

Relationships between Carbon Assimilation, Partitioning, and Export in Leaves of Two Soybean Cultivars¹

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ABSTRACT

To evaluate leaf carbon balance during rapid pod-fill in soybean (*Glycine max* [L.] Merrill), measurements were made of CO₂ assimilation at mid-day and changes in specific leaf weight, starch, and sucrose concentrations over a 9-hour interval. Assimilate export was estimated from CO₂ assimilation and leaf dry matter accumulation. Chamber-grown 'Amsoy 71' and 'Wells' plants were subjected on the day of the measurements to one of six photosynthetic photon flux densities in order to vary CO₂ assimilation rates.

Rate of accumulation of leaf dry matter and rate of export both increased as CO₂ assimilation rate increased in each cultivar.

Starch concentrations were greater in Amsoy 71 than in Wells at all CO₂ assimilation rates. At low CO₂ assimilation rates, export rates in Amsoy 71 were maintained in excess of 1.0 milligram CH₂O per square decimeter leaf area per hour at the expense of leaf reserves. In Wells, however, export rate continued to decline with decreasing CO₂ assimilation rate. The low leaf starch concentration in Wells at low CO₂ assimilation rates may have limited export by limiting carbon from starch remobilization.

Both cultivars exhibited positive correlations between CO₂ assimilation rate and sucrose concentration, and between sucrose concentration and export rate. Carbon fixation and carbon partitioning both influenced export rate via effects on sucrose concentration.

Soybean (*Glycine max* [L.] Merrill) seed growth is almost entirely dependent on assimilate exported from leaves. Sambo, Moorby, and Milthorpe (15) have determined that only about 4% of the carbon imported by the seed is accounted for by fixation of atmospheric CO₂ by the pod.

A proportional relationship between carbon assimilation rate and export rate has been observed in leaves of various plant species (9, 10, 16). Positive correlations between leaf sucrose concentration and export rate have also been reported (9, 16). Silvius, Kremer, and Lee (18), working with soybean, noted a close association between sucrose levels, translocation, and sucrose phosphate synthetase activity. They suggested that sucrose synthesis may potentially limit the transport process and also influence rate of starch accumulation.

In soybean and cotton, measurements of soluble sugars and starch indicate that a substantial portion of assimilated carbon is partitioned into starch during the day and transported out of the leaf at night (3, 5, 23). Increased CO₂ fixation rates by soybean leaves during vegetative growth resulted in increased amounts of starch accumulated, but little or no increase in sucrose concen-

tration (11, 12, 19) or translocation (19). In pod-bearing soybean plants, however, Thorne and Koller (22) found that increased assimilate demand on a source leaf resulted in an increase in CO₂ fixation rate, a decrease in leaf starch concentration, and increases in both sucrose concentration and translocation rate. It is possible therefore, that at different stages of growth and with changing sink demands, there may be significant shifts in carbon flow through the soybean leaf.

The present study was initiated to help assess the potential limitations imposed on soybean seed growth by leaf carbon fixation, partitioning, and export. The general experimental approach was to measure simultaneously the carbon assimilated by the leaf, the partitioning of carbon between starch and sucrose pools, and the export of carbon from the leaf. Measurements of leaf carbon balance were made over a range of assimilation rates to permit an evaluation of the interrelationships among assimilation, partitioning, and export. Two soybean cultivars, differing in pattern of leaf starch accumulation, were evaluated.

MATERIALS AND METHODS

Plant Culture and Light Treatments. 'Amsoy 71' and 'Wells' soybean cultivars were grown in a controlled environment room with a 14-h photoperiod (0800-2200 h), a constant 25 ± 1°C temperature, and a RH of approximately 50%. A mixture of cool-white fluorescent and tungsten-filament incandescent lamps provided a PPFD² of 400 ± 30 μE m⁻² s⁻¹ (400-700 nm) at the level of the leaves selected for analysis. Seeds were planted in 2-L clay pots containing *Rhizobium japonicum*-inoculated greenhouse soil. Plants were thinned to one per pot approximately 1 week after seeding and were watered daily with deionized H₂O. Each plant developed approximately 15 to 20 normal pods.

