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Influence of Assimilate Demand on Photosynthesis, Diffusive Resistances, Translocation, and Carbohydrate Levels of Soybean Leaves[1]

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JOHN H. THORNE AND H. RONALD KOLLER
Department of Agronomy, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Rates of net photosynthesis and translocation, CO_2 diffusive resistances, levels of carbohydrates, total protein, chlorophyll, and inorganic phosphate, and ribulose 1,5-diphosphate carboxylase activity were measured in soybean (*Glycine max* L. Merrill) leaves to ascertain the effect of altered assimilate demand. To increase assimilate demand, the pods, stems, and all but one leaf (the "source leaf") of potted plants were completely shaded for 6 or 8 days and the responses of the illuminated source leaf were monitored. Rate of net photosynthesis in the source leaf of the shaded plants was found to increase curvilinearly to a maximum on the 8th day. The source leaf of the control plants (no sink shading) maintained a constant photosynthetic rate during this period. Vapor-phase resistance to CO_2 diffusion did not vary with treatment, but mesophyll (liquid phase) resistance was significantly lower in the source leaf of the shaded plants.

Starch concentration in the source leaf of shaded plants decreased more than 10-fold during the 8-day shading period. In this same period, sucrose concentration rose nearly 3-fold. Conversely, in the source leaf of the unshaded plants, starch concentration remained high (23% of leaf dry weight) and sucrose concentration remained very low (1.2%). When measured on the 8th day of treatment, translocation rate, ribulose 1,5-diphosphate carboxylase activity, and inorganic phosphate concentration were found to be significantly higher in the source leaf of the shaded plants than in the control source leaf.

When shaded plants were again illuminated, all measured response trends in the source leaf were reversed. These data indicate that assimilate demand has a marked influence on source-leaf photosynthesis and carbohydrate formation and export.

A leaf's photosynthetic potential may be limited by the degree to which photosynthate transport and utilization occurs, for it has often been suggested (15, 24) that fixation of CO_2 may be inhibited when accumulations of photosynthetic products remain in the leaf. Most of the evidence supporting a product-inhibition hypothesis has been produced through manipulation of assimilate sources, sinks, or the translocation pathway between them (15, 17, 24). Evidence obtained from altered plants, however, has been criticized (24) on the basis of induced hormonal, nutritional, and carbohydrate imbalances between sources and sinks.

More recent evidence obtained from unaltered plants lends support to the product-inhibition hypothesis. Chatterton *et al.* (5) demonstrated that leaf photosynthetic rates declined over a 16-hr day as chloroplast starch accumulated. If a cold night prevented the breakdown and translocation of this starch, photosynthesis was additionally reduced the following day when starch levels further increased. Conversely, if the plants were actively tillering, no starch accumulated even though their photosynthetic rates were much higher than nontillering plants (5). In these tillering plants with little chloroplast starch, a cold night had no effect on CO_2 assimilation the following day.

The data of Upmeyer and Koller (29) also suggest an inverse relationship between starch content and rate of net photosynthesis. During 16 hr of light-saturated photosynthesis, starch levels of intact soybean leaves increased more than 3-fold to a maximum just before darkness. Net photosynthetic rate of these leaves remained relatively constant for approximately 10 hr, after which it gradually declined until darkness to about 85% of the midday rate. This decline in net photosynthesis was due to increases in both stomatal and mesophyll resistances to CO_2 diffusion.

Excessive assimilate accumulation and reduced photosynthesis may result from slow translocation rates (4). Rates of translocation have been shown to be correlated with net photosynthesis in major crop plants (14). Thus rapid photosynthesis may require rapid translocation (12–14) as well as rapid utilization of photosynthate (5).

Most investigations of the product-inhibition hypothesis have employed treatments designed to increase leaf assimilates to above normal levels (15, 17, 24). In the present study, the hypothesis was tested by (*a*) experimentally enlarging assimilate demand so that the effect of a reduced carbohydrate level in the source leaf could be studied; and then by (*b*) returning assimilate demand to its original level so that the response of the source leaf to accumulating photosynthate could be observed. Rates of gas exchange, synthesis and export of carbohydrate, and various other source leaf processes were studied while assimilate demands were thus altered.

MATERIALS AND METHODS

Plant Culture. 'Amsoy 71' soybeans (*Glycine max* L.) were grown in a controlled environment room with an 8-hr photoperiod (8:00 AM to 4:00 PM). Light from a mixture of fluor-

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escent and tungsten-filament lamps provided 560 ± 10 μ Einsteins m^{-2} sec^{-1} or approximately 30,000 lux at plant level. Air temperature was maintained at 24.0 ± 0.5 C in the light and 22.5 ± 0.5 C in the dark. A relative humidity of approximately 50% was maintained in the constantly circulated air.

