Effects of Elevated Carbon Dioxide Concentration in the Dark on the Growth of Soybean Seedlings

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Previous work has shown that elevated carbon dioxide (CO₂) concentrations in the dark reversibly reduce the rate of CO₂ efflux from soybeans. Experiments were performed exposing soybean plants continually to concentrations of 350 or 700 cm³ m⁻² for 24 h d⁻¹, or to 350 during the day and 700 cm³ m⁻² at night, in order to determine the importance of the reduced rate of dark CO₂ efflux for plant growth. High CO₂ applied only at night conserved carbon and increased dry mass during initial growth compared with the constant 350 cm³ m⁻² treatment. Long-term net assimilation rate was increased by high CO₂ in the dark, without any increase in daytime leaf photosynthesis. However, leaf area ratio was reduced by the dark CO₂ treatment to values equal to those of plants continually exposed to the higher concentration. From days 14-21, leaf area was less for the elevated night-time CO₂ treatment than for the constant 350 or 700 cm³ m⁻² treatments. For the day 7-21-period, relative growth rate was significantly reduced by the high night CO₂ treatment compared with the 350 cm³ m⁻² continuous treatment. The results indicate that some functionally significant component of respiration was reduced by the elevated CO₂ concentration in the dark.

Key words: Glycine max L. (Merr.), carbon dioxide, plant growth, respiration.

INTRODUCTION

Elevated concentrations of CO₂ in the dark have been shown to decrease CO₂ efflux in several species (e.g. Gale, 1982; Gifford, Lambers and Morrison, 1985; Bunce, 1990; El-Kohen, Pontailler and Mousseau, 1991). The resulting conservation of carbon has been shown to increase the dry mass of alfalfa exposed to elevated CO₂ only during the dark period (Reuveni and Gale, 1985), and a low CO₂ concentration at night decreased the growth of duck weed (Reuveni, Gale and Meyer, 1993). Mechanisms by which elevated CO₂ concentrations might reduce CO₂ efflux in the dark include a stimulation of dark CO₂ fixation and a reduction in flow through the alternative respiratory pathway, in addition to a reduction in coupled respiration. If normal, coupled respiration is actually reduced, this could also eventually have a negative impact on plants. A deleterious effect of elevated CO₂ applied only during the dark would suggest that a functionally significant component of respiration was reduced by the treatment. Previous work with leaves of this species indicated that elevated CO₂ caused a rapid, reversible decrease in respiration (Bunce, 1995). This study therefore examined the growth response of soybean seedlings exposed to elevated CO₂ during the dark.

The effects of high night-time CO₂ concentrations on plants are relevant not only to future atmospheric CO₂ concentrations, but also to current conditions. High night-time CO₂ concentrations routinely occur in agricultural fields on nights with low wind or stable atmospheric conditions (e.g. Allen, 1971; Verma and Rosenberg, 1976).

MATERIALS AND METHODS

Soybean, Glycine max L. (Merr.) cv. Clark, was grown in one controlled environment chamber, with successive experiments at different CO₂ concentration conditions. In all experiments the air temperature was 25 °C day and night, the dew point temperature was 18 °C, and there were 14 h d⁻¹ of light at 1.0 mmol m⁻² s⁻¹ photosynthetic photon flux density at the top of the crop plants from a mixture of high pressure sodium and metal halide lamps. Plants were grown one per pot in 15-cm diameter plastic pots filled with 1.8 dm³ of vermiculite and flushed daily with a complete nutrient solution. Pots were spaced so that plants did not shade each other. The CO₂ concentration of the chamber air was monitored continuously with an absolute infrared analyser (LIRA, MSA Instruments, Pittsburgh PA, USA), which activated solenoid valves to control the injection of pure CO₂ or CO₂-free air. Experiments were performed with the following CO₂ treatments: (a) 350 ± 30 cm³ m⁻² day and night, (b) 700 ± 50 cm³ m⁻² day and night, and (c) 350 during the day and 700 cm³ m⁻² at night. When elevated CO₂ was applied only at night, the injection of CO₂ was terminated about 45 min before the end of the dark period to ensure that the CO₂ concentration would be within the daytime control range when the lights came on. Plants were grown for 21 d from planting. Harvests of five plants were made every 2 or 3 d until 21 d from emergence. At the harvests, leaf area, and leaf, stem and root dry mass of each plant was determined and total dry mass per plant was calculated. Each CO₂ treatment was run twice, and pooled data from the two runs are presented. Relative growth rates

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(RGR), leaf area ratios (LAR) and net assimilation rates (NAR) were calculated from the harvest data (Jones, 1983).

