Carbohydrate Partitioning and the Capacity of Apparent Nitrogen Fixation of Soybean Plants Grown Outdoors

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Abstract
Patterns of leaf carbohydrate partitioning and nodule activity in soybean plants grown under natural conditions and the irradiance level required to produce sufficient carbohydrate to obtain maximum rates of apparent N₂-fixation (acetylene reduction) were measured. Soybean plants, grown outdoors, maintained constant levels of leaf soluble sugars while leaf starch pools varied diurnally. When root temperature was kept at 25°C and shoot temperature was allowed to vary with ambient temperature, the plants maintained constant rates of apparent N₂-fixation and root+nodule respiration. Results from a second experiment, in which the entire plant was kept at 25°C, were similar to those of the first experiment. Shoot carbon exchange rate of plants from the second experiment was light saturated at photosynthetic photon flux densities between 400 and 600 micromoles per square meter per second. When plants were subjected to an extended 40-hour dark period to deplete carbohydrate reserves, apparent N₂-fixation was unaffected during the first 10 hours of darkness, decreased rapidly between 10 and 16 hours, and plateaued at one-third the initial level thereafter. After the extended dark period, plants were exposed to photosynthetic photon flux density from 200 to 1000 micromoles per square meter per second for 10 hours. Photosynthetic photon flux densities of 200 micromoles per square meter per second and greater resulted in maximum leaf soluble sugar content and nodule activity. Leaf starch content increased with irradiance levels up to 600 micromoles per square meter per second with no further increase at higher irradiance levels. Results presented here indicate that maximum nodule activity occurs at irradiance levels that do not saturate the plant's photosynthetic apparatus. This response would allow for maximum N₂-fixation to occur in a nodulated legume during periods of inclement weather.

Symbiotic N₂-fixation in legumes requires significant inputs of carbon substrates to provide energy for nitrogen reduction and acceptor molecules for subsequent transport of reduced N (2, 19). It has been shown that legumes dependent upon N₂-fixation as their source of reduced N require more energy per unit of N incorporated than plants grown on combined N (14, 19). Thus, due to this large carbon requirement, symbiotic N₂-fixation has often been said to be closely coupled to photosynthesize production (1, 8).

The relationship between N₂-fixation, photosynthesis, and growth of the plant on a daily basis is not well understood. It appears that carbon substrates needed for symbiotic N₂-fixation are provided by both current photosynthesis and stored carbohydrate (16, 20). Daily photosynthesis allocation to nodulated soybean root systems also may be similar to that allocated to roots of other plants (3, 30). During the light period, photosynthesis is formed in the leaves and translocated to various sinks throughout the plant for synthesis and maintenance requirements. Excess photosynthesis is subsequently stored as starch in the leaves and then drawn upon to provide energy during the dark cycle of each 24 h photoperiod (4, 20, 25). The control of partitioning in the leaf between reserve and mobile forms of photosynthesis by the environment (4, 5) may also play an important role in carbon allocation to the nodulated root system.

Some data indicate that the daily supply of photosynthesize to root nodules is insufficient for potential maximum rates of N₂-fixation in legumes (9, 16, 23). Demonstrated diurnal variations in soybean root-nodule activity and unused N₂-fixation capacity stress the importance of a current supply of photosynthesize. Maximum N₂-fixation rates apparently occur near the period of maximum irradiance level in the field (9, 23), or the end of the light period under controlled environmental conditions (16). However, there are reports that indicate soybean root nodules can function at a maximum rate that is not limited by shoot CO₂ assimilation. This concept is supported by data showing no diurnal variation in N₂-fixation (17, 20, 29) and a lack of an increase in specific nodule activity after short term increases in photosynthesize production (7, 29). Results in all the above studies were attributed to the constant environmental conditions used throughout the growth and/or assay periods. For example, when shoot temperature was varied between day and night, diurnal variations of nodule activity were measured (20). Sloger et al. (23) also found that nodule activity of field-grown soybean plants was significantly correlated with average air temperature but not with average soil temperature. These data indicate that the translocation of photosynthesize to the nodules rather than photosynthesis per se limited nitrogenase activity. The data also demonstrate that the soybean shoot serves as the primary source of reserve carbohydrate for the plant.

Sheehy et al. (21) first determined the shoot CO₂ assimilation rate of legumes needed to provide sufficient carbohydrate for utilization of all available root-nodule activity when grown under natural illumination. There was no significant difference in root-nodule activity even though shoot CO₂ assimilation rate differed 4-fold for soybean plants. Since neither shoot carbon uptake nor root-nodule activity were determined prior to the manipulation of the plant's photosynthetic rate, it is unknown whether daily photosynthesize supply was sufficient to obtain the maximum rates of apparent N₂-fixation subsequently measured. The saturation of root-nodule activity at a very low shoot assimilation rate suggested that the plant's N₂-fixation apparatus may have...
been adapted to operate at maximum capacity under low light conditions. This may have resulted from the ability of the plant to arrive at a balance between photosynthetic production (S) and respiration (R) under varying light conditions.