Seeds were planted in 12.5-cm diameter pots containing *Rhizobium japonicum* inoculated greenhouse soil. Emerging seedlings were thinned to one per pot 4 days later. Pots were spaced so that the plants did not shade each other and were randomly rearranged each week.

When the plants reached the rapid pod-filling stage (60 days after planting) they were approximately 40 cm tall with 13 to 15 pods on about eight leaf-bearing nodes. Two treatment groups were randomly formed, controls and plants to be shaded. Enclosed in either foil or black plastic cylinders, plants of the shaded group were almost totally shaded with only a single leaf on each plant emerging into the light. This single exposed leaf served as the "source leaf" for the shaded plants. After 6 or 8 days of continuous shading, the cylinders were removed. The source leaf of both control and shaded plants was located at the third node from the apex and was usually the most recent fully expanded leaf.

Gas Exchange. Gas exchange measurements were made with an apparatus similar in basic design to that of Gaastra (8), but modified (18) so that O_2 concentration could be varied. Determination of chamber incoming and outgoing CO_2 and water vapor concentrations were made with a Beckman Model 215A infrared gas analyzer and a Vap-Air Model 84 dew point hygrometer, respectively. Oxygen concentration was monitored by a Beckman Model C2 paramagnetic oxygen analyzer. The waterjacketed acrylic assimilation chamber was similar in design to that of Hardwick, *et al.* (11) and contained an effective air-circulating fan. Air temperature was measured with a shaded thermocouple while internal leaf temperature was estimated with thermocouples pressed against the lower side of the leaf.

A saturating light intensity (2300 ± 100 μEinsteins m^{-2} sec^{-1} [400–700 nm] or 81,000 lux) was provided by nine General Electric Cool Beam 150-w lamps, filtered through 6 cm of water. Air temperature was kept at 24.5 ± 0.5 C and leaf temperature at 25.0 ± 0.5 C by recirculating the chamber-cooling water through a constant temperature water bath. Relative humidity was maintained at approximately 65%.

A source leaf was gently sealed into the chamber and allowed to equilibrate in the light. Steady state gas-exchange measurements on each source leaf were made first in an atmosphere containing 21% O_2 and then immediately in 2% O_2. In both cases the ambient CO_2 concentration was maintained at 300 ± 5 $\mu l\ l^{-1}$. Flow rate was adjusted so that the CO_2 depletion across the chamber did not exceed 50 $\mu l\ l^{-1}$.

Calculations of net photosynthesis, transpiration, and CO_2 diffusive resistances in atmospheres of 21 and 2% O_2 were made according to the methods of Gaastra (8), except for the following. Ambient CO_2 and water vapor concentrations were assumed to be that of air exiting the chamber. Carbon dioxide compensation points (50 $\mu l\ l^{-1}$ in air and 15 $\mu l\ l^{-1}$ in 2% O_2) were used as an estimate of the CO_2 concentration at the chloroplast (6). We did not separate stomatal and external air resistances but refer to these collectively as vapor-phase diffusion resistance. Mesophyll resistance (synonymous with the term residual resistance) was taken as the difference between the total resistance and the vapor-phase diffusion resistances, and was not partitioned further.

RuDP Carboxylase. Starting at 2:00 PM on the 8th day of the experiment, control and shaded plant source leaves were extracted and assayed according to the methods of Bowes, *et al.* (3), modified to include 0.5% polyvinylpyrrolidone in the extraction buffer.

Carbohydrates. Source leaf laminae were cut from shaded and control plants and fresh weights were quickly determined. All harvests were made from 2:00 to 2:30 PM. The freshly cut leaves were placed immediately into an oven at 100 C for 60 min. After continued drying to constant weight at 60 C, the leaves were ground in a small Wiley mill.

Subsamples of the dried, ground tissue were extracted and assayed for glucose, fructose, sucrose, and starch according to the methods of Lechtenberg, *et al.* (19).

Translocation. On the 8th day of the experiment, attached source leaves of shaded and control plants were pulse labeled with 40 μCi of $^{14}CO_2$ in a 0.5-liter waterjacketed acrylic chamber. Light from a General Electric Cool Beam 150-w lamp provided 700 ± 10 μEinsteins m^{-2} sec^{-1} (400–700 nm) during the 5-min exposure to $^{14}CO_2$. A sequence of 8-mm discs was punched from the main lamina of these leaves, one from each leaflet at each sampling time. Punches were taken immediately after labeling and again after 0.5, 1, 2, and 3 hr of translocation in the light. The discs were dropped into cold (−78 C) scintillation vials and freeze dried. The weighed discs were pulverized in their vials, 1 ml of H_2O was added, and the mixture was heated to 100 C for 10 min. After cooling, 1 ml of acetate buffer (pH 4.5) and 1 ml of amyloglucosidase (0.5% w/v) were added. The vials were incubated at 40 C for 44 hr and the cpm determined by liquid scintillation. The cocktail consisted of 8 g of PPO, 100 mg of POPOP, and 1000 ml of cellusolve (2-ethoxyethanol) per liter of toluene.

