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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 (CO_2) concentrations in the dark reversibly reduce the rate of CO_2 efflux from soybeans. Experiments were performed exposing soybean plants continually to concentrations of 350 or 700 cm^3 m^{-3} for 24 h d^{-1}, or to 350 during the day and 700 cm^3 m^{-3} at night, in order to determine the importance of the reduced rate of dark CO_2 efflux for plant growth. High CO_2 applied only at night conserved carbon and increased dry mass during initial growth compared with the constant 350 cm^3 m^{-3} treatment. Long-term net assimilation rate was increased by high CO_2 in the dark, without any increase in daytime leaf photosynthesis. However, leaf area ratio was reduced by the dark CO_2 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 CO_2 treatment than for either the constant 350 or 700 cm^3 m^{-3} treatments. For the day 7–21-period, relative growth rate was significantly reduced by the high night CO_2 treatment compared with the 350 cm^3 m^{-3} continuous treatment. The results indicate that some functionally significant component of respiration was reduced by the elevated CO_2 concentration in the dark.

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

INTRODUCTION

Elevated concentrations of CO_2 in the dark have been shown to decrease CO_2 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 CO_2 only during the dark period (Reuveni and Gale, 1985), and a low CO_2 concentration at night decreased the growth of duck weed (Reuveni, Gale and Meyer, 1993). Mechanisms by which elevated CO_2 concentrations might reduce CO_2 efflux in the dark include a stimulation of dark CO_2 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 CO_2 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 CO_2 caused a rapid, reversible decrease in respiration (Bunce, 1995). This study therefore examined the growth response of soybean seedlings exposed to elevated CO_2 during the dark.

The effects of high night-time CO_2 concentrations on plants are relevant not only to future atmospheric CO_2 concentrations, but also to current conditions. High night-time CO_2 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 CO_2 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 tops of the 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 dm^3 of vermiculite and flushed daily with a complete nutrient solution. Pots were spaced so that plants did not shade each other. The CO_2 concentration of the chamber air was monitored continually with an absolute infrared analyser (LIRA, MSA Instruments, Pittsburgh PA, USA), which activated solenoid valves to control the injection of pure CO_2 or CO_2-free air. Experiments were performed with the following CO_2 treatments: (*a*) 350 ± 30 cm^3 m^{-3} day and night, (*b*) 700 ± 50 cm^3 m^{-3} day and night, and (*c*) 350 during the day and 700 cm^3 m^{-3} at night. When elevated CO_2 was applied only at night, the injection of CO_2 was terminated about 45 min before the end of the dark period to ensure that the CO_2 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 CO_2 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 CO_2 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, CO_2, 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 night-time CO_2 concentrations, CO_2 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 CO_2 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 CO_2 analyser was adjusted weekly, but 1200 h CO_2 concentrations were quite uniform and confirmed that there was no significant drift of the analyser calibration.

RESULTS

The CO_2 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 CO_2 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 CO_2 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 CO_2 concentrations at various days from planting. The number of plants was 5–10 per treatment each day, depending on the day*

	Total dry mass (g)			Leaf area (cm²)		
Day	350/350	350/700	700/700	350/350	350/700	700/700
7	0·157[c]	0·203[a]	0·182[b]	11·1[a]	9·1[a]	10·1[a]
10	0·306[c]	0·352[b]	0·400[a]	42·7[a]	41·1[a]	45·6[a]
12	0·469[c]	0·605[b]	0·682[a]	76·9[ab]	71·0[b]	82·3[a]
14	0·981[a]	1·031[a]	1·059[a]	159[a]	129[a]	143[a]
17	1·60[b]	1·69[b]	2·49[a]	257[a]	212[b]	292[a]
19	2·58[b]	2·10[c]	4·20[a]	374[a]	249[b]	463[a]
21	4·89[b]	4·00[c]	5·80[a]	664[a]	503[b]	653[a]

Note: Within each variable and row, numbers followed by different letters are significantly different at $P = 0{\cdot}05$, using analysis of variance and l.s.d. tests.

