



The Influence of Root Zone Temperature on Photosynthetic Acclimation to Elevated Carbon Dioxide Concentrations

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Received: 7 October 1997 Accepted: 11 February 1998

Soybean (*Glycine max* 'Clark') was grown from germination to 21 d after sowing (DAS) at ambient ($\sim 360 \mu\text{mol mol}^{-1}$) or elevated ($\sim 720 \mu\text{mol mol}^{-1}$) carbon dioxide (CO_2) at either one of two soil temperatures, 25 or 30 °C to determine the influence of root zone temperature on root growth and photosynthetic stimulation at ambient and elevated concentrations of carbon dioxide. Although the photosynthetic rate became less stimulate over time, a significant stimulation of whole plant photosynthesis and plant dry weight was observed at the elevated CO_2 concentration during the experimental period irrespective of soil temperature. At neither carbon dioxide concentration did the warmer soil temperature (30 °C) stimulate whole plant growth compared to a soil temperature of 25 °C, but it did increase root growth relative to shoot (top) growth with a subsequent increase in root/shoot ratio. Increasing soil temperature at either carbon dioxide concentration also significantly stimulated whole plant photosynthetic rate. However, the degree of stimulation was reduced with time irrespective of carbon dioxide concentration so that at 21 DAS no difference in photosynthesis between ambient and elevated soil temperatures was observed. Data from this experiment indicate that for soybean, a higher soil temperature stimulates root/shoot ratio and enhances photosynthetic response to elevated carbon dioxide in the short-term (i.e. days), but increasing root/shoot ratios does not provide a satisfactory explanation of long-term stimulation of photosynthesis at elevated levels of carbon dioxide.

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INTRODUCTION

Plants grown at future levels of elevated carbon dioxide do not always show continued photosynthetic stimulation relative to the ambient CO_2 condition but can, with long-term exposure (days to weeks), become insensitive or acclimate to increasing CO_2 levels (see Kramer, 1981; Wulff and Strain, 1982; Hogan, Smith and Ziska, 1991). In contrast, a few studies have demonstrated continued stimulation of photosynthesis at elevated CO_2 for months or even years (Arp and Drake, 1991; Eamus, Berryman and Duft, 1993). The underlying physiological causes of photosynthetic insensitivity to elevated CO_2 have been the object of ongoing experimentation and speculation, but have only been partially resolved (see Bowes, 1996).

It is thought that, at least in many instances, temporal photosynthetic insensitivity to elevated CO_2 may reflect a restricted capacity to utilize additional carbon. For example, if CO_2 enrichment results in an imbalance between source capacity and sink development, insufficient sinks for available carbohydrate could limit photosynthetic capacity presumably due to feedback inhibition and gene regulation of Rubisco (Stitt, 1991; Webber, Nie and Long, 1994). As a consequence, root growth and development have been hypothesized to play a key role in sustaining photosynthetic stimulation under an elevated CO_2 environment (Rogers *et al.*, 1992; Rogers and Runion, 1994). Roots are a principle, non-photosynthesizing sink for additional carbohydrate. The reported responses of root growth to enriched CO_2 levels have been dramatic (Rogers, Cure and Smith, 1986; Bernston and Woodward, 1992; Prior *et al.*, 1994,

but see also Reddy *et al.*, 1994 and Drennan and Nobel, 1996); and restriction of root growth by small pots or insufficient nutrients has been suggested as an underlying cause of photosynthetic acclimation to high CO_2 concentrations (Arp, 1991; Thomas and Strain, 1991; Sage, 1994).

However, if a given environment restricts root growth resulting in a subsequent decrease in photosynthetic stimulation at elevated CO_2 , then, conversely, environmental or growth conditions which stimulate root growth directly should result in an additional enhancement of photosynthesis at elevated CO_2 concentrations. It has been well established that roots are influenced, in part, by soil temperature with optimal temperatures providing maximum root growth and/or elongation (e.g. Drennan and Nobel, 1996). Therefore, changes in soil temperatures which maximize root growth, especially relative to shoot or vegetative growth, could delay the onset of photosynthetic acclimation and allow continued photosynthetic stimulation in response to CO_2 enrichment.

