Effect of Changes in Shoot Carbon-Exchange Rate on Soybean Root Nodule Activity[1]

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ABSTRACT

The effect of short- and long-term changes in shoot carbon-exchange rate (CER) on soybean (*Glycine max* [L.] Merr.) root nodule activity was assessed to determine whether increases in photosynthate production produce a direct enhancement of symbiotic N_2 fixation. Shoot CER, root + nodule respiration, and apparent N_2 fixation (acetylene reduction) were measured on intact soybean plants grown at 700 microeinsteins per meter per second, with constant root temperature and a 14/10-hour light/dark cycle. There was no diurnal variation of root + nodule respiration or apparent N_2 fixation in plants assayed weekly from 14 to 43 days after planting. However, if plants remained in darkness following their normal dark period, a significant decline in apparent N_2 fixation was measured within 4 hours, and decreasing CO_2 concentration from 320 to 90 microliters CO_2 per liter produced diurnal changes in root nodule activity. Increasing shoot CER by 87, 84, and 76% in 2-, 3-, and 4-week-old plants, respectively, by raising the CO_2 concentration around the shoot from 320 to 1,000 microliters CO_2 per liter, had no effect on root + nodule respiration or acetylene-reduction rates during the first 10 hours of the increased CER treatment. When the CO_2-enrichment treatment was extended in 3-week-old plants, the only measured parameter that differed significantly after 3 days was shoot CER. After 5 days of continuous CO_2 enrichment, root + nodule respiration and acetylene reduction increased, but such changes reflected an increase in root nodule mass rather than greater specific root nodule activity. The results show that on a 24-hour basis the process of symbiotic N_2 fixation in soybean plants grown under controlled environmental conditions functioned at maximum capacity and was not limited by shoot CER. Whether N_2-fixation capacity was limited by photosynthate movement to root nodules or by saturation of metabolic processes in root nodules is not known.

Symbiotic N_2 fixation in legumes requires carbon substrates derived from photosynthate or from stored reserves (9). The substrates provide a source of energy for N_2 fixation and acceptor molecules for transporting reduced N. The original source of such carbon compounds is primarily photosynthetic CO_2 reduction. Claims that photosynthate limits N_2 fixation in soybean (3–5) were supported by long-term CO_2-enrichment studies in field-grown plants. Those experiments showed that CO_2 enrichment increased total N content and promoted both the rate of acetylene reduced/unit nodule mass and the total nodule mass after 7 d (3–5). Such evidence indicates the importance of photosynthate for N_2 fixation, but, without shoot CER[3] measurements and infor-

mation on short-term responses, one cannot determine whether photosynthate production or transport limits N_2 fixation in soybean.

Two types of experiments suggest that photosynthate availability in nodules rather than shoot CER limits N_2 fixation in soybean. Widely different values of soybean shoot CER produced by varying CO_2 concentrations had little effect on the maximum acetylene-reduction rate during a 24-h photoperiod (13). However, because a 40-h dark pretreatment was used to achieve other experimental objectives, it is difficult to extrapolate from those results to normal plants. Grafting techniques that produced soybean plants with 2:1 or 1:1 shoot:root ratios also suggest that photosynthate availability rather than CER limits N_2 fixation, but no direct measurements of shoot CER or photosynthate movement were made (16). Thus, short-term increases in acetylene reduced/g nodule tissue could have been associated with increased photosynthate transport or with a trauma-induced utilization of root carbohydrates.

The purpose of the present study was to test whether increases in soybean shoot CER of normal plants produce short-term changes in root nodule activity. When it was observed that no significant change in root nodule activity could be detected within 10 h after increasing CER, it became important to determine how long-term changes in shoot CER produce the previously reported (3–5) increase in acetylene reduction after 7 d of CO_2 enrichment. The experiments, therefore, also address the question of how an important agronomic plant responds to an increase in CER and achieves a new equilibrium between CO_2 and N_2 reduction.