At the mid-point of the rapid seed growth stage (57 ± 7 d after planting), plants were selected randomly and placed in one of six light treatments (10, 60, 140, 240, 400, or 500 μE m⁻² s⁻¹ at level of sampled leaves) at the beginning of the photoperiod. The higher PPFDs were obtained by raising and lowering plants relative to the light source. The PPFD of 10 μE m⁻² s⁻¹ was obtained in an adjacent darkened compartment of the growth room with the partition slightly ajar. Variation in leaf temperature among treatments was not more than 1.5°C.

Leaf Dry Matter and Carbohydrate Analyses. Two mature leaves per plant, located between the fifth and eighth nodes, were selected for measurements. Samples for leaf dry matter accumulation and carbohydrate content were obtained by removing one randomly selected lateral leaflet from each selected leaf at 1000 h and then removing the remaining lateral leaflet at 1900 h. Areas of lateral leaflets, sampled either at 1000 or 1900 h, were determined using a Hayashi Denko area meter, model AAM-5. Leaflets were then quickly frozen on dry ice and stored

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² Abbreviations: PPFD, photosynthetic photon flux density; CPR, carbohydrate production rate(s).

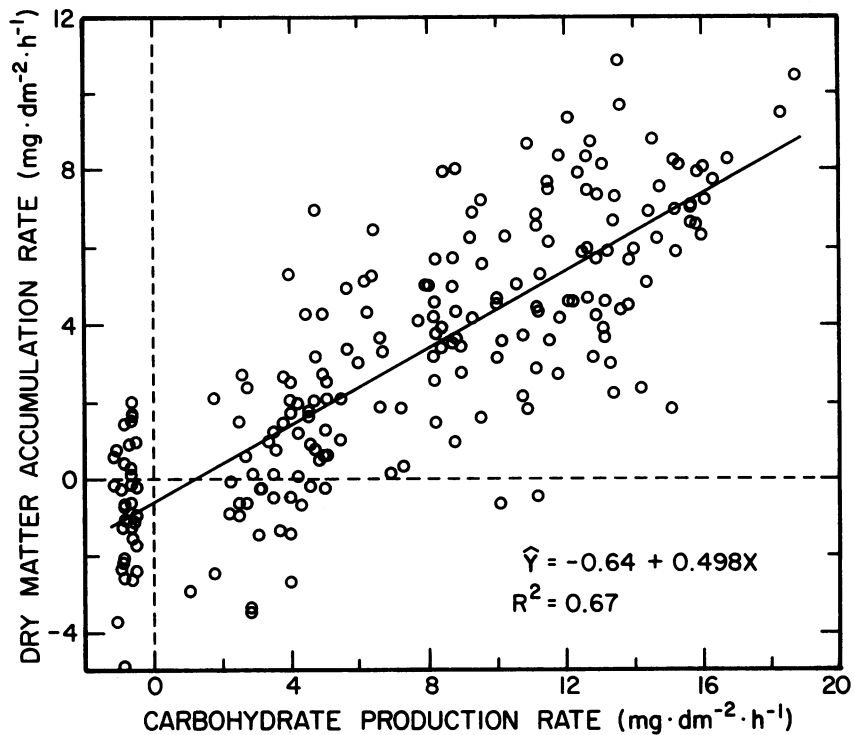


FIG. 1. Relationship between dry matter accumulation rate and carbohydrate production rate in Wells soybean leaves.