On days 14, 18 and 21 from planting, net CO₂ exchange rates at 1200 h were determined for all mainstem leaves larger than 20 cm² area. Exchange rates were measured using a Li-Cor 6200 portable photosynthesis system (LiCor, Inc., Lincoln, USA) under the growth conditions of light, CO₂, temperature, and water vapour pressure. These measurements were made on leaves from five plants per treatment.

In order to document further the existence of high nighttime CO₂ concentrations, CO₂ concentration was monitored in 1993 at the USDA South Farm field site in Beltsville, Maryland, USA. The surrounding vegetation was a mixture of various annual crops, mown lawns or fallow fields for a minimum of 0.5 km in all directions. Air taken from a height of 1.5 m above the soil was pumped continually through an absolute infrared CO₂ analyser (Li-6262, LiCor, Inc., Lincoln NB, USA) which was inside a temperature-controlled shelter, and hourly averages were stored on a datalogger. Photosynthetic photon flux density and wind speed were also logged. The anemometer used had a stalling speed of 0.45 m s⁻¹, and wind speeds from 0 to 0.45 m s⁻¹ were recorded as 0.45 m s⁻¹. Measurements were made continually during Mar. and Aug. The calibration of the CO₂ analyser was adjusted weekly, but 1200 h CO₂ concentrations were quite uniform and confirmed that there was no significant drift of the analyser calibration.

**RESULTS**

The CO₂ concentration at the farm increased at wind speeds below 1 m s⁻¹ both in spring and summer. Wind speeds below 1 m s⁻¹ occurred almost exclusively at night. The mean night-time CO₂ concentration was 420 cm³ m⁻³ in Mar. and 540 cm³ m⁻³ in Aug., with maximum values of about 600 and 800 cm³ m⁻³, respectively. The mean daytime CO₂ concentrations were 355 cm³ m⁻³ in Mar. and 350 cm³ m⁻³ in Aug. The mean wind speed was 1.51 m s⁻¹ in spring and 1.11 m s⁻¹ in summer, although these values are

**Table 1.** Total plant dry mass and leaf area per plant for soybean plants exposed to 350/350, 350/700, and 700/700 cm³ m⁻³ day/night CO₂ concentrations at various days from planting. The number of plants was 5–10 per treatment each day, depending on the day.

<table>
<thead>
<tr>
<th>Day</th>
<th>Total dry mass (g)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>350/350</td>
<td>350/700</td>
</tr>
<tr>
<td>7</td>
<td>0.157a</td>
<td>0.203b</td>
</tr>
<tr>
<td>10</td>
<td>0.309b</td>
<td>0.352b</td>
</tr>
<tr>
<td>12</td>
<td>0.469b</td>
<td>0.605b</td>
</tr>
<tr>
<td>14</td>
<td>0.981b</td>
<td>1.031b</td>
</tr>
<tr>
<td>17</td>
<td>1.60b</td>
<td>1.69b</td>
</tr>
<tr>
<td>19</td>
<td>2.58b</td>
<td>2.10b</td>
</tr>
<tr>
<td>21</td>
<td>4.89b</td>
<td>4.06b</td>
</tr>
</tbody>
</table>

Note: Within each variable and row, numbers followed by different letters are significantly different at P = 0.05, using analysis of variance and l.s.d. tests.

**Table 2.** Analysis of covariance (ANCOVA) for regressions of ln (total plant dry mass) vs. age of soybean plants grown at 350/350, 350/700, and 700/700 cm³ m⁻³ day/night CO₂ concentrations for days 7–21 from planting. Relative growth rate is the slope of the regression.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d.f.</th>
<th>Intercept (± s.e.)</th>
<th>Slope (± s.e.)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regressions</td>
<td>44</td>
<td>-3.554 ± 0.052</td>
<td>0.238 ± 0.004</td>
<td>0.989</td>
</tr>
<tr>
<td>350/350</td>
<td>56</td>
<td>-3.052 ± 0.058</td>
<td>0.210 ± 0.004</td>
<td>0.981</td>
</tr>
<tr>
<td>700/700</td>
<td>44</td>
<td>-3.439 ± 0.042</td>
<td>0.253 ± 0.003</td>
<td>0.994</td>
</tr>
<tr>
<td>ANCOVA</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Slope of 350/350 vs. 350/700; P = 0.0041</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope of 350/350 vs. 700/700; P = 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Leaf area ratio as a function of days since planting in soybeans exposed to day/night CO₂ concentrations of 350/350 (□), 350/700 (△), and 700/700 cm³ m⁻³ (■). Curves are third order polynomials, for illustration only.