MATERIALS AND METHODS

Growth Conditions. 'Clark' soybeans (*Glycine max* [L.] Merr.) were germinated and planted as previously described (18). Plants were grown under controlled conditions at a photon flux density of 700 μE m^{-2} s^{-1} (400–700 nm), a 14-/10-h light/dark photoperiod at 28/25°C, and 70% RH. Root temperatures were maintained at 25 ± 1°C. Carbon dioxide was controlled at either 320 ± 20 or 1,000 ± 75 μl CO_2/l of air by continuous monitoring with an IR gas analyzer. Plants were inoculated with *Rhizobium japonicum* strain USDA 3Ilb110 and watered with a nutrient solution containing 2 mM NO_3^- (18). Under these conditions, soybeans flowered approximately 31 days after planting.

Gas-Exchange Measurements. Rates of apparent whole-plant photosynthesis were measured by differential IR gas analysis in Plexiglas chambers (18) with environmental conditions identical to those under which the plants had grown. Root + nodule respiration was measured with the same system by passing air through the rooting medium which was separated from the shoot system at all times by a Plexiglas lid sealed to the pot. Measurements of apparent N_2 fixation were made on intact plants by determining acetylene-dependent ethylene production, less rigorously termed acetylene reduction (18).

Compositional Analyses. Plants were harvested and dried at

[3] Abbreviation: CER, carbon-exchange rate.

70°C for 48 h. Sucrose, fructose, glucose, pinitol, and starch were measured by gas chromatography (13). Values recorded for sucrose, fructose, glucose, and pinitol were summed to estimate total soluble sugar.

RESULTS

Root + nodule respiration and apparent N_2 fixation showed no significant diurnal variation under normal environmental conditions 2 to 6 weeks after planting (Figs. 1 and 2). When the dark period was extended beyond the customary night period, both root + nodule respiration and apparent N_2 fixation declined dramatically, compared with plants exposed to their normal photoperiod (Fig. 3). When plants were grown under normal CO_2 conditions (320 μl CO_2/l) and then transferred to a low CO_2 concentration (90 μl CO_2/l), a marked diurnal variation in both root + nodule respiration and apparent N_2 fixation was measured 5 d later (Table I).

Increasing shoot CER by raising the CO_2 concentration from 320 to 1,000 μl CO_2/l for 10 h produced no significant change in root + nodule respiration or apparent N_2 fixation in plants of five ages (Table II). When plants were grown at the higher CO_2 concentration for longer periods of time, significant differences in respiration and total acetylene reduction between the two treatments were measured after 5 d (Table III), but there was no significant effect of CO_2 enrichment on specific acetylene-reduction activity (defined as μmol ethylene g^{-1} nodule dry weight h^{-1}).
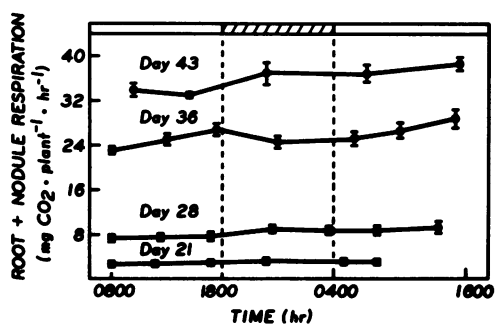

FIG. 1. Diurnal time course of root + nodule respiration at intervals up to 6 weeks after planting. Dark periods are indicated by shading on the upper axis. Each point represents the mean ± SE of four replicate plants. There was no significant diurnal variation of root + nodule respiration in 14-d-old plants.
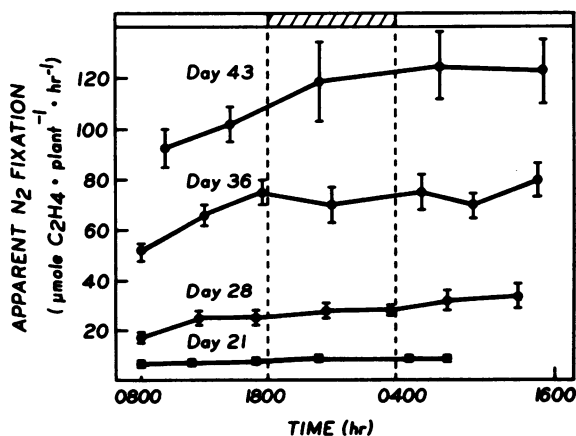

