followed each feeding, during which gas exchange parameters were determined and label was chased out of the initial products of CO_2 fixation. Some experiments were repeated with a 10-min chase to check if this would alter the apparent labeling pattern. No difference between a 5- and 10-min chase was observed. If the assimilation rate before and after the pulse of $^{14}CO_2$ differed by more than 10%, the experiment was not considered a steady state experiment and was not used for analysis. Measurements were made to investigate possible effects of endogenous rhythms. No such effect was observed over the time period used in these experiments, but our protocol was designed to reduce the effects of any potential rhythmicity. Plants were kept in laboratory light (20 μmol photons m^{-2} s^{-1}) until they were placed in the gas exchange chamber. The leaf was illuminated at the indicated light level for 30 min, then measurements were taken. Our data cannot be interpreted to show that no rhythm occurs in beans.

Analysis for Starch and Sucrose. The killing and extraction procedures followed those of Atkins and Canvin (2). The leaf was killed by plunging it into boiling ethanol/water/formic acid; 33/7/2. Leaf areas were between 30 and 50 cm^2 and 80 ml of ethanol solution was used per leaf. Except as noted, the first cm of petiole was included in the extraction. As soon as substantial decoloration had occurred (~15 s), the beaker containing the leaf material and ethanol was put on ice. The leaf was ground in a mortar and pestle, then filtered through diatomaceous earth. The filter cake was washed with water and then transferred to a beaker with 20 ml of citrate buffer (pH 4.5) and 0.5 ml amyloglucosidase (1400 units ml^{-1}, grade V from Sigma). This solution was left overnight in a 55°C oven. Microscopic examination of iodine-stained filter cake showed that this procedure caused all iodine stainable particles to disappear. The filtrate from this procedure contained no sucrose or glucose 6-P, and a large amount of glucose as judged from NADP linked enzymic analysis. This filtrate was presumed to represent the starch fraction of the leaf.

The filtered ethanol solution containing plant extract was dryed at 55°C under an air stream. The water-soluble fraction was obtained by adding water to the ethanol-soluble fraction residue. After filtration a faintly yellow solution remained. A portion of the water-soluble fraction was counted for ^{14}C, the rest was put through ion exchange columns (Dowex 1 Cl form and Dowex 50 formate form) in sequence. The colorless neutral fraction was counted for ^{14}C. In all cases, 200 μl of the sample were put in 5 ml Scinti-Verse II (Fisher Scientific Co.). NADP-linked enzymic assay indicated that sucrose was present in the neutral fraction.

RESULTS

Response to CO_2 Partial Pressure and Light. CO_2 pressure affects photosynthesis in C_3 plants because it is a substrate and because it is a competitive inhibitor of photorespiration. The way that A and sucrose/starch formation ratio were affected by intercellular CO_2 pressure is shown in Figure 1. Because the CO_2 pressure reported is that occurring inside the leaf, these results are not affected by stomatal movements. Each point represents data from a separate leaf since the determination of sucrose/starch ratio required destruction of the sample. At low CO_2 pressure, more carbon was found in sucrose than in starch and the ratio varied strongly with CO_2 concentration below about 150 μbar. As CO_2 pressure increased, both starch and sucrose synthesis rates increased, but the ratio of starch to sucrose synthesis remained approximately constant. In air, the intercellular CO_2 pressure is usually between 200 and 250 μbar. Over this range there was always more starch than sucrose made.

