Effect of Irradiance on Development of Apparent Nitrogen Fixation and Photosynthesis in Soybean

ABSTRACT

Soybeans grown with 2 millimolar NO₃⁻, which optimized apparent N₂ fixation by Rhizobium symbionts, showed significantly different rates of apparent photosynthesis and C₂H₂ reduction during seedling development at two irradiances. Those physiological processes were lower for several weeks in plants grown at 1,500 microEinsteins per meter² per second than in those exposed to 700 microEinsteins per meter² per second. The irradiance-induced retardation was evident in short-term rates of apparent photosynthesis and N₂ fixation, as well as in measures of dry matter and total N accumulation. In spite of their previously inhibited development, plants grown at 1,500 microEinsteins per meter² per second were distinguishable by day 28 from those exposed to 700 microEinsteins per meter² per second in terms of whole-plant CO₂-exchange rate; by day 35 they were identical in terms of whole-plant C₂H₂-reduction rate. On day 38 there was no significant difference in dry weight or N content between treatments. Shifting plants between irradiance treatments on day 21 showed that the higher irradiance also had a short-term inhibitory effect on C₂H₂ reduction. The fact that 16 millimolar NO₃⁻ prevented the continuous exposure to 1,500 microEinsteins per meter² per second from inhibiting apparent photosynthesis suggested that seedlings grown on 2 millimolar NO₃⁻ with Rhizobium were N₂-limited. Although rates of apparent photosynthesis were similar by day 28, the additional week required to produce equal rates of apparent N₂ fixation between irradiance treatments showed that physiological adaptations of shoots, as well as photosynthesis per se, can affect root nodule activity.

Soybean seedlings (Glycine max [L.] Merr.) growing under conditions of low soil N with Rhizobium are forced to allocate limited seed resources to the construction of leaves and root nodules. Reduction of CO₂ and N₂, the primary physiological functions of these two organs, is obviously crucial for the integrated growth of the plant, but how the developing plant reacts to C and N limitations is poorly understood.

To determine whether a seedling is C- or N-limited, one should examine each factor separately, but reported interactions between CO₂ and N₂ reduction complicate the situation. Factors that altered apparent CO₂ reduction in mature plants caused parallel changes in C₂H₂ reduction (14), an indirect assay for N₂ fixation (12). The fact that Rhizobium strains with different capacities for N₂ fixation affected dry matter production in soybeans (15, 23) suggests that the interdependence of CO₂ and N₂ reduction shown in 26-day-old peas (5) also occurs in soybeans. Data are not available on dynamic interactions between CO₂ and N₂ reduction as N₂-fixing legume seedlings develop.

Although the question of whether a seedling is C- or N-limited is complicated by interactions between C and N metabolism, growth conditions can be manipulated to alter either one or both processes. N limitations can be assessed by comparing growth of plants largely dependent on symbiotic N₂ fixation with performance of plants supplied high levels of combined N. C limitations can be identified by varying net CO₂ exchange. Because of the known interdependence of apparent photosynthesis and N₂ fixation, such data must be collected under conditions of soil N availability that allow optimum development of rhizobial N₂ fixation.

Irradiance is one ecologically important factor known to alter apparent photosynthesis in N₂-fixing legumes (3). Classical studies found that high levels of irradiance retarded development of the N₂ fixation capability of soybean seedlings (9, 19). Lacking techniques to record short-term changes in CO₂ and N₂ reduction in 1935, those workers measured soluble carbohydrate and reduced N content. Short-term measures of apparent photosynthesis and N₂ fixation were used in the present study to examine the effects of a moderate (700 μE m⁻² s⁻¹) and a high, potentially saturating (1,500 μE m⁻² s⁻¹) level of irradiance on the initial growth of nodulated soybean seedlings. By comparing combined N conditions that optimized N₂ fixation with 16 mM NO₃⁻ treatments in the presence of two irradiances, we attempted to determine whether Rhizobium bacteria can provide enough N for optimum plant development when soil N is scarce.