To date however, few studies have determined the influence of root growth and/or development on photosynthetic sensitivity to elevated CO_2 over time. It has been shown in previous studies that single leaves of soybean can acclimate photosynthetically to elevated CO_2 concentration (Sicher, Kremer and Bunce, 1995; Bunce, 1997). To understand the role of root growth in photosynthetic acclimation in this species, we used seedlings of soybean to determine whether changes in root growth or root/shoot ratio altered the degree of photosynthetic acclimation in elevated CO_2 grown plants.

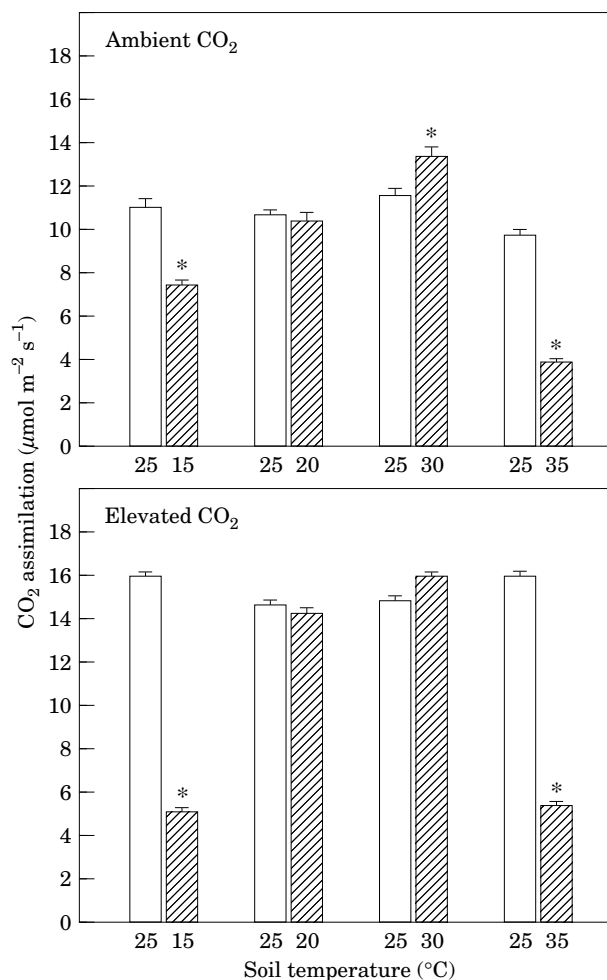


FIG. 1. CO₂ assimilation rates (\pm s.e.) at ambient or elevated CO₂ concentrations for soybean seedlings averaged over a 48 h period at four different soil temperatures. \square , Soil temperatures of 15, 20, 30 and 35 °C; \square ambient control (25 °C) for each soil temperature. * indicates a significant difference ($P \leq 0.05$) in assimilation rates between the ambient condition and a given soil temperature treatment. Student's unpaired *t*-test. See Materials and Methods for additional details.

MATERIALS AND METHODS

Two to three seeds of soybean (*Glycine max* 'Clark', Maturity group IV) were sown in 15-cm diameter plastic pots filled with 1.8 dm³ of vermiculite. 'Clark' seed was obtained from the USDA soybean germplasm collection in Urbana, Illinois. All pots were thinned to one plant within 48 h following emergence. For each experiment, 20–25 pots were assigned to each CO₂ treatment with pots arranged to avoid mutual-shading. All pots were watered daily with complete nutrient solution containing 13.5 mM nitrogen (Robinson, 1984).

Experiments were conducted using controlled environment chambers located at the Climate Stress Laboratory, USDA-ARS, Beltsville, Maryland. The carbon dioxide concentration was controlled for each environment chamber (EGC, Chagrin Falls, Ohio, USA) by flushing the chamber with CO₂-free air, then re-injecting CO₂ to the desired CO₂ concentration. Injection of CO₂ was controlled by an infrared gas analyzer in absolute mode (WMA-2, PP Systems,

Haverhill, MA, USA) which sampled air continuously. The set points for CO₂ control were 360 (ambient) and 720 $\mu\text{mol mol}^{-1}$ (elevated) carbon dioxide. Actual CO₂ concentrations determined at 10 min intervals over 24 h for a 21 d period were 368 ± 14 and 729 ± 16 $\mu\text{mol mol}^{-1}$. In all chambers, plants received 14 h of 1.0 $\text{mmol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) from a mixture of high pressure sodium and metal halide lamps. Day/night air temperature was kept constant at 25 °C, and average daily humidity exceeded 60%. Previous work with soybean has shown these temperature, light and humidity values to be optimal for photosynthesis and growth (Ziska and Bunce, 1997). Temperature, CO₂ concentration and relative humidity were monitored and recorded at 1 min intervals by an EGC network datalogger (EGC Corp., Chagrin Falls, Ohio, USA) in conjunction with a PC.

