THE RESPONSE OF SOYBEAN SEEDLING GROWTH TO CARBON DIOXIDE CONCENTRATION AT NIGHT IN DIFFERENT THERMAL REGIMES

Bunce, J. A.
Climate Stress Laboratory USDA-ARS Beltsville Agricultural Research Center

https://hdl.handle.net/2324/8270

KYUSHU UNIVERSITY

THE RESPONSE OF SOYBEAN SEEDLING GROWTH TO CARBON DIOXIDE CONCENTRATION AT NIGHT IN DIFFERENT THERMAL REGIMES

J. A. BUNCE*

Climate Stress Laboratory, USDA–ARS, Beltsville Agricultural Research Center, 10300 Baltimore Ave., Beltsville MD 20705–2350, USA

BUNCE J. A. *The response of soybean seedling growth to carbon dioxide concentration at night in different thermal regimes.* BIOTRONICS **30**, 15–26. 2001. Carbon dioxide concentration during the dark is often not controlled in investigations of plant growth or in studies of plant responses to elevated $[CO_2]$ in the field. In this work we have investigated whether $[CO_2]$ at night significantly affected the growth of soybean seedlings, and whether this varied with the magnitude of the day/night temperature variation. Plants were grown under day/night $[CO_2]$s of 350/350, 350/700, 700/700 and 700/350 μmol mol^{-1} $[CO_2]$ in controlled–environment chambers under four temperature regimes. Field studies were also conducted using semi–open chambers in which plants were fumigated with air at the ambient $[CO_2]$ or with air at ambient $+350\,\mu$mol mol^{-1} $[CO_2]$ applied only during the day, only at night, or for 24 h per day. When the day $[CO_2]$ was low, elevated $[CO_2]$ at night decreased plant leaf area and dry mass with constant day/night temperatures, but had no effect when there was a diurnal change in temperature. With high $[CO_2]$ in the day, elevated $[CO_2]$ at night decreased plant leaf area and dry mass when the diurnal change in temperature was small, but increased these parameters when the diurnal change in temperature was larger. The data for the field was similar to that obtained in the controlled–environment chambers in the regimes with the larger diurnal temperature changes. No effect of elevated $[CO_2]$ on leaf area or biomass was observed in the field when the elevated $[CO_2]$ treatment was applied only in the daytime. Additionally, the results indicate that a build up of $[CO_2]$ at night in controlled–environment chambers or in the field can significantly reduce plant biomass under some temperature regimes for plants grown at low $[CO_2]$ in the day time. The results indicate the usefulness of controlling $[CO_2]$ at night in studies of plant growth.

Key words: CO_2; growth; photosynthesis; respiration; soybean; temperature

---

*present address: Plant Science Institute, ACSL USDA–ARS, Beltsville Agricultural Research Center 10300 Baltimore Ave. Beltsville MD 20705–2350 USA

INTRODUCTION

Control of $[CO_2]$ at night is often neglected in studies of plant growth. This is true in many growth chamber experiments, where unless CO_2 is actively removed at night, CO_2 produced by plant respiration may accumulate to quite high concentrations (sometimes to $700\,\mu$mol mol^{-1}, in our experience). In some studies simulating increased atmospheric $[CO_2]$, the $[CO_2]$ treatment has been applied only during the daytime (e.g. *7, 8*), either because of the extra expense, or the lack of wind at night to deliver the CO_2 to the plots in free air CO_2 enrichment studies. Additionally, in many field sites and in forest understories, the $[CO_2]$ may normally reach quite high levels during nights when the atmosphere is not well mixed (e.g. *3*). In all of these situations, it may be important to know whether elevated $[CO_2]$ during the dark period has consequences for plant growth.

There have been a few, sometimes conflicting, reports of effects of elevated $[CO_2]$ at night on plant growth. Reuveni and coworkers found growth stimulation in alfalfa (*11*) and in duck weed (*12*) with elevated $[CO_2]$ at night. In soybean seedlings, growth was initially stimulated, but later inhibited by elevated $[CO_2]$ at night (*3*). Elevated $[CO_2]$ at night decreased growth in two of four C_4 species (*16*). These studies all used the current atmospheric $[CO_2]$ as the daytime $[CO_2]$. In *Xanthium strumarium*, growth was unaffected, increased, or decreased when $[CO_2]$ was elevated at night, depending on the daytime $[CO_2]$ and salinity (*13*).

The primary motivation behind the work reported here was to determine whether it made a difference to soybean seedling growth under field conditions if elevated $[CO_2]$ treatments were maintained continually or only in the daytime. We then attempted to duplicate the field results in controlled–environment chambers, and found that the temperature regime was crucial to the plant response. The results have implications not only for elevated $[CO_2]$ investigations, but also for studies in controlled–environment chambers in which plants are grown at ambient $[CO_2]$ but $[CO_2]$ at night is not controlled.