FIG. 2. Diurnal time course of apparent N_2 fixation at intervals up to 6 weeks after planting. Dark periods are indicated by shading on the upper axis. Each point represents the mean ± SE of five replicate plants. There was no detectable acetylene-reduction activity in 14-d-old plants.
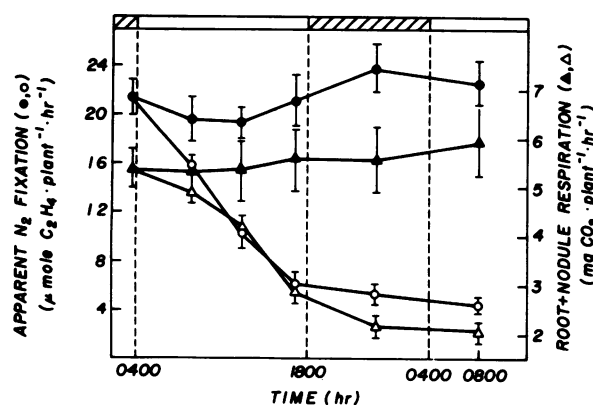

FIG. 3. Apparent N_2 fixation and root + nodule respiration of 21-d-old plants kept in continuous darkness following the normal dark cycle (O, △). Control plants (●, ▲) grown under the normal light/dark cycle (indicated by shading on the upper axis) are included for comparison. Each point represents the mean ± SE of four plants.

Table I. *Diurnal Changes in Root + Nodule Respiration and Apparent N_2 Fixation of Soybeans in a CO_2-Depleted Atmosphere*

Plants were grown at 320 ± 20 μl CO_2/l for 23 d and then maintained at 90 ± 10 μl CO_2/l for 5 d. A 10-h dark period was imposed between 1800 and 0400 h. The rates of root + nodule respiration and apparent N_2 fixation on day 23 for the plants were 8.6 ± 0.2 mg CO_2 plant^{-1} h^{-1} and 20.4 ± 1.3 μmol ethylene plant^{-1} h^{-1}. Shoot CER on day 28 for control plants and plants transferred to low CO_2 were 71.2 ± 2.5 and 18.2 ± 0.9 mg CO_2 plant^{-1} h^{-1}, respectively. Root + nodule respiration and apparent N_2 fixation in control plants on day 28 were 14.7 mg CO_2 plant^{-1} h^{-1} and 35.7 μmol ethylene plant^{-1} h^{-1}. All values are the mean ± SE of three replicate plants.

Time	Root + Nodule Respiration	Apparent N_2 Fixation
h	*mg CO_2 plant^{-1} h^{-1}*	*μmol ethylene plant^{-1} h^{-1}*
1730	8.0 ± 1.5	18.5 ± 2.0
2230	4.7 ± 0.5	13.7 ± 2.0
0330	4.4 ± 0.5	10.3 ± 2.1
0830	7.0 ± 0.8	17.6 ± 2.0

Table II. *Shoot CER, Root + Nodule Respiration, and Apparent N_2 Fixation of Soybean Plants before and after 10-Hour Exposure to 1,000 μl CO_2/l*

Plant Age	Shoot CER		Root + nodule Respiration		Apparent N_2 Fixation	
	Before	After	Before	After	Before	After
days	*mg CO_2 plant^{-1} h^{-1}*				*μmol ethylene· plant^{-1} h^{-1}*	
14	4.4	8.2[a]	0.8	0.9	—[b]	
21	13.7	25.4[c]	3.6	4.4	10.3	9.6
27	55.8	98.3[d]	8.6	9.0	26.1	28.0
36	—[e]		25.0	26.2	75.9	76.2
43			48.5	49.4	116	131

[a] CO_2 treatment effect significant at $p \leq 0.001$ (df = 6).
[b] There was no detectable acetylene-reduction activity in 14-d-old plants.
[c] CO_2 treatment effect significant at $p \leq 0.05$ (df = 6).
[d] CO_2 treatment effect significant at $p \leq 0.01$ (df = 6).
[e] CER was not measured after day 27, because plants were too large for the assay chamber.