Soil temperature was controlled by placing pots within one or two 18 cm diameter PVC containers which circulated water in copper pipes adjacent to the walls of the pots in which the plants were rooted. Water temperature was controlled by a Hotpack refrigerated bath circulator (Hotpack Corp., Philadelphia, PA, USA). Soil temperature was measured using copper constantan thermocouples (with a BAT-12 Physitemp digital thermometer, Clifton, NJ, USA) placed at a depth of 2.5 cm and 5 cm, 2 cm from the edge of the pot. Initial results indicated that for well watered pots, soil temperature between thermocouples deviated by less than 0.1 °C. To determine daily rates of CO₂ exchange, each PVC container was enclosed within a 5l mylar chamber which was held at the growth air temperature. Air of the same carbon dioxide concentration used during growth was obtained by mixing CO₂ free-air with pure carbon dioxide before passing through each chamber. The air stream was humidified by bubbling through a water filled container. Humidity, light and temperature within the smaller measurement chamber were set to match those of the entire growth chamber. A differential infrared CO₂ analyzer (LiCor 6252, Lincoln, NE, USA) measured the net carbon dioxide exchange rate over a 7–9 d period across each of the mylar enclosed chambers. The sensitivity of the analyzer was corrected for the background carbon dioxide concentration. Data for chamber temperature, soil temperature, flow rate and CO₂ concentration were recorded using a micro-logger (21X, Campbell Scientific, Logan, UT, USA) at 10 min intervals.

To determine optimal soil temperature for root growth and photosynthesis, two pots from each of the two CO₂ treatments were placed within one of the mylar/PVC 'mini-chambers' at the desired soil temperature. Because of the limited number of mini-chambers, each experiment was repeated at soil temperatures of 15, 20, 30 and 35 °C and compared to the ambient soil temperature (25 °C). Initiation of 24-h whole plant gas exchange began with unfolding of the first trifoliolate (approx. 12–16 DAS) and was determined on a whole plant basis for 48 h at a given soil temperature. At the end of 48 h plants were harvested, separated into leaves, stems (including petioles) and the roots carefully washed. Leaf area was also measured with a leaf area meter (Li-Cor, model 3100). All plant parts were weighed after drying for 72 h or until a stable dry weight was maintained.

For plants grown at either CO₂ concentration, a 30 °C soil temperature was found to result in both maximum root growth and photosynthesis during a 48 h period. To determine the long-term effect of optimal soil temperatures, the 48 h experiment was repeated for four plants from each of the two CO₂ concentrations at either 25 or 30 °C soil temperatures from 13 to 21 DAS. As with the earlier experiment, 24-h gas exchange and plant dry weight were determined during and after the experimental period, respectively.

The effect of elevated CO₂ on gas exchange parameters and dry weight (including root weight and root/shoot ratio) were analysed using a Student's unpaired *t*-test. Interactions between soil temperature and photosynthesis and final dry weight were analysed by 2 way analysis of variance with means separated by a least squares table (SuperANOVA, Abacus Concepts, Berkley, CA, USA). Unless otherwise indicated, differences were determined as significant at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

For the short-term (48 h) evaluation of different soil temperatures, temperatures within a 5 °C range of ambient soil temperature (25 °C) resulted in optimal CO₂ assimilation rates at either CO₂ concentration (Fig. 1). Temperatures which varied by 10° above or below a 25 °C soil temperature resulted in significant reductions in CO₂ assimilation. For all soil temperatures tested, a temperature of 30 °C resulted in maximum values of assimilation. This assimilation value was significantly greater for the ambient CO₂ concentration when compared to the 25 °C ambient soil temperature (Fig. 1). It is unclear why temperatures 10 °C below the ambient soil temperature in this experiment were sub-optimal since