MATERIALS AND METHODS

Field experiments were conducted at the South Farm of the Beltsville Agricultural Research Center in Beltsville, Maryland, USA. Four identical semi–open chambers were used in multiple experimental runs during June, July and August of 1998, 1999, and 2000. Each chamber was 1.0×1.2 m at the base, and 1 m high. Walls were made of transparent acrylic plastic. A clear polyvinyl chloride film with 1 cm diameter perforations every 10 cm covered each chamber to reduce the intrusion of outside air. Air was blown into the bottom of each chamber at $6\,\text{m}^3\,\text{min}^{-1}$ and evenly distributed with perforated pipe running the length of the chamber. CO_2 was introduced into the inlet blower of some chambers at a rate sufficient to increase the $[CO_2]$ in the chamber by 350 ± 25 μmol mol^{-1} Air samples from each chamber were sequentially pumped to an

absolute infrared carbon dioxide analyzer inside an adjacent air–conditioned shed, and [CO_2] values, shaded air temperatures and photosynthetically active radiation inside and outside the chambers were recorded every 5 min. Chambers were watered as necessary to prevent significant soil water deficits.

Soybean (*Glycine max* L. (Merr.) cv. Clark) seeds were sown in 4 rows 25 cm apart. Plots were thimed shortly after seedling emergence to a density of 25 plants m^{-2}. Six plants from the center of the interior rows of each chamber were harvested at first flowering, which occurred 27 to 35 days after planting in the different experiments. At harvest, total leaf area was determined with a leaf area meter, and leaf and stem dry mass were determined after oven drying at 60℃.

An initial test of chamber uniformity was conducted, with CO_2 constantly added to all four chambers to produce [CO_2]s of 350 μmol mol^{-1} above ambient air. The mean values of shoot dry mass ranged from 2.0 to 2.2 g across chambers, and did not differ between chambers ($P=0.66$), when treating each harvested plant as a replicate in an analysis of variance. In the remainder of the experiments, the four chambers were used to establish four [CO_2] treatments, no added CO_2, +350 μmol mol^{-1} [CO_2] for 24 h per day, +350 μmol mol^{-1} [CO_2] from 5am to 7pm, and +350 μmol mol^{-1} from 7pm to 5am. A total of five experimental runs were conducted over the three summers, with the [CO_2] treatments rotated among chambers. In analysis of this data, each chamber was considered an experimental unit, and the [CO_2] treatments were compared using analysis of variance with n=5.

Soybean plants were also grown in controlled–environment chambers using four day/night [CO_2] treatments, 350/350, 350/700, 700/700 and 700/350 μmol mol^{-1} [CO_2], with a day length of 14 h. Chamber [CO_2] was controlled to ±10 μmol mol^{-1}, except for brief periods when doors were opened to water plants. FTIR purge gas generators (Model 75–62, Whatman, Inc., Tewksbury, MA) were used to provide CO_2– free air to flush the chambers when [CO_2] exceeded the set point. Three experiments were run with constant day/night temperatures of 25/25, 27/22 and 29/19 each ±0.2℃, and with 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation from a mixture of high pressure sodium and metal halide lamps. Dew point temperatures were 18±2℃. Plants were grown in plastic pots containing 4 dm^3 of vermiculite and watered daily with a complete nutrient solution containing 14.5 mM nitrogen (*14*). These three experiments were all conducted in 2 controlled–environment chambers, with [CO_2] treatments rotated between chambers in sequential runs. Each temperature and [CO_2] treatment had four replicate chamber runs. For each chamber run a mean value of total leaf area and leaf, stem and root dry mass was obtained from 8 plants 22 d after planting. The same four [CO_2] treatments were also applied to plants in controlled–environment chambers programmed to approximate the average diurnal pattern of temperature inside the field chambers. This was accomplished by establishing 6 fixed temperature set points through the day (Table 1), and programming the control temperature at any time as a linear interpolation between these set points. The 24 h mean temperature was 25.2℃ for this

J. A. BUNCE

Table 1. Air temperature and photosynthetic photon flux density set points in the program controlling chambers to mimic the temperatures observed in the field chambers. The temperature control point at any given time was a linear interpolation between these set points.

Time (h)	Temperature (°C)	PPFD (μmol m^{-2} s^{-1})
05:00	20.0	450
07:00	27.0	1,000
11:00	34.0	1,500
15:00	34.0	1,000
17:00	23.0	450
19:00	22.0	0

treatment. The mean, maximum and minimum temperatures for this programmed temperature regime duplicated those obtained from the average of the field experiments. In the controlled–environment chambers, radiation was also varied through the day, at three levels (450, 1000, and 1500 μmol m^{-2} s^{-1}) of photosynthetic photon flux density (Table 1). The four [CO_2] treatments were applied simultaneously in four identical chambers, and each treatment was replicated 4 times, with the [CO_2] treatments rotated among the chambers. Mean values of leaf area and leaf, stem and root dry mass at 22 d after planting were obtained from 12 plants per chamber.