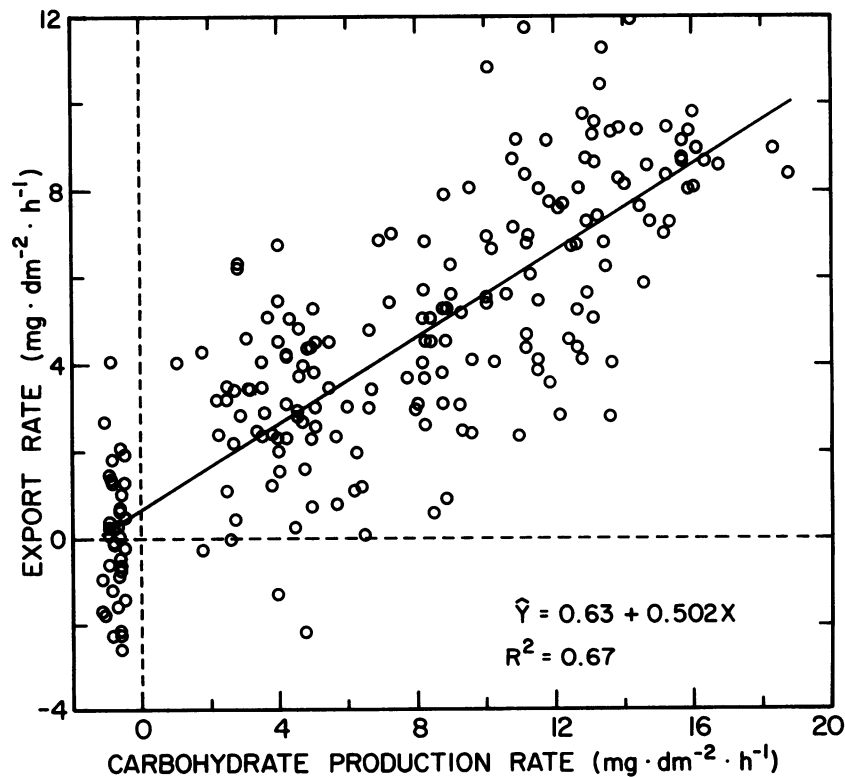


FIG. 2. Relationship between export rate and carbohydrate production rate in Wells soybean leaves.

at -29°C until freeze-dried. After freeze drying, leaflets were weighed and dry matter accumulation rates per unit leaf area calculated. Freeze dried lateral leaflets were then ground to pass through a 1-mm screen in a Wiley mill and the ground tissue stored in glass vials at -29°C until extracted.

Extraction of sucrose was carried out by placing individual tissue samples of 50 mg into plastic centrifuge tubes with 6 ml

of 80% (v/v) ethanol and shaking in a water bath for 18 h at 40°C . After extraction, the supernatant was decanted and the extract centrifuged for 10 min at 6000g. A 1.0-ml aliquot of extract was placed in a 12-ml centrifuge tube and the Chl removed by adding 1.0 ml chloroform, 1.0 ml water, and centrifuging for 10 min at 6000g. A 1.5-ml aliquot of the upper clear phase was used for sucrose analysis. Sucrose concentration was

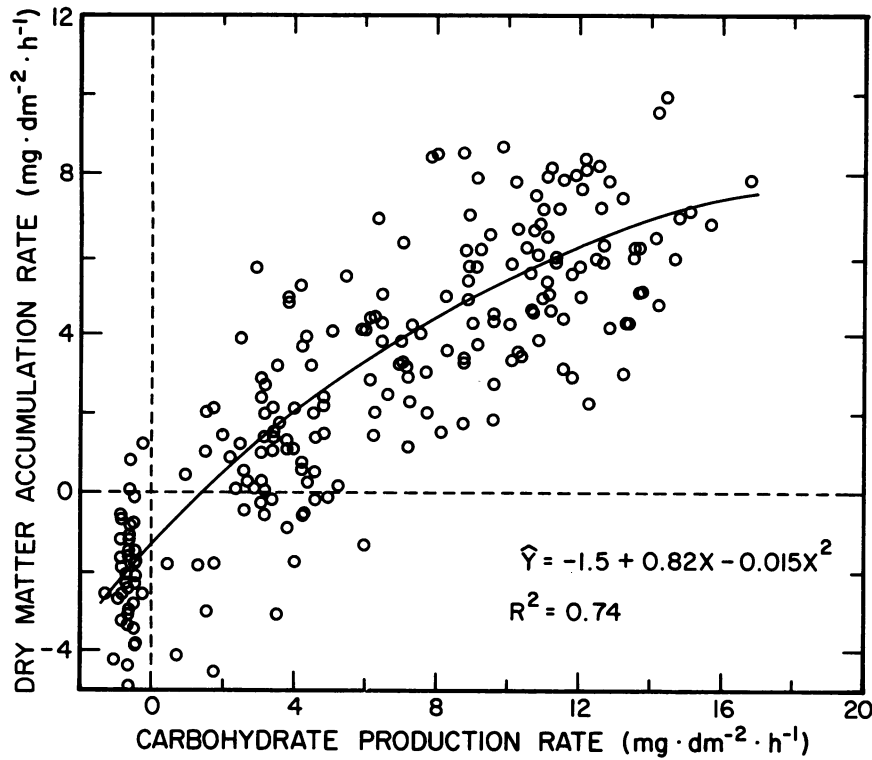


FIG. 3. Relationship between dry matter accumulation rate and carbohydrate production rate in Amsoy 71 soybean leaves.