MATERIALS AND METHODS

Growth Conditions. Soybean [G. max (L.) Merr. var. Clark] seeds were germinated in the dark at 28 C. Day 0 was determined as the day of incubation. On day 3, seedlings were transferred to 15-cm diameter plastic pots containing vermiculite covered with 1 cm white perlite. Pots were placed in growth chambers with a 16:8 h light to dark photoperiod at 28:22 C, 50:75% RH, and a CO₂ concentration controlled at 320 ± 20 μl/liter. The photosynthetic photon flux density (400-700 nm) was 700 or 1,500 μE m⁻² s⁻¹; light was provided by alternating mercury and metal-halide lamps. Root temperatures 5 cm below the surface during the light period were 25 ± 2 C under both irradiances. Plants were watered with a nutrient solution (4) modified to contain 0, 2, 4, 8, or 16 mM NO₃⁻ and, on alternate days, with distilled H₂O. Ontogenetic studies were done on plants that were inoculated with R. japonicum strain USDA 311b110 on day 3 and 10 and watered with 2 mM NO₃⁻ nutrient solution. Pots of plants used for measurements of apparent photosynthesis and N₂ fixation were covered with a Plexiglas lid to permit separate gas-exchange measurements of root and shoot systems.

Gas-exchange Measurements. Rates of apparent whole-plant photosynthesis were measured every 3 or 4 days in a Plexiglas chamber. Temperature, irradiance, and RH in the assay chamber were maintained at levels identical to those under which the plants were grown. CO₂ was monitored with a Beckman model 315A IR

1 This material is based on research supported by National Science Foundation Grant PFR 77-07301.
gas analyzer in an open system (1). CO₂ within the measurement chamber was maintained at 320 ± 15 µg/liter. Repeated measurements of apparent N₂ fixation by roots were made on intact plants using the C₂H₂ reduction assay (22). All other C₂H₂ reduction assays were determined on detached root systems. C₂H₂ was injected into a sealed pot to give a final concentration of 0.10 atm, and samples were taken 10 and 40 min later during the linear phase of C₂H₂ production. C₂H₂ and C₂H₄ were measured by GC (2).

**Compositional Analyses.** Plants were harvested weekly, dried at 70 C for 48 h, and weighed. Total N was determined by Kjeldahl analysis (8). Sucrose, fructose, glucose, and starch were measured by GC (24).

**RESULTS**

Low levels of combined N clearly enhanced apparent N₂ fixation by 35-day-old Clark soybeans grown under 700 or 1,500 µE m⁻² s⁻¹ (Table I). The maximum C₂H₂-reduction rates in plants provided 2 mM NO₃⁻ and the vigor of those plants compared with others grown without NO₃⁻ supported a decision to use 2 mM NO₃⁻ in all studies of seedling development. Higher levels of combined N decreased C₂H₂ reduction and thus were unsuitable for maximizing apparent N₂ fixation in 35-day-old soybeans (Table I). Comparable conclusions could be drawn when data were expressed as C₂H₂ reduction per gram of root nodule tissue.

Through day 14, there was no difference in rate of apparent whole-plant photosynthesis between seedlings growing at 700 and 1,500 µE m⁻² s⁻¹ (Fig. 1). During the next 2 weeks of growth, however, three consecutive measurements showed that plants grown at the lower irradiance had significantly (P ≤ 0.01) higher rates of apparent photosynthesis. On days 28 and 31, there were again no statistical differences in apparent photosynthesis between the two treatments. Apparent photosynthesis was not measured after day 31 because the plants were too large for the assay chamber.

When apparent photosynthesis was expressed on a leaf-area basis, the rates for plants grown at the higher irradiance decreased from day 14 to 20, to levels not statistically different from those of plants grown at the lower irradiance (Fig. 2). That decline in photosynthetic rate corresponded to the period when whole-plant photosynthesis showed no significant increase. Rates of whole-plant photosynthesis increased dramatically for both sets of plants during the subsequent 12 days. The decreases in apparent photosynthesis per unit leaf area from day 28 to 31 coincided with a doubling of the relative leaf growth rate of both experimental sets of plants.