In addition to the harvest data, midday values of CO_2 assimilation rates were measured under the midday growth conditions on recently fully expanded leaves on day 21 after planting in each of the experiments in controlled–environment chambers.

RESULTS

The daytime [CO_2] in the field experiments averaged 353 μmol mol^{-1}, and was quite consistent from day to day. The [CO_2] increased at night, with a larger increase on nights with low wind speed. The mean [CO_2] of ambient air at night was 474 μmol mol^{-1}, with means ranging from 397 to 568 μmol mol^{-1} on different nights. The air temperature in the chambers averaged 2°C higher than that of outside air, with differences as large as 5°C in full sunlight. These are larger temperature differences than we obtain in fully open topped chambers, and can be attributed to the perforated tops used to prevent intrusion of outside air. In the field experiments, the total leaf area and shoot dry mass were highest for the chambers given elevated [CO_2] continuously, and were not significantly different for the other [CO_2] treatments (Fig. 1). Specific leaf area was about 20% lower for both treatments with elevated [CO_2] in the daytime than for the two treatments with ambient [CO_2] in the daytime.

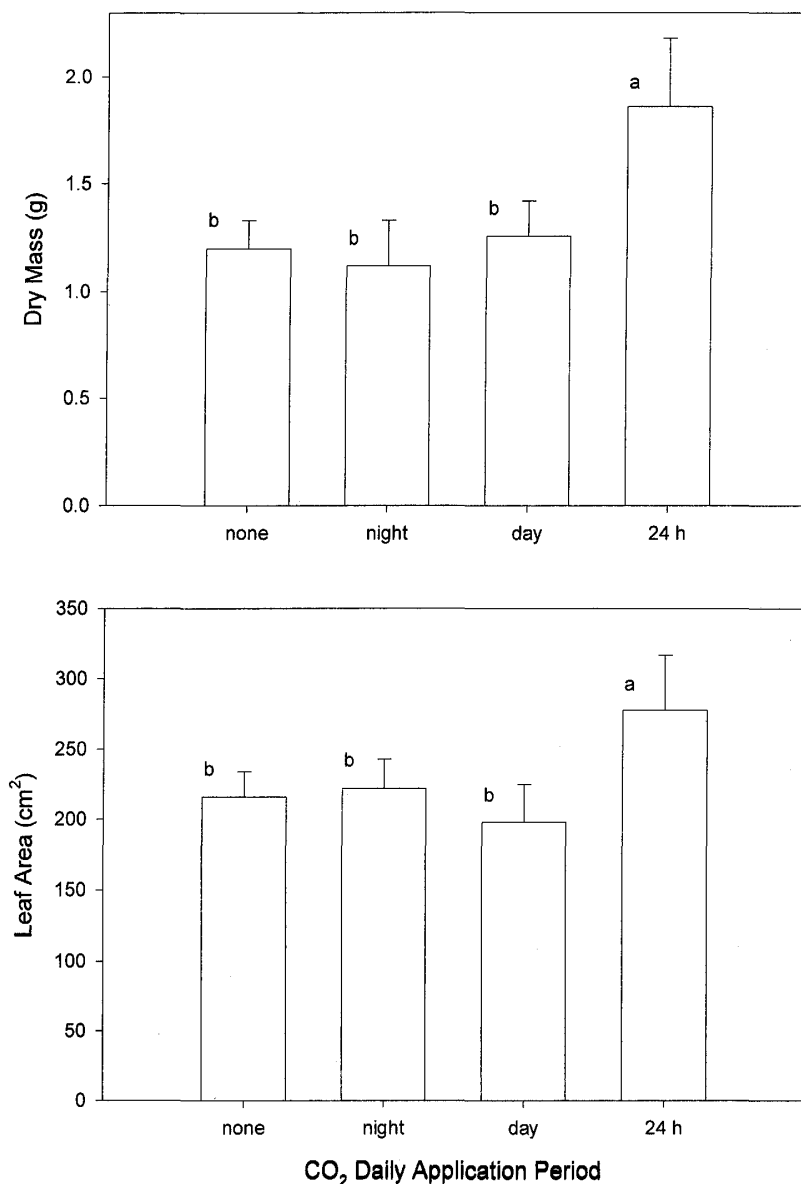In the controlled–environment chambers, the effect of the four [CO_2]

Fig. 1. Total shoot dry mass and leaf area at first flowering for soybean plants grown in the field in semi-open chambers with ambient + 350 μmol mol^{-1} [CO$_2$] applied during the day, night, both day and night, or neither. Error bars represent SE for n=5. Different letters signify values which differ at P=0.05, using Fischer's protected LSD tests.