All of the following determinations were performed on freeze dried and ground source leaf tissue harvested from control and shaded plants on the 8th day of the experiment.

Chlorophyll. Approximately 100-mg subsamples of prepared source leaf tissue were weighed into conical centrifuge tubes and vortexed with 10 ml of absolute methanol for 1 min. After 30 min of dark extraction at 25 C, the tubes were centrifuged and Chl *a* and *b* concentrations were determined with a Beckman Acta III spectrophotometer according to the methods of Arnon (1).

Protein. The methanol-insoluble pellets of the Chl assay were resuspended in 5 ml of 0.1 N NaOH, incubated 20 min with stirring, and centrifuged. The supernatant was decanted and aliquots were diluted appropriately for total protein determination by the Lowry procedure (20).

Inorganic Phosphorus. Inorganic P was extracted from subsamples of prepared source leaves according to the methods of Hall and Hodges (10) and assayed by the Fiske-SubbaRow procedure (7).

Electron Microscopy. Electron microscopy was used to determine the location, quantity, and relative size of starch grains in source leaves harvested at 2:30 PM on the 6th day of the experiment. Sections of control and shaded plant source leaves were fixed, embedded, sectioned, and stained according to the procedures of Meek (22). Sections were examined with a Phillips-300 electron microscope.

RESULTS

CO_2 Assimilation. Photosynthetic rates of source leaves were reversibly altered by the shading treatment imposed (Fig. 1). These rates increased significantly ($P < 0.05$) from 37.9 to 56.7 mg dm^{-2} hr^{-1} by the 8th day of continuous shading. Measured in 2% O_2, this increase was from 54.2 to 81.1 mg dm^{-2} hr^{-1}. During this 8-day period, source leaf photosynthetic rates among controls did not significantly change from rates of about 42 mg dm^{-2} hr^{-1} in air and 60 mg dm^{-2} hr^{-1} in 2% O_2 (Fig. 1).

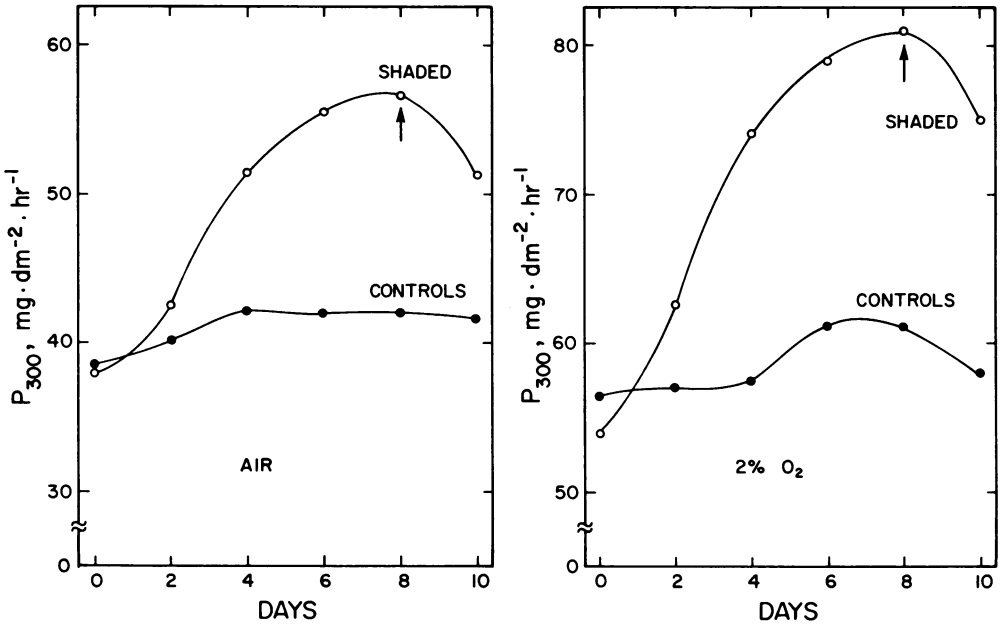

FIG. 1. Light-saturated photosynthetic rates of source leaves of controls and shaded plants measured in atmospheres of 300 μl l^{-1} of CO_2 and 21% (air) or 2% oxygen. Shaded plants were uncovered on the 8th day, as indicated by arrows. Points represent the means of three plants. $S_{\bar{x}}$ = 3.7 in air, 5.0 in 2% O_2.