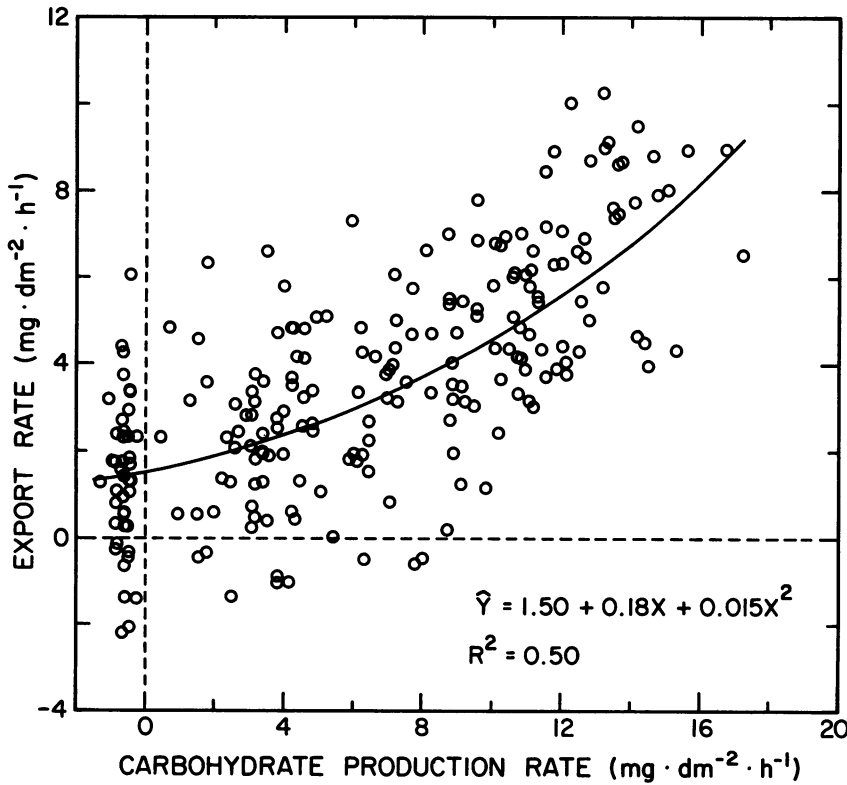


FIG. 4. Relationship between export rate and carbohydrate production rate in Amsoy 71 soybean leaves.

determined using the resorcinol test (1) after free fructose was destroyed with 1.0 N NaOH.

The starch-containing residue from the ethanol extraction was dried at 55°C, then gelatinized in 5 ml H₂O for 2 h in a boiling water bath. Upon removal, 5 ml of 0.1 M acetate buffer (pH 4.5), 25 mg amyloglucosidase (Sigma Chemical Company), and 5 ml

water were added. Gas release stoppers were inserted and the contents incubated at 55°C for 24 h. Following incubation, the contents were mixed and filtered (Whatman No. 42 filter paper). The resulting glucose concentration was measured with a glucose oxidase enzyme system (Statzyme, Worthington Diagnostics NR:27633). Starch equivalents were obtained by multiplying by