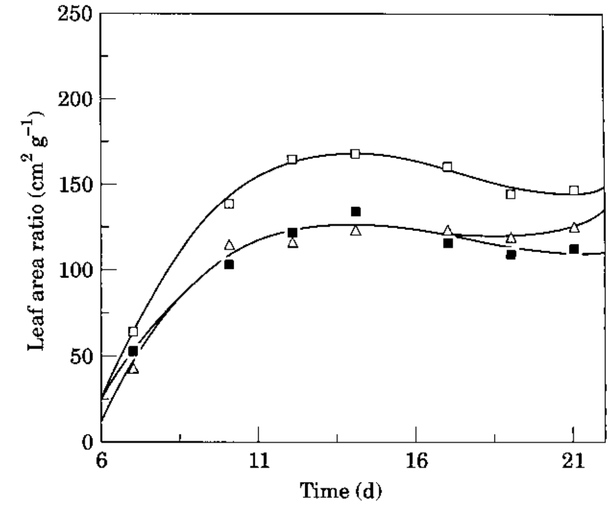

FIG. 1. Leaf area ratio as a function of days since planting in soybeans exposed to day/night CO_2 concentrations of 350/350 (□), 350/700 (△), and 700/700 cm³ m⁻³ (■). Curves are third order polynomials, for illustration only.

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 CO_2 concentrations for days 7–21 from planting. Relative growth rate is the slope of the regression*

Treatment (cm³ m⁻³)	d.f.	Intercept (±s.e.)	Slope (±s.e.)	r^2
Regressions				
350/350	44	−3·554±0·052	0·238±0·004	0·989
350/700	56	−3·052±0·058	0·210±0·004	0·981
700/700	44	−3·439±0·042	0·253±0·003	0·994
ANCOVA				
Slope of 350/350 *vs.* 350/700: $P = 0{\cdot}0041$				
Slope of 350/350 *vs.* 700/700: $P = 0{\cdot}0001$				

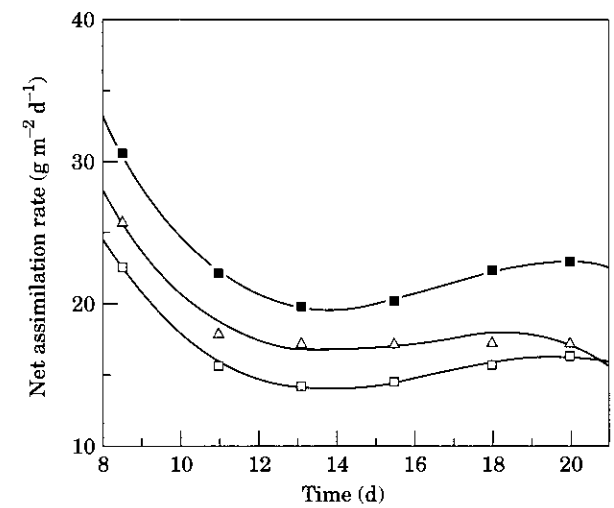

FIG. 2. Net assimilation rate as a function of days since planting in soybeans exposed to day/night CO_2 concentrations of 350/350 (□), 350/700 (△), and 700/700 cm³ m⁻³ (■). Curves are third order polynomials, for illustration only.