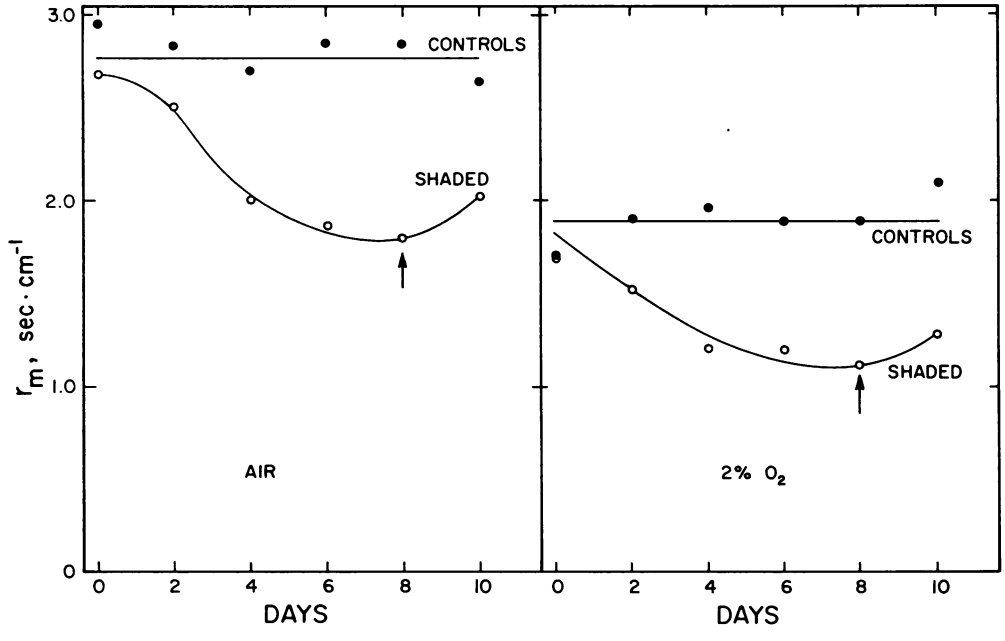

FIG. 2. Mesophyll resistances of source leaves of control and shaded plants calculated from photosynthetic and transpiration data obtained in atmospheres of 300 μl l^{-1} of CO_2 and 21% (air) or 2% oxygen. In both cases, shaded plants were uncovered on the 8th day (arrow). Points represent the means of three plants. $S_{\bar{x}}$ = 0.29 in air, 0.19 in 2% O_2.

When the shaded plants were uncovered on the 8th day, their source leaf photosynthetic rates dropped rapidly. As before, the controls continued unchanged.

Diffusive Resistances. Vapor-phase resistance to CO_2 diffusion into source leaves of shaded and control plants was not significantly different on any day of the experiment and averaged 1.76 sec cm^{-1} in both air and 2% O_2 atmospheres.

By the 8th day, the source leaves of control and shaded plants differed significantly ($P < 0.05$) in estimated mesophyll resistance to CO_2 transfer, whether measured in air or 2% O_2 (Fig. 2). Values obtained in 2% O_2 were always significantly ($P < 0.01$) lower than those obtained in air. Mesophyll resistance of control source leaves measured in both air and 2% O_2 remained essentially constant throughout the experiment. In source leaves of shaded plants, however, mesophyll resistance decreased to a minimum on the 8th day and increased again when the plants were uncovered.

Carbohydrates. Concentrations of sucrose (Fig. 3) and starch (Fig. 4) changed dramatically when the plants were shaded. From an initial concentration of 23% of dry weight, source leaf starch declined to 1.8% by the 9th day of continuous sink shading. If the plants were unshaded on the 6th

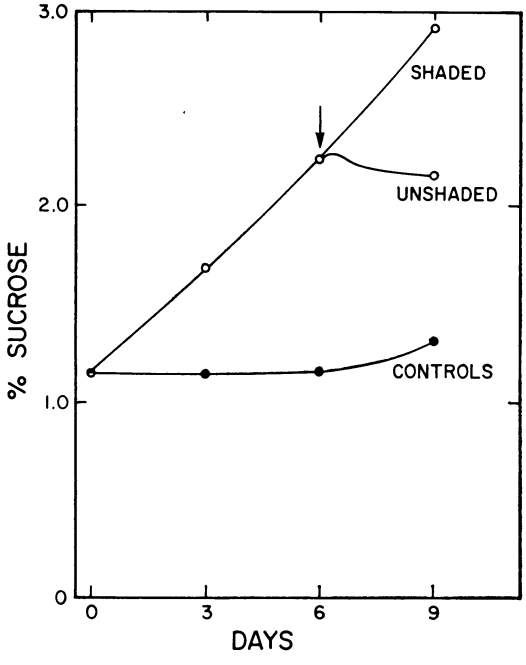

FIG. 3. Sucrose concentrations in source leaves of controls and shaded plants. Shaded plants were either uncovered on the 6th day (arrow) or continuously shaded. Points represent the means of four plants. $S_{\bar{x}} = 0.14$.