The irradiance environment of plants grown in the glasshouse is complex. Instantaneous PPFD changes constantly depending upon solar angle and cloud cover, and as a result, total integrated PPFD varies daily. The question of how a symbiotic legume acclimates to such a complex environment and what influence this has on the capabilities of the N₂-fixing apparatus is unknown. This purpose of this study was to determine how acclimation of nodulated soybean plants to a natural environment affects regulation of photosynthetic partitioning and N₂-fixation. Initially, relationships among daily carbon uptake of the shoot, leaf carbohydrate partitioning, and root-nodule activity were determined. Subsequently, irradiance level and leaf carbohydrate status needed to produce maximum nodule activity in carbohydrate-depleted soybean plants were measured.

MATERIALS AND METHODS

Soybeans (Glycine max L. Merr. cv. Clark) were germinated in the dark at 25°C. Three days after imbibition, seedlings were inoculated with a slurry of Rhizobium japonicum USDA strain 110 and transferred to 13 cm diameter pots containing vermiculite. For determination of intact plant root-nodule activity, some pots were covered with a Plexiglas lid which was perforated to accommodate one plant per pot and allow for watering with either a nutrient solution modified to contain 2 mM KNO₃ (27) or distilled H₂O on alternate days. Plants in both covered and uncovered pots were then randomly placed approximately 30 cm apart on platforms outdoors where they remained throughout the experimental period. During this period, PPFD, ambient air temperature, and pot temperature were monitored with a data logger.

One experiment was conducted during the late spring and one during the late summer in Baton Rouge, LA. Each time, diurnal changes in photosynthetic partitioning in the leaves and root-nodule activity were determined in 35-d-old plants grown outdoors. During the late spring, the roots of four separate plants were kept at 25 ± 2°C. Temperature control was accomplished by enclosing the four pots in a Plexiglas chamber equipped with a heat exchanger coupled to a water bath.

Diurnal activity of the roots and nodules was determined in a manner similar to that described by Sheehy et al. (21). The pots which had previously been fitted with perforated Plexiglas covers were first sealed with a pliable nontoxic sealant (Mortite caulking cord, Mortell Co., Kankakee, IL). Root and nodule respiration was monitored by passing air at a constant flow rate through the sealed pots to an automatic gas sampling system. This system consisted of solenoid valves operated by a cam timer which sampled air in each pot every 3 min. A complete cycle in which each replicate plant was sampled lasted 30 min. CO₂ concentration was measured with an ADC Mark II IR gas analyzer. PPFD was measured at the top of the plant using a LI-COR model 185B quantum sensor. Temperature, irradiance, and output from the IR gas analyzer were recorded every minute with a data logger. At approximately 4 h intervals during the diurnal period, air flow through the pots was interrupted, and the reduction of acetylene to ethylene over a 20 min period was determined as previously described (27). Shoot carbon exchange rates during the day were determined using an open system similar to that described by Williams and Phillips (27). Irradiance levels were varied by the use of neutral density shade screens.

Plants were harvested at 4 h intervals to determine diurnal changes in nonstructural carbohydrate composition in the leaves.

Leaves were separated from the rest of the plant and oven dried at 75°C for 48 h. Leaf tissue was finely ground and a subsample analyzed for starch and soluble sugar content using the method described by Upmeyer and Koller (25).

The irradiance level required to produce and allocate carbohydrate sufficient to obtain maximum root-nodule activity was determined by first extending the normal dark period for 40 h to deplete carbohydrate reserves. The dark period was extended by taking plants to the laboratory and placing them in darkened cabinets. Plants were then exposed to stepped increases in irradiance provided by 1000 W metal-halide lamps. During exposure, respiration of the roots and nodules was monitored. After 10 h, plants were assayed for acetylene reduction on detached root systems and the leaves removed and analyzed for starch and soluble sugar content.

RESULTS

Irradiance levels and ambient air temperatures varied considerably both times the experiment was conducted (Figs. 1 and 2).