Table III. *Root + Nodule Respiration and Apparent N_2 Fixation of Soybeans Exposed to a CO_2-Enriched Atmosphere*

Plants were grown at 320 µl CO_2/l continuously or transferred to 1,000 µl CO_2/l 23 d after planting. Values represent the mean of five replicate plants. Shoot CER of soybeans exposed to 1,000 µl CO_2/l was 73% greater on day 2 and 58% greater on day 5 than was that of plants grown at 320 µl CO_2/l. Specific activity of apparent N_2 fixation on day 6 was 186 ± 6 and 198 ± 18 µmol ethylene g^{-1} nodule dry weight h^{-1} at 320 and 1,000 µl CO_2/l, respectively.

Time after Transfer	Root + Nodule Respiration		Apparent N_2 Fixation	
	320	1,000	320	1,000
	µl CO_2/l			
days	*mg CO_2 plant^{-1} h^{-1}*		*µmol ethylene·plant^{-1} h^{-1}*	
0	9.7	9.6	21.9	19.4
2	11.2	11.5	26.6	28.3
3	11.4	13.4	28.4	33.8
5	14.7	18.1[a]	35.7	49.7[b]
6	18.3	23.1[b]	51.2	66.9[b]

[a] CO_2 treatment effect significant at $p \leq 0.01$.

[b] CO_2 treatment effect significant at $p \leq 0.05$.

Table IV. *Dry Weight, Soluble Sugar, and Starch Content of 29-Day-Old Soybeans*

Plants were grown continuously at 320 µl CO_2/l or transferred on day 23 to 1,000 µl CO_2/l. Values are the mean of five replicate plants on a dry-weight basis.

Plant Organ	Dry Weight		Soluble Sugar		Starch	
	320	1,000	320	1,000	320	1,000
			µl CO_2/l			
	g/plant		*mg/g*			
Leaves	1.76	2.92[a]	43.9	43.6	27.9	77.8[b]
Stem	0.80	0.95[b]	33.0	32.0	2.4	5.9
Root	1.01	1.16	12.1	15.4	0.9	1.4
Nodules	0.27	0.34[b]	13.6	10.7	7.4	7.7

[a] CO_2 treatment effect significant at $p \leq 0.01$.

[b] CO_2 treatment effect significant at $p \leq 0.05$.

Significant effects of CO_2 on dry matter accumulation of leaves, stems, and nodules were measured after 6 d (Table IV). However, carbohydrate analyses showed that only the leaves of CO_2-enriched plants had a significantly greater concentration of nonstructural carbohydrates and that the increase resulted from starch accumulation (Table IV).

Thirty-five-d-old soybean plants, pretreated for 2 d with 1,000 µl CO_2/l and then kept in darkness following the normal dark cycle, maintained acetylene reduction equal to or greater than initial rates for 19 h after the lights normally would have come on (Fig. 4). Control plants not exposed to CO_2 enrichment responded in a manner similar to that of younger plants, with apparent N_2 fixation declining soon after lights normally would have come on (Fig. 4 *versus* Fig. 3). In contrast, plants that were grown with 1,000 µl CO_2/l for 28 d maintained normal acetylene-reduction rates for only 5 h during an extension of the normal dark period (Fig. 5).

DISCUSSION

Results from this study clearly demonstrate that soybean root nodules on intact, growing plants can reduce acetylene at a stable rate without diurnal fluctuations. That conclusion is consistent with studies of soybeans examined at one stage of development
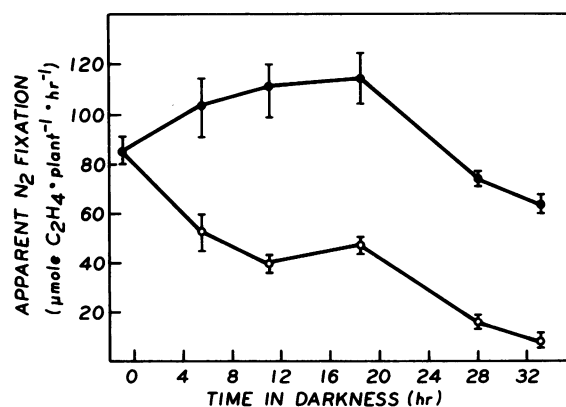