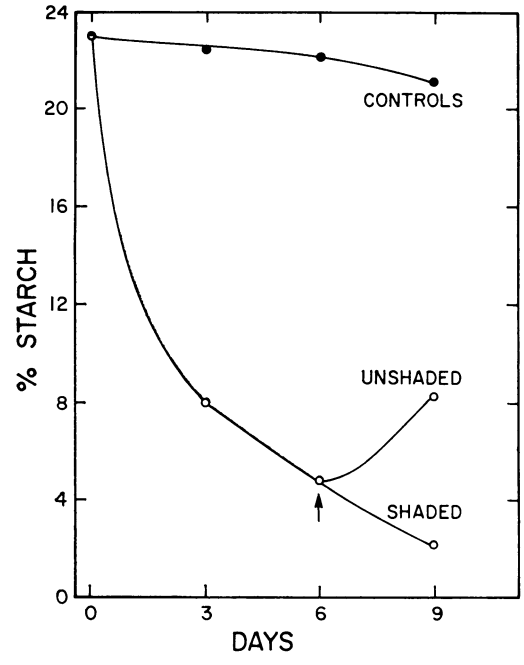

FIG. 4. Starch levels in source leaves of controls and shaded plants. Shaded plants were either uncovered on the 6th day (arrow) or continuously shaded. Points represent the means of four plants. $S_{\bar{x}} = 0.81$.

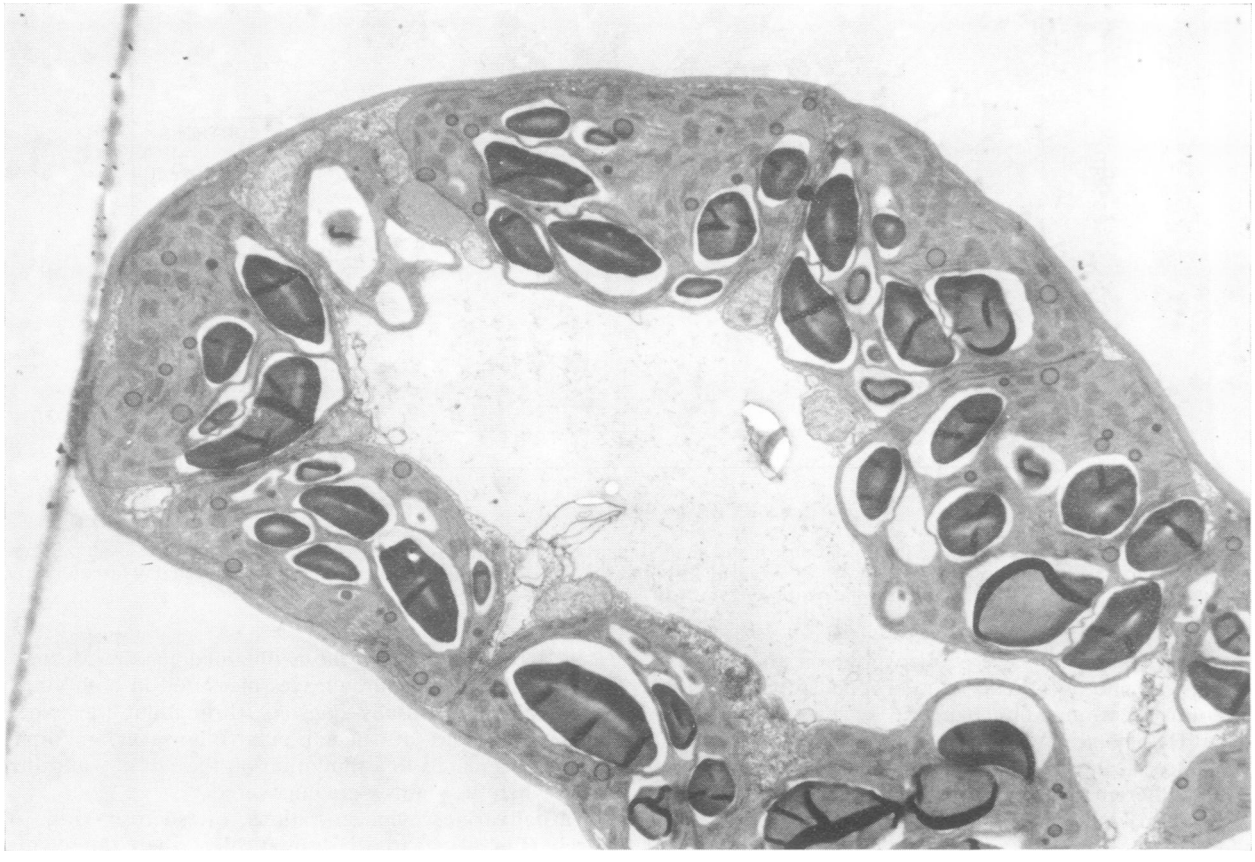

FIG. 5. Electron micrograph of soybean chloroplasts in a source leaf mesophyll cell of a control plant sampled on the afternoon of the 6th day. Note the large centripetal accumulation of starch. × 9,500.