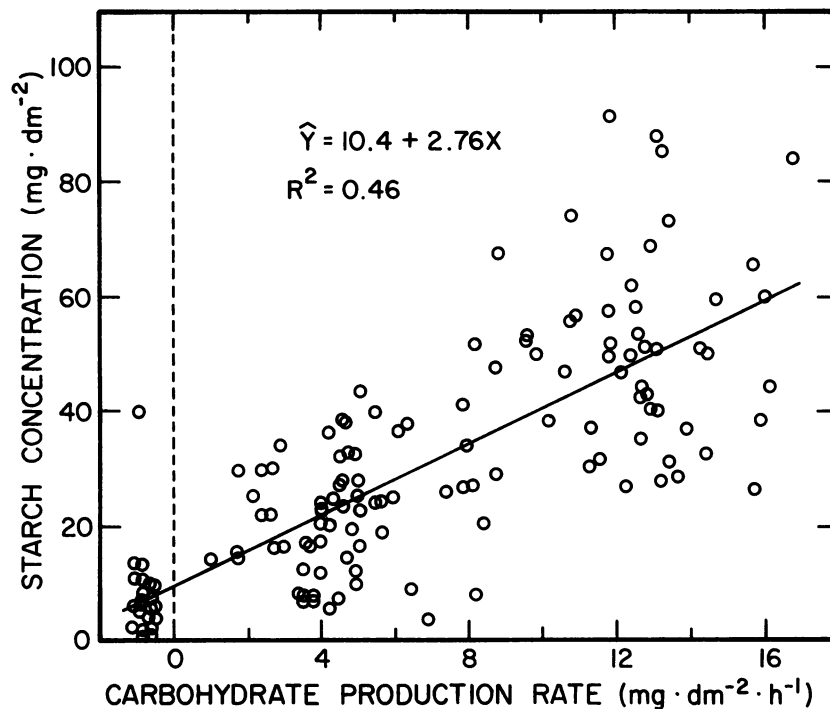


FIG. 5. Influence of carbohydrate production rate on starch concentration at 1900 h in Wells soybean leaves.

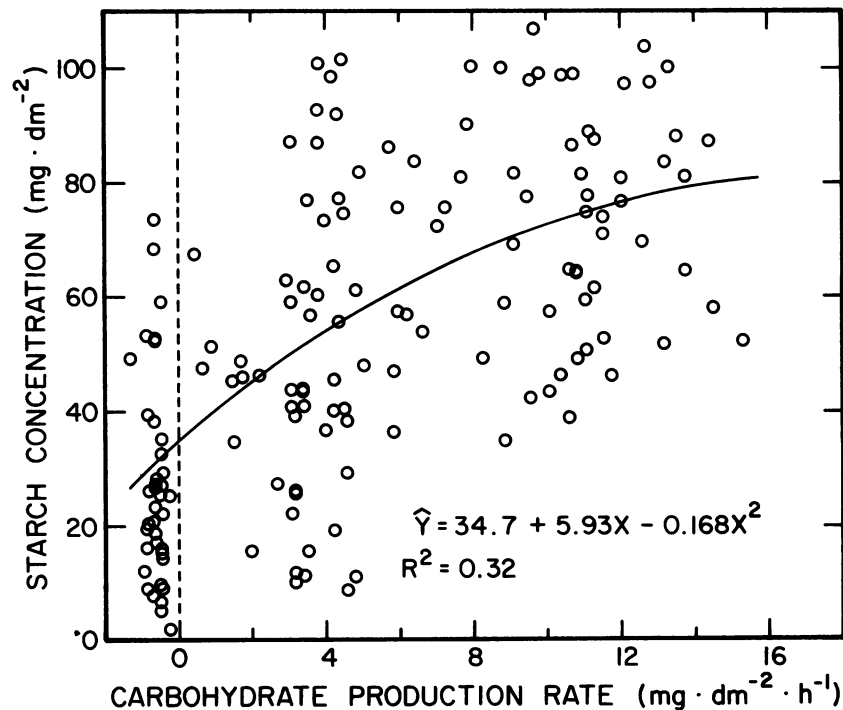


FIG. 6. Influence of carbohydrate production rate on starch concentration at 1900 h in Amsoy 71 soybean leaves.

0.9.

Gas Exchange Measurements. Net CO_2 exchange rates were measured on terminal leaflets of the selected leaves at approximately 1400 h. This was the mid-point of the 9-h experimental period. Results of previous studies (14, 23) indicate that soybean leaf CO_2 fixation rate would be expected to remain constant during this 9-h period. Measurements were made using a clamp-on chamber similar to that described by Catsky and Slavik (2). Air containing about $300 \mu\text{l CO}_2 \text{l}^{-1}$ was supplied to the chamber from a compressed air cylinder at a flow rate of approximately

0.9 l min^{-1} . Prior to entering the chamber, the air was humidified by passing part of the stream over the surface of water in an insulated flask. RH was maintained at approximately 50%. CO_2 uptake was measured as the difference between entering and exiting CO_2 concentrations using an Ethyl Intertech Corporation IR gas analyzer (model URAS 2). Leaf temperature was measured using a thermocouple pressed to the underside of the leaf and connected to a Leads and Northrup Numatron (model 914). Leaf temperature in the chamber was $26 \pm 2^\circ\text{C}$. Dewpoints of entering and exiting air streams were monitored by two General