![Fig. 1. Relationships among ambient air temperature, root-zone temperature, and solar radiation (A), leaf carbohydrate partitioning (B), root-nodule respiration (C), and apparent N₂ fixation (D) in 35-d-old soybean plants with and without controlled root-zone temperature. Data were collected during the month of May. Vertical bars indicate the se of the mean.](https://example.com/fig1.jpg)
However, there was no apparent diurnal variation in either root+nodule respiration or acetylene reduction when the root system was maintained at a constant temperature. There was a diurnal pattern of leaf starch accumulation with a maximum concentration measured at 1800 h, but only a slight variation in leaf soluble sugar content occurred. Uncontrolled root-zone temperature varied between 19 and 37°C during the course of the day (Fig. 1). Root+nodule respiration had a $Q_{10}$ of approximately 4 from 0800 to 1400 h when root-zone temperature increased from 20 to 35°C. Subsequently, respiration dropped almost 3-fold to 22 nmol CO$_2$ plant$^{-1}$ s$^{-1}$ while the root-zone temperature increased to 37°C. When temperature was not controlled, there was a significant decrease in apparent N$_2$-fixation after the root-zone temperature exceeded 35°C. Although the root-zone temperatures had decreased to 25°C by 1900 h, apparent N$_2$-fixation remained depressed and constant until 0600 h. In contrast, when root-zone temperature was controlled at 25°C, apparent N$_2$-fixation rates were constant throughout the experimental period.

Light saturation of whole plant apparent photosynthesis occurred at 600 μmol m$^{-2}$ s$^{-1}$ when measured with 33-d-old plants (Fig. 3). The whole plant carbon exchange rate at 200 μmol m$^{-2}$ s$^{-1}$ was approximately 50% of the light saturated values.

Plants subjected to an extended dark period of 40 h after the normal photoperiod exhibited no significant change in acetylene reduction until 14 h into the extended dark treatment (Fig. 4). This corresponded approximately to the time when the normal photoperiod would have begun outdoors. There was no further decrease in apparent N$_2$-fixation with an additional 20 h of darkness.

When plants were exposed to various irradiance levels following 40 h of darkness, root+nodule respiration reached a maximum approximately 3 to 4 h after exposure to light regardless of irradiance level (data not shown). For plants grown during the spring or fall, an irradiance level of 200 μmol m$^{-2}$ s$^{-1}$ for 10 h after a 40 h dark period significantly increased the rate of apparent N$_2$-fixation (Table I; Fig. 5). However, irradiance treatments above 200 μmol m$^{-2}$ s$^{-1}$ did not produce significant increases in nodule activity. Leaf starch content in the spring study increased with increasing irradiance levels up to 1000 μmol m$^{-2}$ s$^{-1}$ (Table I). Leaf starch content did not increase with increased irradiance above 600 μmol m$^{-2}$ s$^{-1}$ in the fall study.
Table 1. Effect of Various Irradiance Levels for 10 h on Leaf Soluble Carbohydrates and Apparent N₂-fixation Determined on Detached Roots of Soybean Plants Grown Outdoors during May, after a 40 h Dark Pretreatment. Plants Were 35 d Old at the Start of the Dark Pretreatment.

<table>
<thead>
<tr>
<th>Irradiance (µmol m⁻² s⁻¹)</th>
<th>Sol. Sugars (mg g⁻¹ dry wt)</th>
<th>Starch (mg g⁻¹ dry wt)</th>
<th>Acetylene Reduction (µmol plant⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.5</td>
<td>25.1</td>
<td>1.7</td>
</tr>
<tr>
<td>200</td>
<td>41.6</td>
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</tr>
<tr>
<td>600</td>
<td>45.7</td>
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<td>14.9</td>
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<tr>
<td>1000</td>
<td>43.0</td>
<td>134.3</td>
<td>10.1</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*This treatment represents plants that have been in darkness for 40 h.

Fig. 5. Response of apparent N₂-fixation and carbohydrate partitioning to increased irradiance levels following 40 h in darkness. Plants were grown outdoors during September and were 35 d old at the beginning of the extended dark period. Acetylene reduction measurements were conducted on detached roots. The zero irradiance treatment represents plants that have been in darkness for 40 h. Vertical bars indicate the se of the mean.

DISCUSSION

Nodules of soybean plants grown outdoors reduced acetylene and respired CO₂ at a constant rate when the temperature of the root system remained constant. These results occurred despite changes in irradiance levels and shoot temperature throughout the day. These results are similar to a controlled-environment study (29) but differ from the results of Huck et al. (11) where diurnal changes in root respiration occurred despite controlled root-zone temperature. Huck et al. (11) and others (20, 23) have suggested that the acetylene reduction and respiratory activity of nodules also responds to shoot temperature, perhaps resulting from temperature effects on vein loading and carbohydrate translocation from the shoot to nodules. Vein loading and translocation in the phloem of wheat plants, however, is largely unaffected by temperatures from 20 to 40°C (26). Similar translocation-temperature response curves also have been shown with bean plants (10, 24). In the present study, ambient air temperatures were within limits that probably would not decrease the export of carbohydrates out of the leaves or other storage organs of the soybean plant (Figs. 1 and 2).