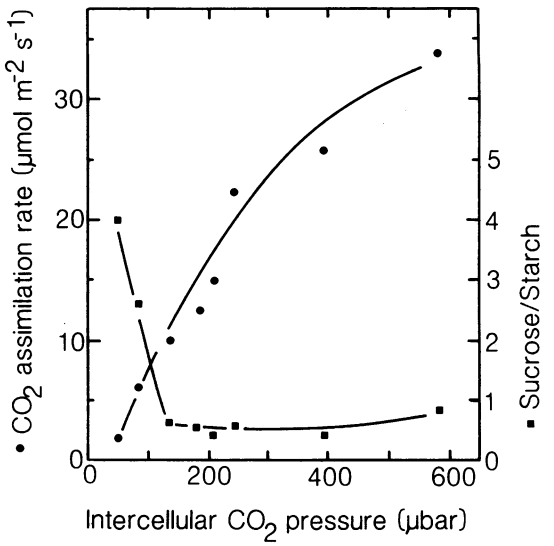

FIG. 1. Response of CO_2 assimilation rate and sucrose/starch ratio in leaves of *Phaseolus vulgaris* to intercellular CO_2 pressure. Light intensity was 1000 μmol m^{-2} s^{-1}. Leaf temperature was 27°C, water vapor pressure difference, leaf to air, was 8–10 mbar. Each pair of data points are from a separate leaf because of the destructive sampling. The data was determined beginning with high CO_2 in the morning and ending with the low CO_2 leaves in the afternoon. On other occasions this protocol was reversed; no difference could be discerned in the results.

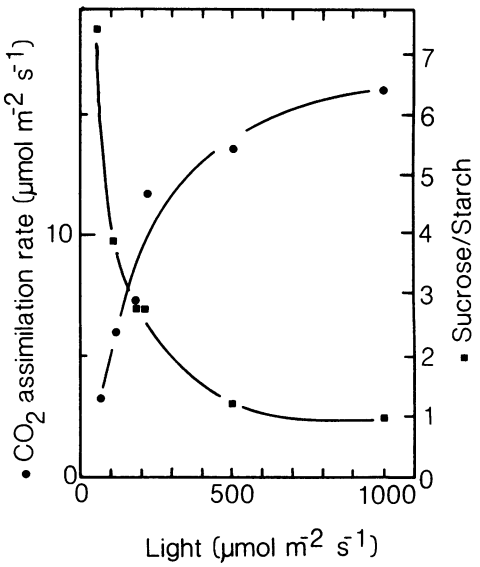

FIG. 2. Response of CO_2 assimilation rate and sucrose/starch ratio in leaves of *Phaseolus vulgaris* to light (photosynthetic photon flux areal density). The ambient CO_2 pressure was 300 μbar, C_i was uncontrolled and varied between 200 and 220 bar. Other conditions were as described for Figure 1.

The response of assimilation to light intensity at normal CO_2 and O_2 pressure is shown in Figure 2. When the assimilation rate was low, over seven times more carbon went into sucrose than into starch. As observed for the CO_2 response, increased assimilation rate was accompanied by increased starch and sucrose synthesis rate, starch more than sucrose. Roughly equal amounts

of starch and sucrose were measured in leaves labeled in one-half full sunlight (which saturated photosynthesis, data not shown).

At low CO_2 pressure, as much as 80% of the newly fixed carbon was ionic (*i.e.* did not pass through Dowex 1 and Dowex 50 columns, Table I) indicating that this carbon remained in carbon reduction or carbon oxidation cycle intermediates or that it was used to make amino acids. At higher CO_2 pressure only 10 to 20% of the newly fixed carbon was in this fraction. In contrast, when the assimilation rate was low because the light intensity was low this pool never exceeded 30%.

If we assume that the ^{14}C activity in the water-soluble plus starch fractions represents the total incorporation of ^{14}C, then the proportion of total counts in the starch or sucrose fraction multiplied by the assimilation rate that had occurred would be the rate of starch or sucrose synthesis. The rates of sucrose synthesis and starch synthesis for all of the light and CO_2 response experiments are plotted as functions of assimilation rate in Figure 3. Linear regression lines were determined by the method of least squares. For sucrose synthesis the slope was 0.35 and the y intercept was 0.3 ($r^2 = 0.83$). In other words, about 35% of newly fixed carbon was found in the sucrose pool at all CO_2 assimilation rates. For starch the slope was 0.56 and the x intercept was 3.9 ($r^2 = 0.94$) when the points below 5 μmol m^{-2} s^{-1} CO_2 are neglected. In other words, when the assimilation rate was below 3.9 μmol m^{-2} s^{-1} no starch was being formed but starch synthesis accounted for 56% of all carbon fixed in excess of 3.9 μmol m^{-2} s^{-1}. Separate regressions for all light and all CO_2 data were not significantly different for either the starch or sucrose data (the null hypothesis that the slopes of the regressions were equal was not rejected at P = 0.5 and 0.01).

Table I. *Proportion of Label in Sucrose Plus Starch*

Light was 1000 μmol photons m^{-2} s^{-1} (400–700 nm).