From day 18 to 31, the rate of apparent N₂ fixation, measured as C₂H₂ reduction, in plants grown at 700 µE m⁻² s⁻¹ was significantly greater than the rate in plants grown at the higher irradiance (Fig. 3). Such rates, however, were not statistically different between the two treatments from day 35 to 39. The variation in apparent N₂ fixation rates between the two experimental sets for at least 13 days was not due primarily to differences in nodule development. Nodule mass and number per plant were remarkably uniform between irradiance treatments. With the exception of nodule mass on day 18 (low irradiance: 69 mg/plant; high irradiance: 41 mg/plant, P ≤ 0.05), those parameters did not vary significantly between irradiance treatments at any time during the study. Because of that fact, C₂H₂ reduction per gram nodule dry weight was significantly less between day 18 and 31 for plants grown at the higher irradiance (Fig. 4).

Long-term C and N assimilation (Table II) reflected short-term measurements of apparent photosynthesis and C₂H₂ reduction (Figs. 1, 3). From day 18 to 31, the dry weight and N content of plants grown at 700 µE m⁻² s⁻¹ were significantly greater (P ≤ 0.05) than those of plants grown at 1,500 µE m⁻² s⁻¹. Data from the harvest on day 38 indicate that during the previous 7 days, net dry matter production and N₂ fixation were greater for the plants grown at 1,500 µE m⁻² s⁻¹. Nonstructural carbohydrate contents of plants grown at 700 and 1,500 µE m⁻² s⁻¹ were 4.5 and 6.5%, respectively, on day 18. The harvest on day 18 was during the period of visible, and measured (Table II), N stress of plants grown at 1,500 µE m⁻² s⁻¹. Those same plants showed no significant increase in the rate of apparent photosynthesis between day 14 and 20 (Fig. 1).

On day 21 in other experiments, five plants in each irradiance treatment were exchanged with those grown under the other irradiance. Transferring plants from 700 to 1,500 µE m⁻² s⁻¹ caused a decrease in apparent N₂ fixation rate relative to those maintained at 700 µE m⁻² s⁻¹ (Fig. 5a). Plants moved from high to low irradiance became greener within 4 days and soon showed higher rates of C₂H₂ reduction than plants kept continuously at

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**Table I. The Effect of Nitrate on C₂H₂ Reduction by Excised Root Systems of 35-day-old Soybean Plants Grown at Two Irradiances**

<table>
<thead>
<tr>
<th>Irradiance</th>
<th>Nitrate Concentration (mm)</th>
<th>µmol C₂H₄ plant⁻¹ h⁻¹</th>
<th>LSD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>700</td>
<td>43.1</td>
<td>73.6</td>
<td>50.2</td>
</tr>
<tr>
<td>1,500</td>
<td>12.7</td>
<td>76.9</td>
<td>49.4</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Apparent photosynthetic rates for plants grown at 700 (●) or 1,500 (○) µE m⁻² s⁻¹ with 2 mM NO₃⁻ and *R. japonicum* 311b110. Each point represents the mean ± se of five plants. The same plants were measured throughout the experimental period. Photosynthetic rates for plants grown 16 mM NO₃⁻ and grown at 700 (■) or 1,500 (□) µE m⁻² s⁻¹ represent the mean of at least three measurements.
DISCUSSION

The results obtained in the present growth chamber studies support and extend earlier reports on soybean plants grown in containers outside (9, 19). In both types of studies, plants grown under high irradiance showed a period of N stress characterized by chlorosis and stunted growth. In both cases, lower irradiance or adequate amounts of combined N corrected the problem. Results from the present study, which include short-term rates of apparent CO₂ and N₂ reduction, alter the classical interpretation of this phenomenon.