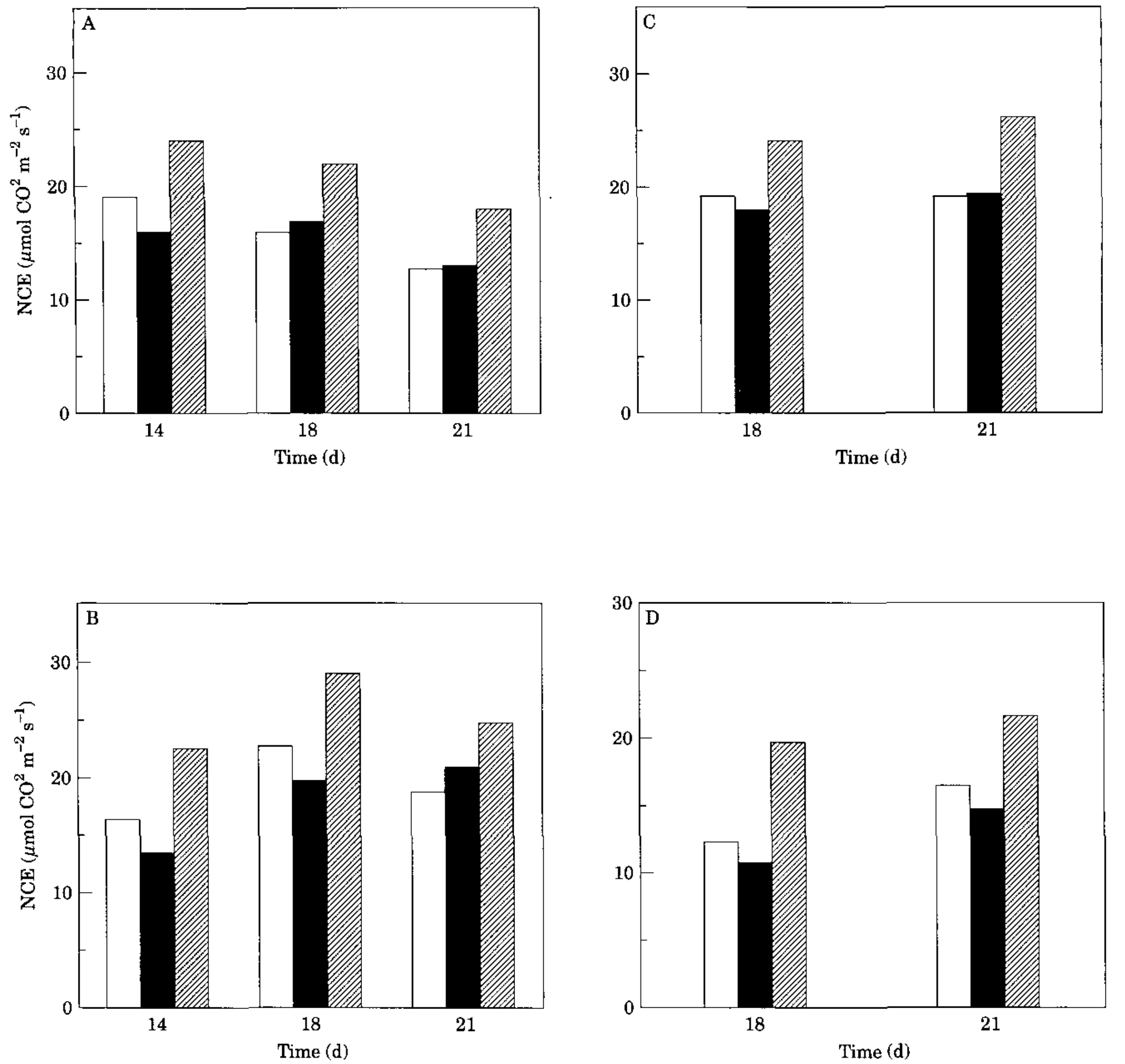

FIG. 3. Single leaf rates of net CO_2 exchange (NCE) under the growth conditions for soybeans exposed to day/night CO_2 concentrations of 350/350, 350/700, and 700/700 $cm^3 m^{-3}$. A, Primary leaves; B, first trifoliate leaves; C, second trifoliate leaves; D, third trifoliate leaves. □, 350/350; ■, 350/700; ▨, 700/700 $cm^3 m^{-3}$.

inflated because of the stalling of the anemometer at low wind.

There was no difference among CO_2 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 CO_2 at night resulted in greater total plant dry mass but not leaf area (Table 1). The total plant dry mass of the 350/700 $cm^3 m^{-3}$ treatment was intermediate between the 350 and the 700 $cm^3 m^{-3}$ treatments on days 10 and 12 (Table 1). No differences occurred between CO_2 treatments on day 14 for total mass or leaf area. The total dry mass of plants given elevated CO_2 only at night was less than the low CO_2 plants after day 14 (Table 1). Leaf area was relatively more decreased by the high night-time CO_2 treatment than was dry mass. Leaf area ratio was lower throughout the experiment in the 350/700 and 700 $cm^3 m^{-3}$ treatments than in the 350 $cm^3 m^{-3}$ treatment (Fig. 1). Leaf mass to total mass and root mass to total mass were unaffected by CO_2 treatment (not shown). Relative growth rate was significantly less in the 350/700 $cm^3 m^{-3}$ treatment than the 350 or 700 $cm^3 m^{-3}$ treatments (Table 2). Net assimilation rate was increased by high night-time CO_2 over most of the growth period and was increased further by the continuous high CO_2 treatment (Fig. 2).

Leaf photosynthetic rates were slightly less in plants exposed to elevated CO_2 only at night compared with those kept at 350 $cm^3 m^{-3}$ 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 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ 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 Reuveni 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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