C_i	A	Sucrose + Starch/Total
μbar	*μmol m^{-2} s^{-1}*	*ratio*
50	1.8	0.2
85	6.2	0.5
135	10.1	0.6
180	12.5	0.8
210	15.0	0.8
245	22.4	0.8
395	25.9	0.8
585	33.9	0.9

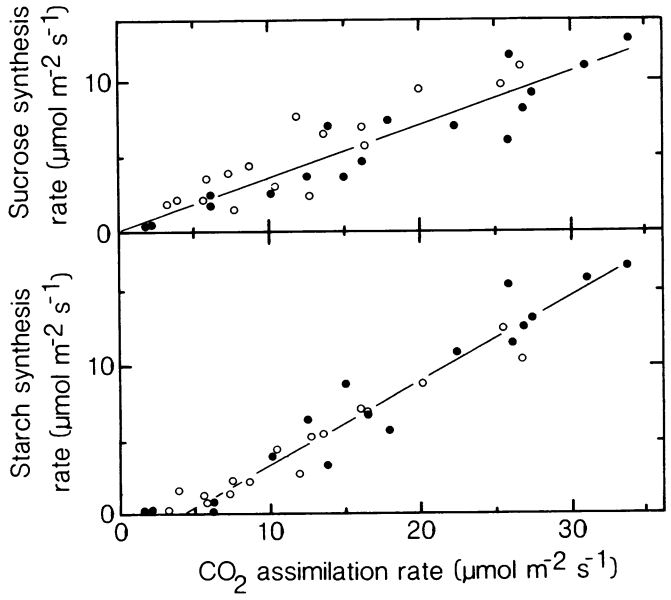

FIG. 3. Rate of starch and sucrose synthesis from CO_2 as affected by the rate of CO_2 assimilation. One mol is equal to 1 mol of CO_2 which ends up as starch or sucrose. Assimilation was varied by light (O) or CO_2 (●).

Application of ABA. ABA is well known as a hormone that causes stomatal closure and hence a reduction in CO_2 supply. In addition, Raschke (14) and Cornic and Miginiac (3) report that ABA can reduce the photosynthetic capacity of intact leaves apart from its effect on stomata. Huber *et al.* (11) has reported that ABA-fed leaves lost some sucrose phosphate synthase activity. We tested the effect of feeding ABA to leaves on the ratio of sucrose/starch formation rate.

Since detached leaves had to be used in this study we first checked that detached leaves behaved as attached leaves. We found that the amount of ^{14}C in the petiole + axillary bud of a leaflet fed $^{14}CO_2$ was very low. In one experiment we found 2% of the total radioactivity in the petiole of an attached leaf; all other measurements were lower. Detached leaves never had any radioactivity in the petiole. We also found no radioactivity in the feed water of detached leaves. The ratio of sucrose/starch formation in four attached leaves was found to be 1.00 ± 0.12 and in four detached leaves 0.84 ± 0.10 (average ± SE). As expected, there was a measurable decrease in the proportion of label going to the sucrose pool in detached leaves but over the time period used the effect of detaching leaves from plants was not large.

To assess the effect of ABA on the sucrose/starch synthesis ratio, a leaf was detached from the plant and the petiole was put in distilled H_2O. The response of the assimilation rate to intercellular CO_2 pressure was determined. A solution of 5×10^{-6} M ± ABA was then substituted for the distilled H_2O and gas exchange was monitored until assimilation was reduced by about 30% at a limiting intercellular CO_2 pressure. The leaf was then labeled as described and analyzed for sucrose/starch synthesis ratio. Control leaves were treated similarly but without feeding ABA. The proportion of label found in sucrose and starch for these leaves is listed in Table II. There was essentially no difference in the proportion of starch and sucrose being formed in ABA fed *versus* control leaves.

Effect of Oxygen. Photosynthesis of leaves of *Phaseolus vulgaris* at high light and 500 μbar intercellular CO_2 pressure can sometimes be inhibited by switching to low O_2 pressure. Sharkey (16) has postulated that this is the result of an imbalance in the phosphate metabolism. We determined the relative rates of starch and sucrose synthesis for leaves in this condition. For this experiment each leaf was measured at low and normal O_2 levels and during this experiment each leaf was found to have a higher rate of photosynthesis at normal O_2 than at low O_2. Data shown in Table III indicate that although switching to low O_2 lowered the

Table II. *Label in Starch and Sucrose as Affected by ABA*

	A	C_i	Sucrose	Starch
	μmol m^{-2} s^{-1}	*μbar*	*%*	
5×10^{-6} M + ABA	16.3	242	38	53
Control	23.8	253	39	49

Table III. *Per Cent Label in Sucrose and Starch as Affected by O_2 under Conditions where O_2-Stimulated Photosynthesis C_i was 500 μbar*

O_2	A	Sucrose	Starch
%	*μmol m^{-2} s^{-1}*	*%*	
19	28	41	48
3	25.8	38	46

rate of assimilation, it had no effect on the relative rate of sucrose and starch synthesis.