FIG. 4. Apparent N_2 fixation of 35-d-old soybean plants during an extended dark treatment following the normal night period. Plants were either grown at 1,000 µl CO_2/l for 2 d before the initiation of the experiment at time 0 (●) or kept at 320 µl CO_2/l throughout (○). The first value represents the mean ± SE of six plants (three from each treatment) taken 30 min before the growth chamber lights were to have come on. Subsequent values represent the mean ± SE of five replicate plants.
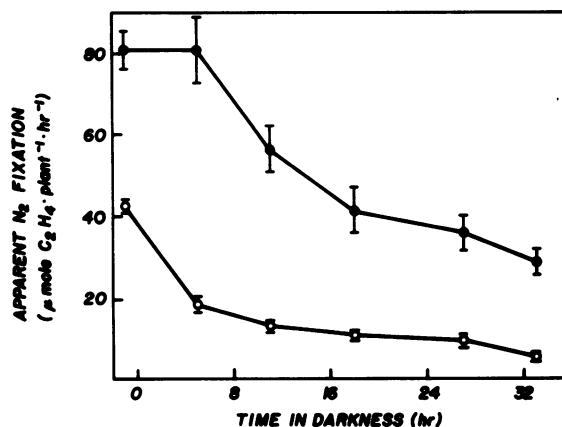

FIG. 5. Apparent N_2 fixation of 28-d-old soybean plants during an extended dark treatment following the normal night period. Plants were grown continuously in 1,000 µl CO_2/l (●) or in 320 µl CO_2/l (○) from time of planting. Time 0 represents the end of the normal night period. Each point represents the mean ± SE of five replicate plants. Specific activities of acetylene reduction for high and control CO_2 treatments at the start of the experiment were 223 ± 13 and 238 ± 27 µmol ethylene g^{-1} nodule dry weight h^{-1}, respectively.

under constant temperatures (2, 12) but contrasts with observations by other workers when diurnal temperatures varied. Neither root + nodule respiration nor acetylene reduction showed any significant decline during the night period in plants assayed weekly between 14 and 43 d after planting (Figs. 1 and 2). Such fluctuations would be expected if carbon substrates available to roots and nodules declined below a critical level at any time during each 24-h period. That assumption is supported by the observation that acetylene reduction and root + nodule respiration declined significantly when the normal dark period was extended (Fig. 3) and by the demonstration that those parameters declined significantly during a normal night period when plants were shifted from 320 to 90 µl CO_2/l for 5 d (Table I). Such data support the concept that the nodule carbohydrate pool is limited but do not indicate whether an increase in CER could enhance N_2 fixation.

Increasing CER by providing 1,000 rather than 320 µl CO_2/l had long-term, but not short-term, effects on root nodule activity. Significant amounts of new photosynthate move to soybean roots within 1 h after exposure to $^{14}CO_2$ (6, 11), and, even in carbohy-

drate-depleted soybeans, maximum rates of acetylene reduction were evident after fewer than 4 h in the light (13). Nevertheless, large increases in CER produced by CO_2 enrichment of air surrounding shoots of plants of five ages, including two measurements made after anthesis, had no significant promotive effect on root + nodule respiration or acetylene reduction within 10 h (Table II). When the CO_2-enrichment treatment was extended, however, significant increases in root + nodule respiration and acetylene reduction were measured after 5 d (Table III). Unlike previously reported studies (3–5), the increase in acetylene reduction after 6 d of CO_2 enrichment reflected primarily nodule growth (Tables III and IV). Although Sheehy et al. (14) found a significant positive correlation between whole plant CER and acetylene reduction, those alfalfa plants with greater CER values also had greater plant and root nodule dry weights. In the present experiments, no difference was measured in acetylene reduction values between control and experimental plants until plants with greater CER values had significantly greater nodule dry weight. The slight increase in acetylene-reduction rate/g nodule dry weight associated with CO_2 enrichment (Tables III and IV) might have developed into a significant difference later and, therefore, may be comparable to previous results (3–5). These data emphasize that root nodules of soybean plants do not always have excess acetylene-reduction capacity that can be activated by CER values greater than those typically experienced by the plants. Whether the situation reported after 7 d of CO_2 enrichment for field-grown soybeans (3–5) actually reflected an excess of acetylene-reduction capacity on day 0 can be determined only by the type of data in Table III.