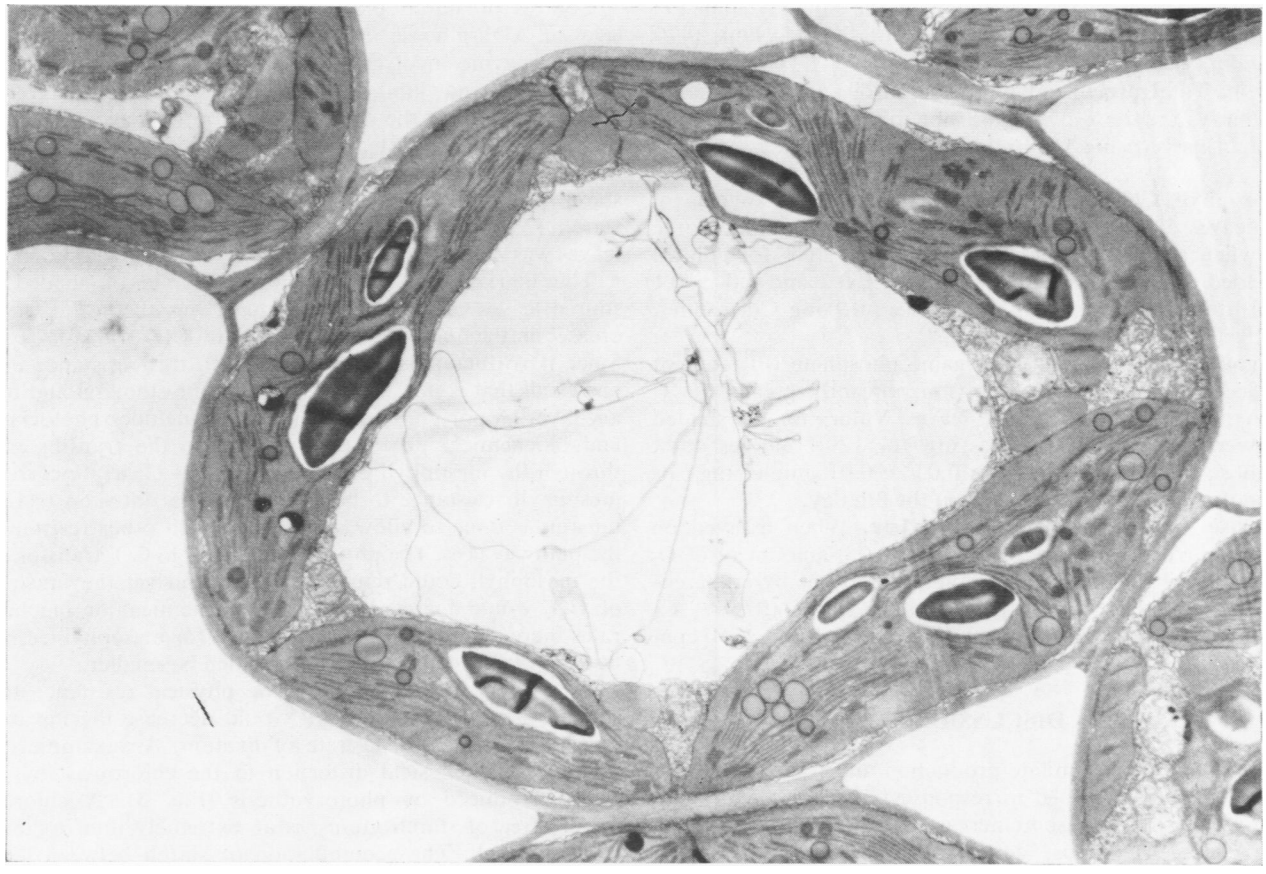

FIG. 6. Electron micrograph of soybean chloroplasts in a source leaf mesophyll cell of a shaded plant sampled on the afternoon of the 6th day. Note the absence of many large starch grains. × 11,000.

day, the rapid depletion of starch ceased and slow accumulation occurred (Fig. 4).

Starch concentration in the control source leaves remained high throughout the experiment. Figures 5 and 6 illustrate chloroplast starch-content differences between control and shaded plant source leaves on the afternoon of the 6th day.