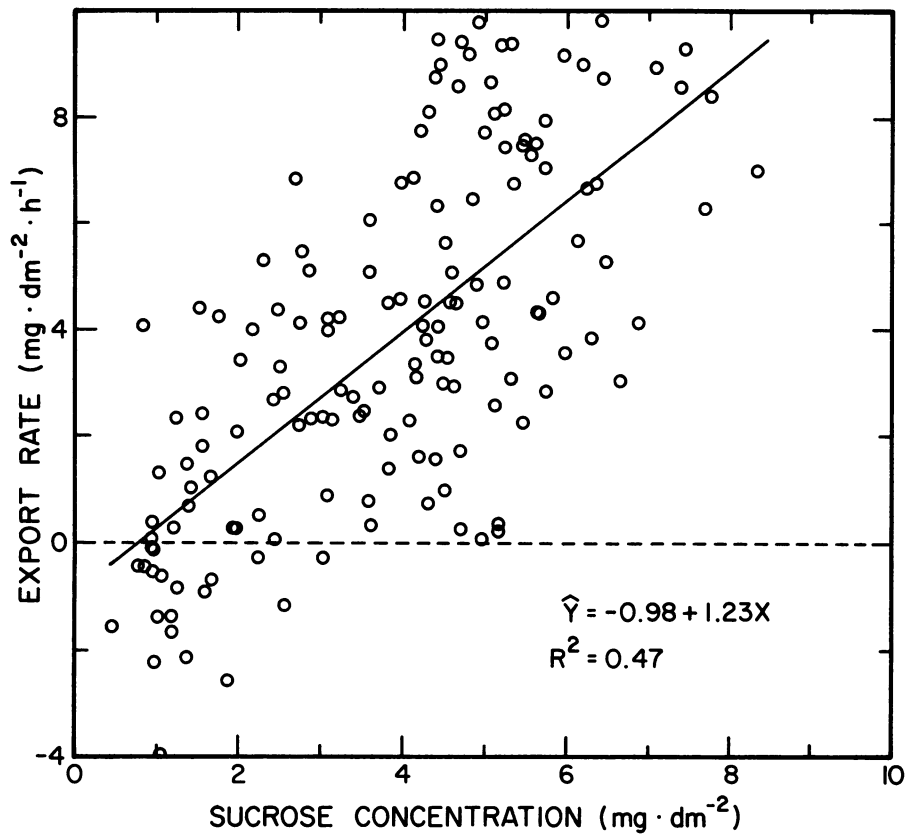


FIG. 7. Relationship between export rate and sucrose concentration in Wells soybean leaves.