Root nodule activities during an extended dark period also differed greatly between plants given short- or long-term CO_2 enrichment treatments. Plants exposed to 1,000 μl CO_2/l for 2 d showed no decline in acetylene-reduction activity of nodules during the first 19 h of an extended dark treatment following the normal night period (Fig. 4). When plants were grown for 28 d in 320 or 1,000 μl CO_2/l treatments, acetylene-reduction activity declined significantly during 11 h of a similar dark treatment (Fig. 5). The latter type of results have been used to indicate that plants grown under constant environmental conditions were in a steady state of substrate production and use on a 24-h basis (7). Although the total acetylene-reduction activity of plants exposed to the higher CO_2 level for 28 d was nearly twice that of plants grown with a normal CO_2 concentration, the specific activity of the root nodules did not differ significantly between the two treatments. That observation is consistent with the data in Table III and contrasts with the response reported for CO_2-enrichment treatments of older soybeans (3–5).

The present experiments indicate that, under constant environmental conditions, soybean root nodule activity was not limited by whole-plant photosynthate production. Instead, the soybean plants under the normal light/dark cycles and CO_2 concentrations maintained a daily balance between substrate production and utilization similar to that reported by McCree and Kresovich (7). The term, utilization, in this context refers to both growth and maintenance uses of photosynthate. Short-term changes from the normal conditions, such as changes in ambient CO_2 concentrations or extension of the dark period, altered that balance. Long-term increases in shoot CER by CO_2 enrichment stimulated the plants to readjust and to develop a new equilibrium between daily substrate production and utilization. Thus, under controlled conditions, increases in whole-plant CER did not directly affect root nodule activity but did alter the 24-h balance between substrate production and utilization, so that plant and nodule growth was stimulated and N_2 fixation on a whole plant basis increased. Data

from subterranean clover grown under constant conditions (15) indicate that a similar 24-h balance may occur in that species when it is dependent on symbiotic N_2 fixation. Nodule activity in peas, however, can be more directly dependent on current photosynthetic activity (10). The concept of a 24-h balance between substrate production and utilization in soybeans grown under controlled environmental conditions is consistent with diurnal patterns of leaf carbohydrate level (17) and evidence for programmable rates of starch accumulation reported by Chatterton and Silvius (1). Some of the differences between the short-term responses of nodule activity to changes in photosynthesis reported in the present research and those reported by others (3–5, 8, 16) may be a function of the relative capacity of the plant to arrive at a balance between photosynthate production and utilization under varying field conditions, compared to controlled conditions. Similarly, correlations between short-term measurements of CER and root nodule activity (14) probably reflect a longer-term equilibrium between photosynthate production in the leaves and utilization by the rest of the plant, including the nodules.

Results from this study clearly establish three points. First, soybean root nodules on intact, growing plants can function at a maximum rate that is not limited by shoot CER. Second, root nodule acetylene-reduction activity of growth-chamber-grown plants is in equilibrium with shoot CER and other plant processes and cannot be perturbed by short-term increases in shoot CER. Third, long-term enhancement of shoot CER produces a coordinate increase in plant growth, nodule growth, and symbiotic N_2 fixation after several days. These results do not argue against such applied objectives as increasing photosynthetic efficiency or decreasing photorespiration, but they indicate that increases in N_2 fixation resulting from a greater shoot CER will be balanced by shoot and nodule growth during vegetative and early reproductive stages of development. Whether translocation or partitioning of photosynthate to root nodules is a major factor limiting symbiotic N_2 fixation is not addressed by these results and deserves further study.

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