Sucrose concentration in the source leaf of shaded plants increased in a manner paralleling the photosynthetic increase. From a concentration of 1.2% originally, it increased to 2.9% on the 9th day. If the plants were unshaded on the 6th day, the rapid accumulation of sucrose ceased and the concentration remained virtually unchanged at 2.2% for the rest of the experiment (Fig. 3).

Glucose and fructose concentrations remained constant throughout the experiment, not varying significantly with treatment or time. Concentrations of glucose and fructose were 0.5% and 0.4% respectively, for both control and shaded plant source leaves.

Translocation. Apparently responding to the increased photosynthetic rate and sucrose level of the shaded plant source leaves, translocation on the 8th day was much faster than controls (Fig. 7). In 1 hr, the control source leaves translocated 32% of the initial pulse of 11,080 cpm/mg leaf dry weight. By 3 hr, 52% of the pulse had been exported from these leaves. The source leaves of shaded plants fixed an initial pulse of 14,300 cpm/mg and within 1 hr had exported over 56%. After 3 hr, over 75% of the initial pulse was gone.

Specific Leaf Weight. The mean specific leaf weight of the control source leaves (580 ± 10 mg dm^{-2}) was significantly

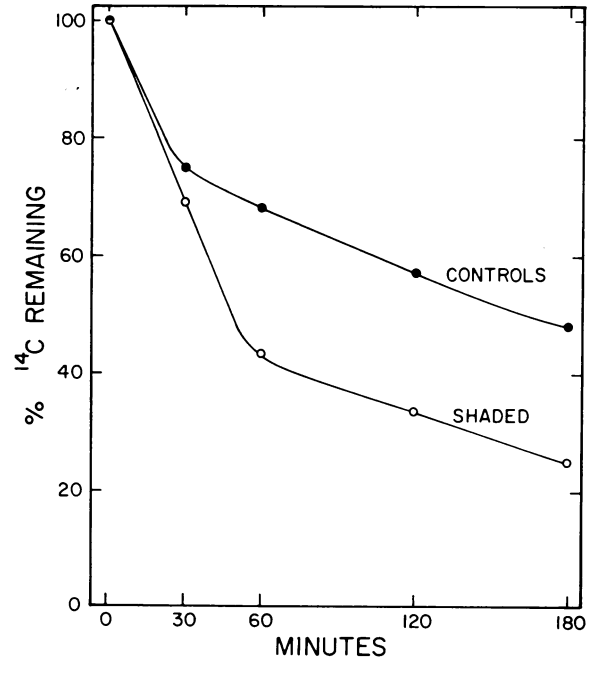

FIG. 7. Rate of ^{14}C-assimilate translocation from source leaves labeled on the 8th day of the experiment. Source leaves of shaded plants initially fixed a 22% larger pulse than those of controls. Points represent the means of five plants.

($P < 0.01$) greater on the afternoon of the 8th day than the specific leaf weight of the source leaves of shaded plants (470 ± 6 mg dm^{-2}).

Protein. Total protein content of control and shaded plant source leaves on the 8th day did not differ significantly on a dry weight basis, being 161 ± 11 and 161 ± 9 mg g^{-1}, respectively.

Chlorophyll. Chlorophyll *a* and *b* concentrations of control source leaves did not differ from those of shaded plant source leaves when assayed on the 8th day. These values for control and shaded plant source leaves were 5.1 ± 0.2 and 5.0 ± 0.1 mg Chl *a* dm^{-2}, and 1.6 ± 0.1 and 1.7 ± 0.1 mg Chl *b* dm^{-2}, respectively.

Inorganic Phosphate. The inorganic phosphate (Pi) content of shaded plant source leaves was significantly greater ($P < 0.01$) than that of control source leaves. Values for the shaded plants were 0.29 ± 0.03 μmoles mg^{-1} or 129.1 μmoles dm^{-2}, while those for the controls were 0.05 ± 0.01 μmoles mg^{-1} or 31.0 μmoles dm^{-2}, when measured on the 8th day.

Ribulose 1,5-Diphosphate Carboxylase. When assayed on the 8th day, a significantly greater ($P < 0.05$) amount of $^{14}CO_2$ was assimilated per unit time on a leaf area basis by crude enzyme extracts from shaded plant source leaves (976 ± 63 cpm dm^{-2} min^{-1}) than from control source leaves (680 ± 50 cpm dm^{-2} min^{-1}).

DISCUSSION

The efficiency of assimilate production and translocation by the source leaves increased in response to experimental shading, apparently in response to increased assimilate demand (17). Concurrently, the sucrose content increased and the starch content decreased. The almost total disappearance of source leaf starch among the shaded plants was apparently responsible for the lower specific leaf weight of these leaves compared to the controls. By the 8th day, the source leaf of the shaded plants had not only been depleted of previously stored starch, but normal diurnal accumulation of starch was also reduced. These source leaf responses were reversed, however, when the plants were unshaded and thus demand for source leaf assimilate was reduced.