DISCUSSION

The rate of sucrose synthesis was a linear function of the rate of CO_2 assimilation with a slope of 0.35 when assimilation rate was varied by changes in light intensity or CO_2 supply. Servaites and Geiger (15) obtained similar data with sugar beet except that in their data the slope was 0.18.

The rate of starch synthesis was also linear with CO_2 assimilation rate but the line was steeper (slope = 0.56) and offset such that below 4 μmol m^{-2} s^{-1} no carbon was found in the starch pool. By observing leaf carbon balance, Ho (7) found that at low rates of carbon assimilation leaves of *Lycopersicon esculentum* lost carbon, presumably because transport exceeded assimilation. Neither the analysis of recent photosynthate (this study) nor analysis of carbon balance (7) can give information about how net starch formation is regulated so that it becomes zero at positive assimilation rates. However, Stitt and Heldt (17) suggest that starch synthesis and degradation can occur simultaneously and that the rate of each process is regulated by the level of phosphate inside the chloroplast. Because of this simultaneous synthesis and breakdown, at low rates of photosynthesis some carbon originally used in starch synthesis could end up in the sucrose pool.

When the rate of photosynthetic CO_2 assimilation was above 10 μmol m^{-2} s^{-1}, the rate of starch formation was always between one and two times the rate of sucrose formation. Sucrose and starch accounted for 80 to 90% of the recent photosynthate. Because the feeding time was short (15 min) less than 2% of the label ever left the leaf lamina and so measurements made on leaves represented the fate of recent photosynthate accurately.

When CO_2 assimilation fell below 10 μmol m^{-2} s^{-1}, roughly one-third of the maximum capacity, relatively less photosynthate went to starch. When the rate of photosynthesis was low because of low light, starch plus sucrose synthesis still accounted for over 70% of the recent photosynthate. When the rate of assimilation was low because of low intercellular CO_2 pressure, starch and sucrose accounted for as little as 20% of recent photosynthate. In one of the repetitions of this experiment, a 20-min pulse and 10-min chase were used and still only 30% of the recent photosynthate appeared in starch plus sucrose when A was less than 3 μmol m^{-2} s^{-1}, indicating that the results are probably not an artifact caused by slow turnover time of the phosphorylated intermediate pools. This result could be caused by the increased flux through the photorespiratory pool at low C_i resulting in more carbon draining into amino acids. A high rate of photorespiration could also result in a large Pi pool, at the expense of the PGA pool, since one and two-thirds PGA are required to make RuBP and oxygenation of RuBP causes the production of one and one-half PGA. The extra phosphate is released inside the chloroplast by the action of phosphoglycolate phosphatase (18). A large Pi pool and small PGA pool would inhibit starch and sucrose formation.

When photosynthesis becomes inhibited by low O_2 in the presence of high CO_2 it is believed to result from an imbalance of sugar phosphate metabolism in photosynthetic carbon metabolism (16). Regardless of the mechanism, our results show that under this condition neither starch nor sucrose synthesis are preferentially affected.

ABA can inhibit phloem loading (20) and ABA fed leaves can have a lower extractable sucrose phosphate synthase activity (11). The ABA effect on A can only be seen in intact leaves (3, 14) which might be expected if the effect of ABA were to inhibit the production or use of sucrose and so cause feedback inhibition of photosynthesis. However, we found that both starch and sucrose synthesis rates were lower in ABA fed leaves than in control leaves. If sucrose synthesis or use were the primary target of ABA then starch should have become the predominant product. Instead the results suggest that ABA causes an inhibition in the rate of assimilation in some way that does not alter the balance between starch and sucrose synthesis directly. It is possible that ABA has direct, independent effects on both starch and sucrose synthesis but this seems less likely than an effect on photosynthesis before the starch-sucrose branch point. The effects of ABA on sucrose phosphate synthase and phloem loading could be either consequences of the effect of ABA on photosynthesis or separate effects of ABA.