Optimum activity of apparent N₂-fixation in nodulated soybean plants occurs at root temperatures between 20 and 30°C (6, 9). Previous studies (13, 18) have demonstrated that the temperature optimum of R. japonicum USDA strain 110 occupies a very narrow range. Strain 110 also was found to be very sensitive to high temperatures (>30°C) regardless of host genotype. In the present study, there was no significant decrease in apparent N₂-fixation when root-zone temperature reached 35°C at 1400 h (Fig. 1). Subsequent to this measurement, however, there was a significant decrease in both root+nodule respiration and acetylene reduction. The decrease in root+nodule respiration from 1400 to 1600 h occurred without a concomitant decrease in pot temperature. It is unknown whether the continued high pot temperature from 1400 to 1600 h and/or other related variables such as plant water status were responsible for both decreases.

The pattern of nonstructural carbohydrate content throughout the day (Figs. 1 and 2) resembled that found in soybeans grown under controlled environmental conditions (5, 25). Maximum starch content measured at 1800 h both days was similar to maximum values obtained after carbohydrate depleted plants had been held at a constant irradiance level for 10 h (Fig. 5; Table 1). This probably was due to the similarities in length of the normal photoperiod (2 h) and the photoperiod of Figs. 1 and 2 and the time used for the constant irradiance experiments. Chatterton and Silvius (4) showed that the rate of starch accumulation in fully expanded soybean leaves was a function of the duration of the photosynthetic period. They also reported that lowering the irradiance level did not change the partitioning of photosynthate as long as the duration of the photosynthetic period remained the same (5). In this study, a decrease in irradiance level below the light saturation level of 600 µmol m⁻² s⁻¹ (Fig. 3) resulted in a significant decrease in starch content when compared after 10 h in the light (Table 1). Differences in results between the two studies may have been due to differences in tissues sampled (all leaves on a plant versus only fully expanded leaves), sinks present (nodulated versus nonnodulated plants), or the effect of a 40 h dark period before the resumption of the light period.

It is not surprising that patterns of leaf carbohydrate accumulation and root-nodule activity in this paper were similar to those obtained with plants grown under controlled environmental conditions. The light saturation curve of shoot CO₂ uptake indicated that irradiance levels greater than 600 µmol m⁻² s⁻¹ did not result in greater carbon assimilation for the plant (Fig. 3) or increased leaf starch content (Fig. 5). Thus, during the fall study, plants were grown in an environment outdoors that probably could be described as a constant 600 µmol m⁻² s⁻¹ for 8 h daily under our growth conditions (approximately number of hours above the light saturation point for an individual soybean plant during September in southern Louisiana). In addition, daily solar radiation received in Baton Rouge was nearly constant for all but one of 9 d preceding the fall study. This would be similar to growing plants in a controlled environment growth chamber at a near constant irradiance level and photoperiod.

Recent studies have demonstrated that plants grown under constant environmental conditions maintain a balance between substrate production and utilization on a 24 h basis (15). They are characterized by a rapid decrease of respiratory CO₂ efflux just subsequent to an extension of the normal dark period (15). This concept has been used to describe the growth of nodulated legumes in growth chamber experiments (22, 29). It could be concluded that soybean plants used in this study also were in a steady state of substrate production and utilization even though they were grown in a fluctuating light and temperature environment. When the normal dark period was extended (Fig. 4) there was a significant decrease in apparent N₂-fixation after the time sunrise naturally would have occurred. It appeared that the pool
of storage carbohydrates was nearly exhausted during the normal dark period with that remaining unable to support further maximum nodule activity. Thus, results from this and growth chamber studies indicate that in addition to temperature, the maintenance of a constant, maximum rate of nodule activity during the vegetative stage of soybean growth is dependent upon the current day's supply of photosynthate. Photosynthate is partitioned in the leaves during the light period such that the pool of soluble sugars is enough to support maximal nodule activity of the plant even under low light conditions. Continued maximum activity of metabolic processes during the dark period is dependent upon the amount of excess photosynthate stored as starch during the day.

Two recent studies (7, 12) have suggested that photosynthate is inefficiently partitioned in nodulated soybean. The fact that exposure to a low irradiance level (Table I; Fig. 5) and low rates of shoot CO2 assimilation (21) produced maximum nodule activity in soybean plants does not support that suggestion. It would appear that the physiological processes (N2-fixation) of the vegetative soybean plant are structured to maintain maximum activity if alterations in shoot CO2 uptake occur, for example during short periods of inclement weather. This mechanism would allow for the plant an efficient means to provide for maximum nitrogen assimilation by the root nodules. This would be especially important during seedling growth of legumes under low soil N conditions since growth often is limited by N availability at this stage (28).

Acknowledgments—We would like to thank Drs. D. A. Phillips and D. J. Longstreth for reviewing the manuscript.

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