Fred et al. (9) contended that photosynthesis of plants grown in full sunlight was greater than that of plants grown in the shade and thus would accumulate an excess of carbohydrate. That interpretation was supported by data showing that for the one date analyzed, plants grown in full sunlight had a higher percentage of soluble carbohydrate content than did the shaded plants (19). A similar relationship was observed on day 18 in the present experiments, but data in Figure 1 show clearly that excess soluble carbohydrate was not caused by a greater rate of net CO₂ exchange in plants grown at the higher irradiance. The accumulation of soluble carbohydrates in such plants may be due to the transient N deficiency, in as much as others have shown that plants under N stress accumulated soluble carbohydrates in both leaves and stems (10, 21).

The lower rate of apparent photosynthesis by plants grown at 1,500 µE m⁻² s⁻¹ (Fig. 1) probably was due to a greater N deficiency (Table II). Percentage N content, which can be calcu-

Fig. 2. Apparent photosynthesis of plants grown at 700 (○) or 1,500 (□) µE m⁻² s⁻¹. Rates were determined by dividing whole-plant photosynthetic rates reported in Figure 1 by total leaf area of the individual plant.

Fig. 3. Apparent nitrogen fixation, assayed by C₂H₂ reduction on intact root systems, of plants grown with 2 mM NO₃⁻ and R. japonicum 311010 at 700 (○) or 1,500 (□) µE m⁻² s⁻¹. Each point represents the mean ± se of five plants. Data were collected from the same plants assayed for apparent photosynthesis (Fig. 1).

Fig. 4. Apparent nitrogen fixation per g nodule dry wt of soybeans grown at 700 (○) or 1,500 (□) µE m⁻² s⁻¹. Each point represents the mean ± se of five plants. Assays were conducted on detached roots.
photosynthesis and seedling development were N-limited.

The detrimental effects of high irradiance on the photosynthetic capacity of N-deficient plants also must be considered. Photodestruction of light-harvesting pigments occurs at high irradiance (6, 11). Medina (17) demonstrated that N stress at high light levels was more deleterious than the same stress imposed at a lower irradiance. Thus, the obvious foliar chlorosis and lower rates of photosynthesis by N-stressed plants in the present study could have resulted in part from high irradiance. Two points support this suggestion: (a) plants grown with 2 mM NO₃⁻ became visibly greener within 4 days when transferred from high to low irradiance on day 21; and (b) plants given 16 mM NO₃⁻ did not show any detectable adverse effects at 1,500 μE m⁻² s⁻¹ (Fig. 1, Table II). Additional insight is provided by comparing apparent photosynthesis data expressed on a whole-plant and a leaf-area basis for nodulated plants grown at the higher irradiance (Figs. 1, 2). From day 14 to 20, there was no significant increase in whole-plant photosynthesis (Fig. 1), but a 130% increase in leaf area during that period (39-90 cm²) decreased the net CO₂ exchange per dm² leaf area (Fig. 2). That change in leaf area, as well as further increments, appeared to increase auto-shading and may have decreased the stress caused by high irradiance.

Partitioning of recent photosynthesis also may have affected root nodule activity. On day 28, there was no significant difference between the photosynthetic rates of the two light treatments (Fig. 1), but apparent N₂ fixation of plants grown at the higher irradiance was only half that of plants grown at 700 μE m⁻² s⁻¹, and it remained statistically lower until day 35 (Fig. 3). Those results, as well as differences in specific activity (Fig. 4), suggest that partitioning of recent photosynthesize, and not merely its production, may have limited N₂ fixation. Irradiance has been shown to affect partitioning of ¹⁴C-photoassimilate to pea root nodules (25). Powell and Ryle (21) showed that N deficiency changed partitioning of assimilates in *Lolium temulentum* and that shaded plants responded differently from those exposed to full light. Thus, a difference in partitioning of recent photosynthesis by the plants grown at 1,500 μE m⁻² s⁻¹, relative to those at 700 μE m⁻² s⁻¹, may have been caused by a more severe N stress and the higher irradiance level.