This inverse relationship between starch concentration and photosynthetic rate has also been observed by Chatterton *et al.* (5). Their data suggest that photosynthesis is normally somewhat reduced by the diurnal accumulation of starch, severely reduced by large induced accumulations of starch, and stimulated by conditions (*e.g.*, tillering) in which little starch accumulates. Control leaves in the present study assimilated CO_2 at normal rates (6) while containing 23% starch. When the starch concentration of source leaves was experimentally reduced to less than 2%, the rate of net photosynthesis increased by more than 25%. Concurrently, the rate of translocation from these leaves also increased, consistent with the close correlation established between rates of translocation and net photosynthesis (14). The increased sucrose concentration of these leaves had little adverse effect on the photosynthetic rate, perhaps because the sucrose was not in the chloroplasts (9).

In comparison to the shaded plant source leaves, it would appear that the normal photosynthetic rate of the controls was 25% reduced under conditions that permit high starch content and that the rates achieved when this level of starch was reduced are more nearly their true potential. This is consistent with the observation by Maggs (21) that "leaves normally operate at levels well below the maxima of which they are capable."

Control of starch biosynthesis is known to occur at the level of ADP-glucose pyrophosphorylase (25). The regulation of this enzyme involves allosteric activation by glycolytic intermediates and inhibition by Pi (27). The nearly 4-fold greater Pi level of the shaded plant source leaves than that of the controls is consistent with the low starch levels in these leaves, because Pi stimulates the enzymatic degradation by starch phosphorylase while it inhibits the synthesis of further starch (25, 27). The origin of the extra Pi in these source leaves was not determined.

The marked rise in photosynthetic rate of shaded plant source leaves observed in this study was the result of a decrease in the mesophyll resistance to CO_2 transfer (Fig. 2). Since it is obtained by subtraction (8), this resistance encompasses all that is not accounted for by the stomatal and boundary-layer (vapor phase) resistances and includes photochemical and biochemical processes unrelated to the transfer of CO_2 through the mesophyll cell (16). It is thus clearly incorrect for mesophyll resistance to be expressed as a diffusion resistance, but this is done to allow comparison with other resistances in the pathway (16). The physical resistance to CO_2 transfer across the mesophyll could remain constant and yet the inward flux of CO_2 would increase if any photochemical or biochemical rates increased. The estimate obtained for mesophyll resistance to CO_2 transfer and fixation would then be smaller.

Conversely, any increase in the physical resistance to CO_2 transfer across the mesophyll would decrease the inward flux of CO_2 and likewise, its rate of fixation. An example of this might be the physical distortion of the chloroplast by starch grains produced by photosynthesis (Fig. 5). A chloroplast, largely free of starch grains, is an extremely thin organelle in the living cell. The accumulation of starch between lamellae so distorts the chloroplast that it approaches the shape of a sphere (32). Rackham (26) has shown that the effective path length (as calculated from electron micrographs) of CO_2 diffusion to the grana is affected by the presence or absence of starch grains in the chloroplast. The starch distortion of control chloroplasts, seen in Figure 5, appears to support his prediction, although the centripetal arrangement of these grains permits large peripheral areas to be totally devoid of starch.

The presence of many large starch grains could also possibly decrease photosynthesis (and thus increase estimates of mesophyll resistance) if they effectively shaded the lamellae (31). However, the photochemical component was eliminated from the estimates of mesophyll resistance reported here by saturating photosynthesis with light.

Also affecting estimates of mesophyll resistance is the activity of RuDP carboxylase. The activity of this enzyme (as well as net photosynthesis) has been shown to be stimulated by plant hormones, whether applied exogenously (28, 30), or produced in response to plant manipulation (23, 30). In the present study, plant shading stimulated source leaf RuDP carboxylase activity. Thus, the photosynthetic response of the source leaf may have resulted from a change in hormone distribution pattern. In addition to mesophyll resistance, the magnitude of stomatal resistance to CO_2 diffusion is hormonally influenced (23). However, in the present study the absence of a change in vapor phase resistance suggests that source leaf stomata were not influenced by plant shading.

The results of this study suggest that sink demands for photosynthate have a marked influence on source leaf photosynthesis and subsequent carbohydrate formation and export. In regard to the control mechanism, sucrose has apparently been eliminated as a possible mass-action regulator of photosynthesis. The role of starch level as a regulator of photosynthetic rate is questionable, although these data do not conclusively

eliminate this possibility. The mobilization of Pi and the increased activity of RuDP carboxylase suggest a source-sink hormonal interaction.

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