treatments on growth depended on the amount of diurnal variation in temperature. At the constant 25°C temperature, the treatments with 700 μmol mol^{-1} [CO$_2$] at night had lower total leaf area and dry mass than the treatments with 350 μmol mol^{-1} [CO$_2$] at night, for both daytime [CO$_2$] conditions (Fig. 2). The same pattern of response to [CO$_2$] at night occurred in the 27/22°C day/ night temperature regime, although the treatment differences were considerably
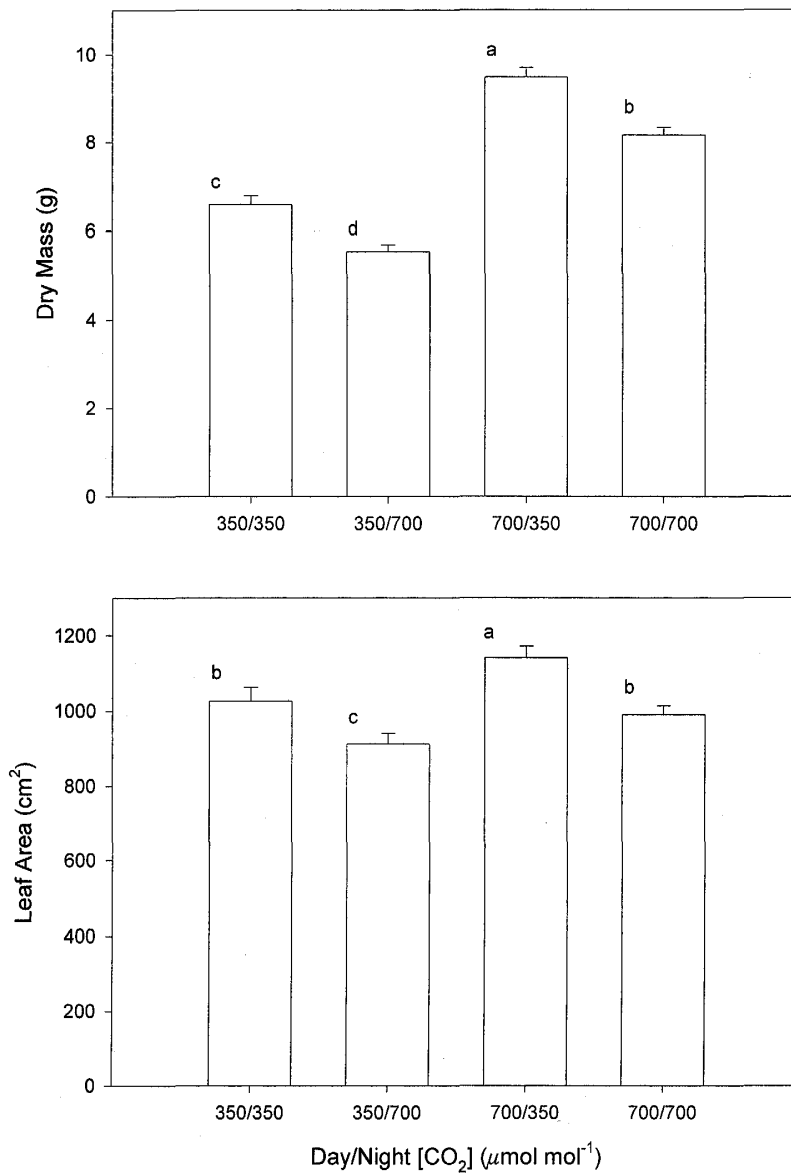
Fig. 2. Total plant dry mass and leaf area at 22 d from planting for soybean plants grown with four day/night $[CO_2]$ treatments, at a day/night temperature of 25/25°C. Error bars represent SE for n=4 chamber runs. Different letters signify values which differ at P=0.05, using Fischer's protected LSD tests.