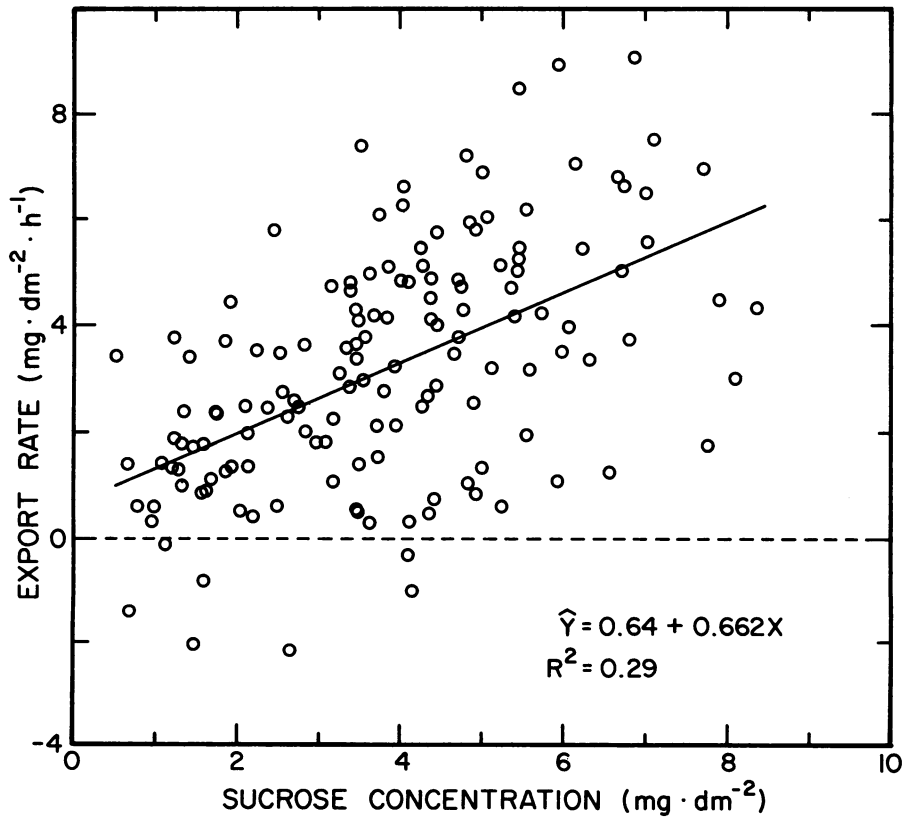


FIG. 8. Relationship between export rate and sucrose sucrose concentration in Amsoy 71 soybean leaves.

Table I. Influence of Photosynthetic Photon Flux Density on Leaf Carbohydrate Production Rates of Two Soybean Cultivars

Cultivar	CPR at Following PPF D ($\mu\text{E m}^{-2} \text{s}^{-1}$)					
	10	60	140	240	400	500
	<i>mg CH₂O dm⁻² h⁻¹</i>					
Wells	-0.7 ^a	2.4	4.3	7.9	12.7	14.9
Amsoy 71	-0.6	1.5	3.7	6.6	11.1	12.5

^a Each value is mean of at least 20 leaves.

Eastern dewpoint hygrometers (model 1201). The chamber was placed on the terminal leaflet and held perpendicular to the light source. Measurements of net CO₂ exchange and temperature were made after a 2- to 3-min equilibrium period. Calculation of net CO₂ exchange rates were made using the methods of Gastra (7) and Sestak *et al.* (17).

Estimation of Assimilate Export. Estimation of assimilate export from the leaf was made using the method of Terry and Mortimer (21). The rate of export *T* was determined using the relationship,

$$T = P - A,$$

where *P* is the calculated rate of carbohydrate production due to CO₂ fixation and *A* the rate of accumulation of dry matter. It was assumed that the dry matter changes in the leaf were attributable to changes in carbohydrate-type compounds.

In order to express CO₂ exchange rate and transport rate in the same units, mg CO₂ dm⁻² h⁻¹ was converted to mg CH₂O dm⁻² h⁻¹ since a large proportion of leaf organic matter is represented by this empirical formula. This was done by multiplying the CO₂ exchange rate by 0.68, the molar ratio of the two forms of carbon.

Statistical Analysis. A completely randomized design was used and analysis of the data was made using the least squares method of multiple regression (20). All final regression equations selected were significant at *P* < 0.01. To examine the possibility that a curvilinear relationship existed, a stepwise regression procedure was used to test the significance of each additional order of the polynomial equation. Selection of parameters included in the equations were based on the significance of the partial regression coefficient (*P* < 0.05) and significant reduction in the residual sums of squares.

To test for significant differences between cultivars, analyses of covariance using the partitioning of the cross-product was used (20). Significant differences imply that regression and correlation coefficients provide estimates for distinct populations.

RESULTS

Average CPRs in the different light treatments are shown in Table I. At 60 $\mu\text{E m}^{-2} \text{s}^{-1}$ and above, Wells had slightly higher CPRs than Amsoy 71. This difference may be due in part to the lower CO₂ stomatal diffusion resistance found in Wells (data not shown). At 10 $\mu\text{E m}^{-2} \text{s}^{-1}$, CPRs for both cultivars were slightly negative. Positive CPRs in the treatments ranged from 19 to 117% of the CPR at the light intensity at which the plants had been grown for Wells, and from 12 to 112% for Amsoy 71.

In leaves of Wells, dry matter accumulation rate (Fig. 1) and export rate (Fig. 2) increased as CPR increased. Net accumulation of dry matter occurred at CPRs greater than 1.3 mg CH₂O dm⁻² h⁻¹. At zero rate of carbohydrate production, the export rate was 0.64 mg CH₂O dm⁻² h⁻¹ and was maintained at the expense of dry matter.

In leaves of Amsoy 71, dry matter accumulation rate (Fig. 3) and export rate (Fig. 4) also increased with CPR. However, the

statistical significance of the second-order term in the equations suggests that there was deceleration in dry matter accumulation rate and acceleration in export rate as CPR increased. Net accumulation of dry matter in Amsoy 71 occurred at CPRs above 1.9 mg CH₂O dm⁻² h⁻¹. At a carbohydrate production rate of zero, the rate of export was approximately 1.5 mg CH₂O dm⁻² h⁻¹ (more than twice that in Wells) and, as in Wells, maintained at the expense of dry matter.