**Fig. 2.** Net assimilation rate as a function of days since planting in soybeans exposed to day/night CO₂ concentrations of 350/350 (□), 350/700 (△), and 700/700 cm³ m⁻³ (■). Curves are third order polynomials, for illustration only.
inflated because of the stalling of the anemometer at low wind.

There was no difference among CO₂ treatments in the controlled environment chamber in the timing of seedling emergence (day 4) nor in the dry mass at emergence (not shown). On days 7, 10 and 12, elevated CO₂ at night resulted in greater total plant dry mass but not leaf area (Table 1). The total plant dry mass of the 350/700 cm³ m⁻² treatment was intermediate between the 350 and the 700 cm³ m⁻² treatments on days 10 and 12 (Table 1). No differences occurred between CO₂ treatments on day 14 for total mass or leaf area. The total dry mass of plants given elevated CO₂ only at night was less than the low CO₂ plants after day 14 (Table 1). Leaf area was relatively more decreased by the high night-time CO₂ treatment than was dry mass. Leaf area ratio was lower throughout the experiment in the 350/700 and 700 cm³ m⁻² treatments than in the 350 cm³ m⁻² treatment (Fig. 1). Leaf mass to total mass and root mass to total mass were unaffected by CO₂ treatment (not shown). Relative growth rate was significantly less in the 350/700 cm³ m⁻² treatment than the 350 or 700 cm³ m⁻² treatments (Table 2). Net assimilation rate was increased by high night-time CO₂ over most of the growth period and was increased further by the continuous high CO₂ treatment (Fig. 2).

Leaf photosynthetic rates were slightly less in plants exposed to elevated CO₂ only at night compared with those kept at 350 cm³ m⁻² on the majority of measurement times.
(Fig. 3). Higher rates occurred in the constant 700 cm$^3$ m$^{-3}$ treatment, because those rates were measured at the elevated daytime CO$_2$ concentration. The mean rates averaged over all leaves and measurement times were 16.3 μmol CO$_2$ m$^{-2}$ s$^{-1}$ for the 350/700 cm$^3$ m$^{-3}$ treatment, 17.2 for the 350 treatment, and 23.2 for the 700 cm$^3$ m$^{-3}$ treatment.

DISCUSSION

The stimulation in biomass caused by elevated CO$_2$ during the dark period which we found in the early phase of growth is similar to the results Reuvendi and Gale (1985) obtained with alfalfa. Because total leaf area and leaf photosynthetic rates were not increased by elevated night-time CO$_2$ in our study, reduced night-time CO$_2$ efflux (Bunce, 1990) is the probable cause of the increased NAR and increased biomass.

After 14 d, elevated CO$_2$ at night reduced leaf area growth, and resulted in less rapid biomass accumulation. The lower LAR of plants at high night-time CO$_2$ was evident even before 14 d, suggesting that the detrimental effect of high night-time CO$_2$ on leaf area partitioning occurred throughout the experiment. The generally slightly lower leaf photosynthesis rates in the high night-time CO$_2$ treatment may also have contributed to slower growth. Reduced photosynthesis following high night-time CO$_2$ treatment has been previously reported for soybean (Bunce, 1992). It is interesting that there was no difference in LAR between plants given continuously elevated CO$_2$ and those given high CO$_2$ only at night. The reduction in LAR and specific leaf area commonly observed at elevated CO$_2$ is therefore not simply a function of increased photosynthesis.

Because elevated CO$_2$ during the dark period can reduce plant growth, the reduction in dark CO$_2$ efflux reflects a decrease in some functionally important component of respiration. Night-time CO$_2$ concentrations under field conditions are extremely variable, but can reach 800 cm$^3$ m$^{-3}$, Such high concentrations may have significant effects on plant growth even under current atmospheric conditions.

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LITERATURE CITED