smaller than at constant 25°C and not significant in some cases (Fig. 3). With increased amplitude of diurnal temperature change, a different response pattern occurred. At 29/19°C day/night temperature, total leaf area and plant mass were less for the 700/350 μmol mol^{-1} $[CO_2]$ treatment than for the 700/700 μmol mol^{-1} $[CO_2]$ treatment, while at the lower day $[CO_2]$, 700 μmol mol^{-1} $[CO_2]$ at night had no significant effect on growth (Fig. 4). The same pattern of response
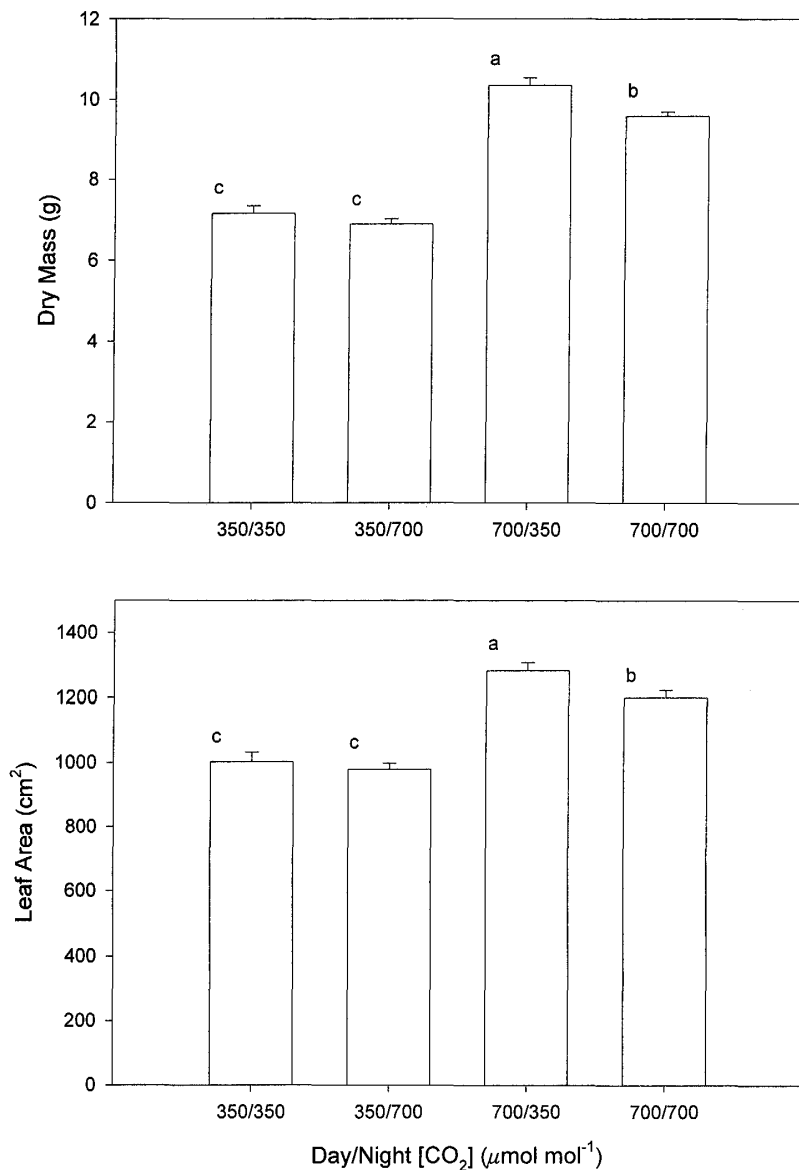
Fig. 3. As in Figure 2, except with a day/night temperature of 27/22°C.

occurred in the temperature regime mimicking the field temperatures, but in this case no increase in leaf area occurred in response to elevated $[CO_2]$ applied only during the day (Fig. 5).

Leaf CO_2 assimilation rates measured under the midday growth conditions were higher at 700 than $350\,\mu\text{mol mol}^{-1}$ daytime $[CO_2]$ (Table 2). At constant 25°C, high $[CO_2]$ at night reduced assimilation rates, but this did not occur when the temperature changed diurnally (Table 2).
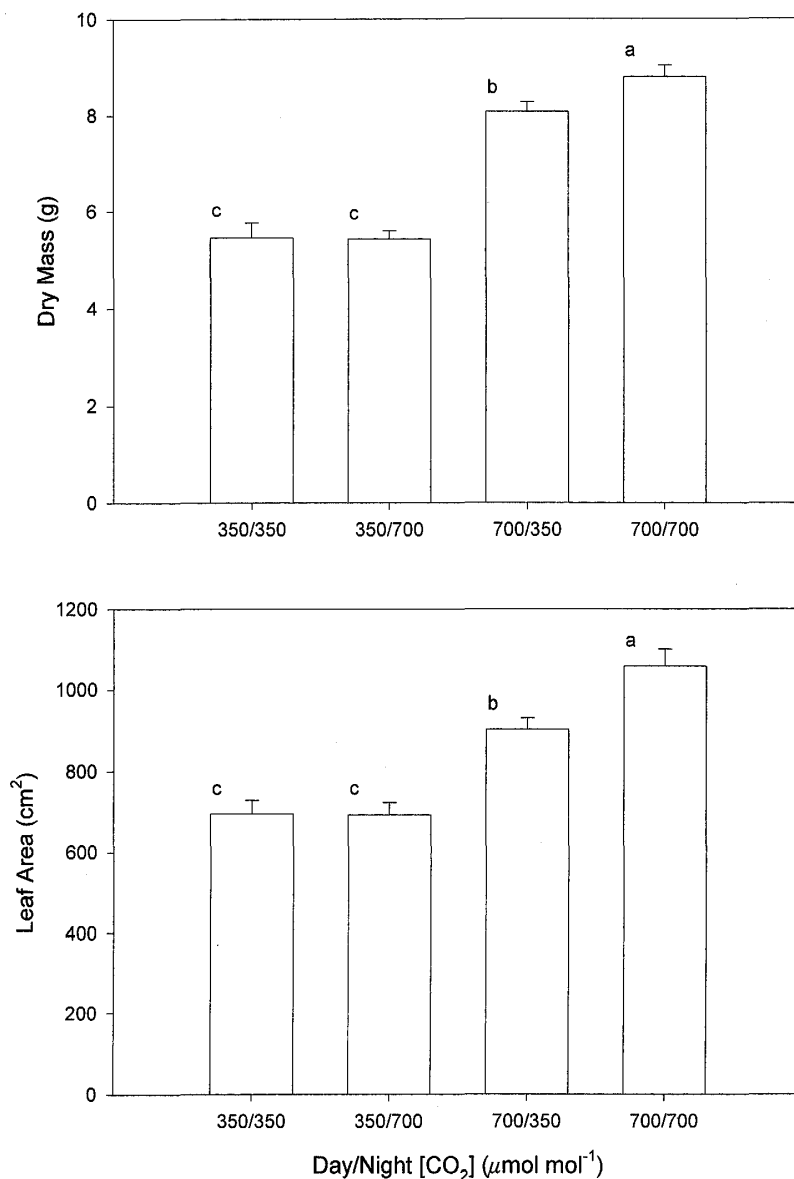
Fig. 4. As in Figure 2, except with a day/night temperature of 29/19°C.