Concentrations of starch and sucrose were measured in leaflets sampled at 1900 h. In both Wells (Fig. 5) and Amsoy 71 (Fig. 6), starch concentration increased with increasing CPR. Although the concentration of starch was highly variable, correlation and regression coefficients estimated distinct populations (*P* < 0.01) for Wells and Amsoy 71. Amsoy had higher concentrations of starch at all CPRs.

Sucrose concentrations at 1900 h were found to be positively correlated with CPR for both cultivars (*r* = 0.72 and 0.69 for Wells and Amsoy 71, *P* < 0.01). The relationships between export rate and sucrose concentration in Wells and Amsoy 71 are shown in Figures 7 and 8, respectively. In both cultivars, export rate increased with increasing sucrose concentration. However, correlation and regression coefficients estimated distinct populations for Wells and Amsoy 71 (*P* < 0.01). This implies that a difference exists between the cultivars in the sensitivity of export rate to sucrose concentration, with Wells being more sensitive than Amsoy 71.

DISCUSSION

In the pod-bearing plants of this study, increased carbon fixation rates led to increases in both export rate (Figs. 2 and 4) and starch concentration (Figs. 5 and 6) of the leaves. However, Silviu, Chatterton, and Kremer (19) found that when leaves of vegetative soybean plants acclimated to one PPF D were placed under a higher PPF D, increased carbon fixation rates resulted only in increased leaf starch concentration, with no increase in translocation. It is probable that partitioning of carbon between the carbohydrate pools of the leaf and export rate are functions of the changing sink demands associated with stage of growth.

Different abilities of the cultivars to maintain export rates under limiting CPRs may be due in part to the difference in leaf starch concentration. Under conditions leading to limited fixation, Ho (9) found that translocation rates in tomato were maintained at the expense of starch reserves. Under these conditions, export rates are controlled by the mechanism which regulates the remobilization of starch reserves. Charles-Edwards and Ho (4) and Habeshaw (8) suggest that the rate of starch remobilization is constant. Thus, under conditions of limited fixation, translocation rates are dependent on the rate of starch breakdown. In the present study, at carbohydrate production rates near zero, Amsoy 71 had nearly 3 times the leaf starch concentration (*cf.* Figs. 5 and 6) and approximately twice the export rate (*cf.* Figs. 2 and 4) of Wells. It may be postulated that Amsoy 71, which had a much higher leaf starch concentration, was better able to remobilize starch reserves and hence maintain a minimum export rate dependent on the rate of remobilization. The low leaf starch concentration in Wells may have limited export by limiting remobilization of starch, which in turn would reduce the amount of carbon available for export.

Wells and Amsoy 71 both exhibited positive linear correlations between export rate and leaf sucrose concentration (Figs. 7 and 8). Hence, sucrose concentration appeared to be a factor associated with rate of export. Carbon fixation rate and carbohydrate partitioning both influenced export rate through effects on sucrose concentration. The linear relationship also suggests that physiological processes involved in export of sucrose from the leaf were not saturated.

The apparent greater increase in export rate per unit increase

in sucrose concentration in Wells (*cf.* Figs. 7 and 8) may possibly be explained by cultivar differences in partitioning of sucrose within the leaf. Leaf sucrose can be compartmentalized into histological and intracellular components consisting of labile and nonlabile pools (6, 13). Only the labile sucrose pool directly affects translocation rate. Wells may partition a greater proportion of sucrose into the labile pool than Amsoy 71, resulting in the greater increase in export rate per unit increase in sucrose concentration.

Dry matter accumulation rate (Figs. 1 and 3) and export rate (Figs. 2 and 4) both increased as CPR increased in each cultivar. The relative amounts of assimilate partitioned into dry matter (storage) and export also changed as CPR changed. However, the statistical significance of the curvilinearity in dry matter accumulation rate and export rate in Amsoy 71, but not in Wells, suggests that the patterns of assimilate partitioning in the two cultivars differ. The difference between the cultivars in the concentration of starch in the leaf supports this hypothesis. The possibility that Wells and Amsoy 71 leaves have different patterns of assimilate partitioning implies that differences may exist between the cultivars in the regulation of partitioning.

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