The adaptation of leaves to different irradiance is a complex phenomenon involving anatomical, physiological, and biochemical changes (7, 13, 20). The present study demonstrates that irradiance-induced changes in soybean leaves also affected symbiotic N₂ fixation. Plants transferred from low to high irradiance on day 21 went through a prolonged period when C₂H₄ reduction rates were significantly below those measured in plants maintained at 700 μE m⁻² s⁻¹ (Fig. 5a). Foliar adaptation to high light presumably requires extra energy for changes in cell structure and enzyme content. Because such adaptations occurred in the plants transferred to the higher irradiance and the plants showed an inhibition of C₂H₄ reduction activity relative to the opposite transfer (Fig. 5b), it is tempting to relate these observations in a causal manner. Plants transferred from low to high irradiance may not have had the energy reserves to produce high light leaves and still maintain high rates of N₂ fixation.

The concept that development of nodulated soybean seedlings is limited by available N requires clarification. One might argue that the N limitation of *Rhizobium*-dependent seedlings reflects an inadequate supply of reduced carbon compounds. The circularity of such reasoning is shown by the promotive effect of 16 mM NO₃⁻ on apparent photosynthesis (Fig. 1). One way to avoid confusion is to recognize that, although the total plant is N-limited because it fails to utilize available N₂, root nodules may be C-limited. A more appropriate question is not whether a seedling is C- or N-limited, but rather how seedlings maintain C and N sufficiency in all plant parts. Diurnal fluctuations of foliar starch (26) and amino acid output by roots (18) have been reported.

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### Table II. Plant Dry Weight and N Content of Soybeans Grown at Two Levels of Irradiance with *R. japonicum* 311b110 and 2 mm NO₃⁻

<table>
<thead>
<tr>
<th>Age (days)</th>
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<th>700</th>
<th>1,500</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>700</td>
<td>1,500</td>
<td>700</td>
<td>1,500</td>
</tr>
<tr>
<td>Age</td>
<td>Dry wt</td>
<td>N Content</td>
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<td>N Content</td>
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<tr>
<td></td>
<td>g plant⁻¹</td>
<td>mg plant⁻¹</td>
<td>g plant⁻¹</td>
<td>mg plant⁻¹</td>
</tr>
<tr>
<td>10</td>
<td>0.27</td>
<td>12.0</td>
<td>0.26</td>
<td>12.3</td>
</tr>
<tr>
<td>18</td>
<td>1.08</td>
<td>28.7</td>
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</tr>
<tr>
<td>24</td>
<td>3.11</td>
<td>81.9</td>
<td>2.43</td>
<td>64.0</td>
</tr>
<tr>
<td>31</td>
<td>8.39</td>
<td>233</td>
<td>6.69</td>
<td>181</td>
</tr>
<tr>
<td>38</td>
<td>17.60</td>
<td>519</td>
<td>18.40</td>
<td>517</td>
</tr>
</tbody>
</table>

* Values differed significantly between irradiance treatments at P ≤ 0.05 or 0.01, respectively, in a two-tailed t test.

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**Fig. 5.** Apparent nitrogen fixation of intact plants grown at (a) 700 (●) or (b) 1,500 (O) μE m⁻² s⁻¹ and transferred (---) to the alternate irradiance on day 21. Each point represents the mean ± se of at least four plants.

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Plants (16). The previously reported dependence of apparent photosynthesis on N supplied from either N₂ or NH₄⁺ in peas (4, 5) is supported in the present study by data from plants given 16 mM NO₃⁻ (Fig. 1). The dramatically higher apparent photosynthesis in plants given 16 mM NO₃⁻ and the reversal in irradiance effect between N treatments (Fig. 1) indicate that...
Controlling the partitioning of those compounds will determine whether a seedling increases its photosynthetic capacity or N2 fixation capability by constructing new leaves or root nodules, respectively. The adaptability of soybeans in dealing with those problems over a period of days was demonstrated by the present study. Relating such results to short-term changes (18, 26) and to long-term responses (9, 19) will clarify mechanisms determining plant development.

LITERATURE CITED
15. Mayer RJ, WH Heil 1978 Mutant strains of Rhizobium japonicum with increased ability to fix nitrogen for soybean. Science 201: 448-450