DISCUSSION

The data from the field experiments indicate that the response of soybean growth to elevated $[CO_2]$ at approximately double the current atmospheric concentration was quite different depending on whether the elevated $[CO_2]$ was applied continuously or only in the daytime. Only the continuously elevated $[CO_2]$ treatment had increased dry mass and leaf area at flowering. Clearly the continuously elevated $[CO_2]$ treatments are a closer simulation of projected atmospheric $[CO_2]$ conditions. Work is currently underway to determine

Table 2. Rates of CO_2 assimilation measured under the midday growth conditions of second trifoliolate leaves of soybean. SE are given, for n=4 chamber runs. Values within columns followed by different letters are different at P=0.05, using Fischer's protected LSD tests.

Day/Night [CO₂]	Day/Night Temperature (°C):			
	25/25	27/22	29/19	34/19*
(μmol mol^{-1})	Assimilation Rate (μmol m^{-2} s^{-1}):			
350/350	26.3±0.7c	23.7±0.8b	22.8±0.7b	38.4±0.9b
350/700	22.4±0.5d	22.7±0.6b	22.0±0.9b	37.6±0.6b
700/350	43.6+0.9a	32.7±1.0a	27.5±0.6a	47.1+1.1a
700/700	39.1±1.1b	32.8+1.0a	28.7±1.3a	48.8+0.8a

*These are maximum/minimum temperatures for this regime. Day and night temperatures in this treatment changed gradually, as defined in Table 1. In this temperature regime the midday PPFD was 1500 μmol m^{-2} s^{-1}, compared with 1000 μmol m^{-2} s^{-1} in the other treatments.

whether the different effects on plant leaf area and dry mass observed here at flowering have consequences for seed yield responses to elevated [CO₂]. With more extreme day/night [CO₂] treatments than used here, Griffin et al. (6) found no seed yield increase in soybeans if elevated [CO₂] was given only during the daytime, but this was related to reproductive failure rather than whole plant biomass differences.

Work in the controlled-environment chambers indicated that the pattern of plant response to the day/night [CO₂] treatments observed in the field could be closely approximated by using diurnal changes in temperature similar to those occurring in the field. The similar patterns of plant responses to the [CO₂] treatments under the thermal regime with large step changes in temperature (Fig. 4) and the more gradually changing temperatures (Fig. 5) suggests that the rate of temperature change was not crucial to the response observed, although this aspect was not specifically investigated. The pattern of plant response to the day/night [CO₂] treatments was different when there was no diurnal temperature change. The build up of [CO₂] at night in controlled-environment chambers is likely to be larger with warm night temperatures, which in these experiments were the conditions under which growth was inhibited by high [CO₂] at night. Similar reductions in plant growth with high [CO₂] at night have been reported in two of four C₄ species examined (16), and in *Xanthium strumarium* under salt stress (13), when plants were grown with approximately the current atmospheric [CO₂] in the daytime.