Heldt *et al.* (6) showed that the rate of starch synthesis is low very early in the day until the sucrose pool size had reached some level higher than the dark pool size. In the experiments we are reporting, bean leaves made starch only after the rate of assimilation exceeded 4 μmol m^{-2} s^{-1}.

Feedback inhibition by sucrose or starch accumulation has been postulated for many years. Very little evidence has been found in favor of this idea, though longer term 'feedback' regulation of photosynthesis has been demonstrated (reviewed by Sharkey [16]). Our data show no evidence of feedback inhibition of photosynthesis in the range of conditions covered in our experiments.

LITERATURE CITED

1. AMIR J, J PREISS 1982 Kinetic characterization of spinach leaf sucrose-phosphate synthase. Plant Physiol 69: 1027–1030.
2. ATKINS CA, DT CANVIN 1971 Photosynthesis and CO_2 evolution by leaf discs: gas exchange, extraction and ion-exchange fractionation of ^{14}C-labelled photosynthetic products. Can J Bot 49: 1225–1234
3. CORNIC G, E MIGINIAC 1983 Nonstomatal inhibition of net CO_2 uptake by (±) abscisic acid in *Pharbitis nil.* Plant Physiol 73: 529–533
4. CSÉKE C, BB BUCHANAN 1983 An enzyme synthesizing fructose 2,6-biphosphate occurs in leaves and is regulated by metabolite effectors. FEBS Lett 155: 139–142
5. DOEHLERT DC, SC HUBER 1983 Regulation of spinach leaf sucrose phosphate synthase by glucose-6-phosphate, inorganic phosphate, and pH. Plant Physiol 73: 989–994
6. HELDT HW, A GARDEMANN, R GERHARDT, B HERZOG, M STITT, W WIRTZ 1984 The regulation of CO_2 fixation and of sucrose synthesis in plants. *In* C Sybesma, ed, Advances in Photosynthesis Research, Vol III. Martinus Nijhoff/Dr W Junk, The Hague, pp 617–624
7. HO LC 1976 The relationship between the rates of carbon transport and of photosynthesis in tomato leaves. J Exp Bot 27: 87–97
8. HUBER SC 1983 Role of sucrose phosphate synthase in partitioning of carbon in leaves. Plant Physiol 71: 818–821
9. HUBER SC 1981 Inter- and intra-specific variation in photosynthetic formation of starch and sucrose. Z Pflanzenphysiol 101: 49–54
10. HUBER SC 1981 Inter-specific variation in activity and regulation of leaf sucrose phosphate synthetase. Z Pflanzenphysiol 102: 443–450
11. HUBER SC, DC DOEHLERT, TW RUFTY, PS KERR 1984 Regulation of sucrose phosphate synthase activity in leaves. *In* Advances in Photosynthesis Research, Vol III. Martinus Nijhoff/Dr W Junk, The Hague, pp 605–608
12. HUBER SC, DW ISRAEL 1982 Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max* Merr.) leaves. Plant Physiol 69: 691–696
13. PREISS J 1982 Regulation of the biosynthesis and degradation of starch. Annu Rev Plant Physiol 33: 431–454
14. RASCHKE K 1982 Involvement of abscisic acid in the regulation of gas exchange: Evidence and inconsistencies. *In* PF Wareing, ed, Plant Growth Substances. Academic Press, New York, pp 581–590
15. SERVAITES JC, DR GEIGER 1974 Effects of light intensity and oxygen on photosynthesis and translocation in sugar beet. Plant Physiol 54: 575–578
16. SHARKEY TD 1985 Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Bot Rev 51: 53–105
17. STITT M, HW HELDT 1981 Simultaneous synthesis and degradation of starch in spinach chloroplasts in the light. Biochim Biophys Acta 638: 1–11
18. TOLBERT NE 1971 Microbodies peroxysomes and glyoxysomes. Annu Rev Plant Physiol 22: 45–74
19. VON CAEMMERER S, GD FARQUHAR 1981 Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376–387
20. VREUGDENHIL D 1983 Abscisic acid inhibits phloem loading of sucrose. Physiol Plant 57: 403–467