Removal of CO₂ from chambers at night is often done by passing chamber air over soda lime beds (e.g. 6), which is cumbersome. The FTIR purge gas generators used here operate from the building compressed air supply, are self regenerating, and require little maintenance. Each purge gas generator can
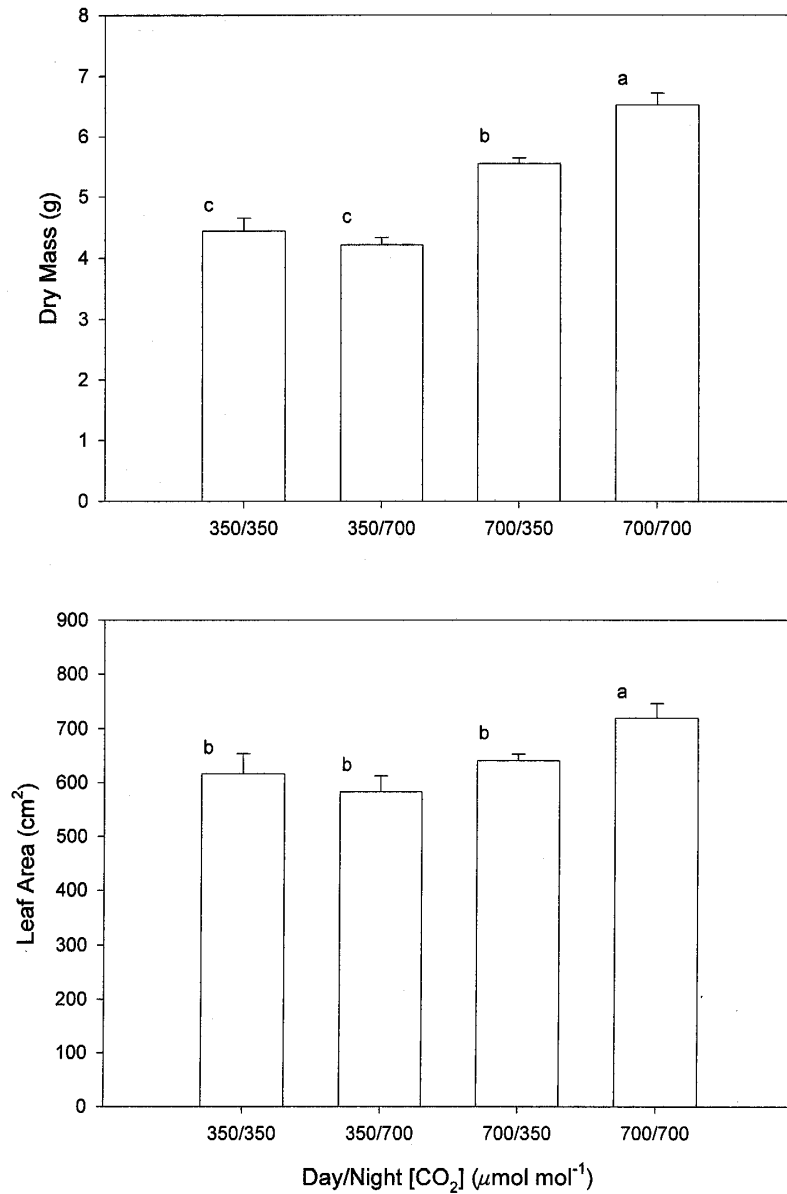
Fig. 5. As in Figure 2, except with gradually changing day and night temperatures defined in Table 1.

deliver up to 60 liters min^{-1} of scrubbed air, which in our experience can keep $[CO_2]$ at night at $350\,\mu mol\ mol^{-1}$ in a $2.9\ m^2$ chamber (EGC M–32, Environmental Growth Chambers, Chagrin Falls, Ohio) full of plants in a room with an ambient $[CO_2]$ as high as $500\,\mu mol\ mol^{-1}$. This would vary with the leakiness of the chamber.

The reduction in plant growth by elevated $[CO_2]$ at night observed when there was no diurnal temperature change was associated with lower single leaf rates of CO_2 assimilation, which have been previously reported for this species

(2, 15). Only for this thermal regime did single leaf CO_2 assimilation rates correlate with the responses of plant growth to $[CO_2]$ at night. This suggests that photosynthetic responses did not predominately control the plant growth responses to $[CO_2]$ at night for all thermal regimes.

Beyond effects on CO_2 assimilation rate, mechanisms by which $[CO_2]$ at night may influence plant growth have not been established, but effects on respiration could be important. High $[CO_2]$ often decreases plant respiration (1, 4, 5, 6, 9, 13), which could conceivably either increase plant dry mass by conservation of carbon or decrease dry mass if a growth–limiting process depends on respiration rate. There is evidence that respiration may sometimes be limited by the demand for energy for growth processes and sometimes by substrate availability (10). Respiration rate could be differently affected by $[CO_2]$ in these situations, and we have found a larger effect of $[CO_2]$ on respiration under conditions of limiting substrate caused by prolonged darkness (4). Whether the suggested differences in the control of respiration have any relationship to the observed contrasting effects of $[CO_2]$ at night on plant growth has not been investigated. Reuveni et al. (13), found that, for salinized plants, high $[CO_2]$ at night decreased growth when daytime $[CO_2]$ was low, but increased growth with high daytime $[CO_2]$. They suggested that with low daytime $[CO_2]$ and photosynthesis, high $[CO_2]$ at night repressed functionally important respiration, decreasing growth, while with higher photosynthesis, high $[CO_2]$ at night repressed respiration that was functionally not important, and increased plant growth by conserving carbon. With regard to the data presented in this paper, it seems possible that with a large diurnal temperature change, most respiration may occur at the higher day temperature, and high $[CO_2]$ at night might then reduce respiration without slowing growth–limiting processes, and increase dry mass by conserving carbon. With constant temperature, respiratory processes would occur at a constant rate, and high $[CO_2]$ at night could reduce functionally important respiration, and inhibit growth.

Regardless of the cause of responses of plant growth to $[CO_2]$ at night, the data presented indicate that plant growth may differ depending on whether elevated $[CO_2]$ is given only in the daytime or for 24 h per day. It also indicates that controlling $[CO_2]$ during the dark may be important for plant growth studies in controlled environment chambers, where $[CO_2]$ would otherwise increase during the dark period. A large diurnal variation in temperature was required to reproduce the pattern of response to $[CO_2]$ at night observed in the field.

REFERENCES

1. Baker J. T., Allen L. H. Jr., Boote K. J. and Pickering N. B. (2000) Direct effects of atmospheric carbon dioxide concentration on whole canopy dark respiration of rice. *Global Change Biol.* **6**, 275–286.
2. Bunce J. A. (1992) Light, temperature and nutrients as factors in photosynthetic adjustment to an elevated concentration of carbon dioxide. *Physiol. Plant.* **86**, 173–179.

3. Bunce J. A. (1995) Effect of elevated carbon dioxide concentration in the dark on the growth of soybean seedlings. *Ann. Bot.* **75**, 365–368.

4. Bunce J. A. (2001) Effects of prolonged darkness on the sensitivity of leaf respiration to carbon dioxide concentration in C_3 and C_4 species. *Ann. Bot.* in press.

5. Gale J. (1982) Evidence for essential maintenance respiration of leaves of *Xanthium strumarium* L. at high temperature. *J. Exp. Bot.* **33**, 471–476.

6. Griffin K. L., Sims D. A. and Seemann J. R. (1999) Altered night–time CO_2 concentration affects the growth, physiology and biochemistry of soybean. *Plant Cell Environ.* **22**, 91–99.

7. Luscher A., Hendrey G. R. and Nosberger J. (1998) Long–term responsiveness to free air CO_2 enrichment of functional types, species and genotypes of plants from fertile permanent grassland. *Oecol.* **113**, 37–45.

8. Mauney J. R., Kimball B. A., Pinter P. A. Jr., LaMorte R. L., Lewin K. F., Nagy J. and Hendrey G. R. (1994) Growth and yield of cotton in response to a free–air carbon dioxide enrichment. *Agric. For. Meteorol.* **70**, 49–67.

9. Mousseau M. (1993) Effects of elevated CO_2 on growth, photosynthesis and respiration of sweet chestnut (*Castanea sativa* Mill.). *Vegetatio* 104/105, 413–419.

10. Noguchi K. and Terashima I. (1997) Different regulation of leaf respiration between *Spinacia oleracea*, a sun species, and *Alocasia odora*, a shade species. *Physiol. Plant.* **101**, 1–7.

11. Reuveni J. and Gale J. (1985) The effect of high levels of carbon–dioxide on dark respiration and growth of plants. *Plant Cell Environ.* **8**, 623–628.

12. Reuveni J., Gale J. and Mayer A. M. (1993) Photosynthesis, respiration and dry matter growth of *Lemna gibba* L. as affect by day/night carbon dioxide regimes. *Current Topics in Plant Physiol.* **8**, 201–206.

13. Reuveni J., Gale J. and Zeroni M. (1997) Differentiating day from night effects of high ambient [CO_2] on the gas exchange and growth of *Xanthium strumarium* L. Exposed to salinity stress. *Ann. Bot.* **79**, 191–196.

14. Robinson J. M. (1984) Photosynthetic carbon metabolism in leaves and isolated chloroplasts from spinach plants grown under short and intermediate photosynthetic periods. *Plant Physiol.* **75**, 397–409.

15. Sicher R. C., Kremer D. F. and Bunce J. A. (1995) Photosynthetic acclimation and photosynthate partitioning in soybean leaves in response to carbon dioxide enrichment. *Photosyn. Res.* **46**, 409–417.

16. Ziska L. H. and Bunce J. A. (1999) Effect of elevated carbon dioxide concentration at night on the growth and gas exchange of selected C_4 species. *Aust. J. Plant Physiol.* **26**, 71–77.