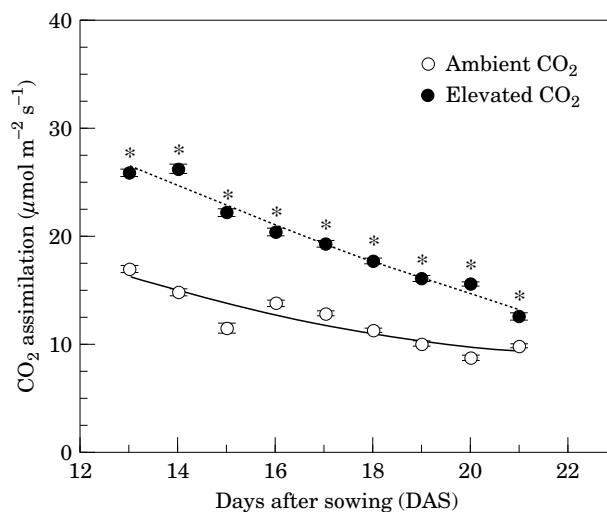


Fig. 2. Photosynthesis (determined as CO₂ assimilation) for whole plants of soybean calculated on a leaf area basis at current and doubled CO₂ concentrations. Each data point represents 84 10 min averages taken from 0800 to 2200 h. Data for a given CO₂ concentration were averaged for both soil temperatures (i.e. 25 and 30 °C). Data were obtained from 13–21 d after sowing (DAS). * indicates a significant difference ($P \leq 0.05$) in CO₂ assimilation relative to the ambient CO₂ concentration using a Student's unpaired *t*-test. Bars = \pm s.e.

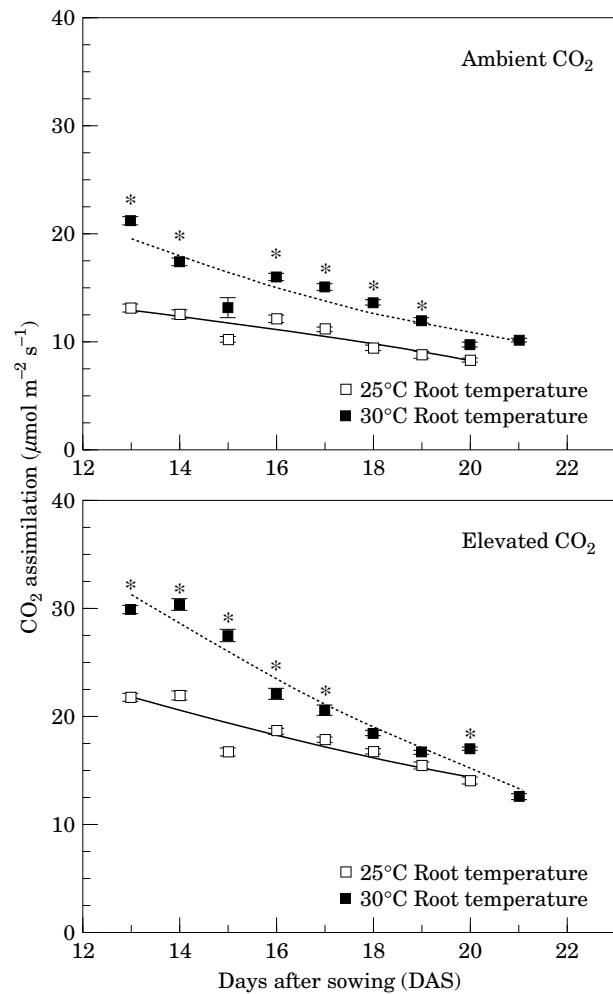


Fig. 3. As for Fig. 2, but separating assimilation as a function of root temperature (25 and 30 °C) at a given CO₂ growth concentration. * indicates a significant differences ($P \leq 0.05$) in assimilation rates between the two soil temperatures at a given DAS. Student's unpaired *t*-test. Bars = \pm s.e.

such temperatures probably occur *in situ*; however, it is likely that for periods longer than 48 h, some adaptation to soil temperature takes place.

For the longer-term (i.e. 13–21 DAS) experiment, CO₂ assimilation (averaged for soil temperatures of 25 and 30 °C) was significantly increased for the entire measurement period at the elevated CO₂ concentration (Fig. 2). However the relative stimulation of assimilation by enriched CO₂ declined with time and was only half that of the initial stimulation (e.g. 52 vs. 27% at 13 and 21 DAS, respectively). The higher soil temperature (30 °C) resulted in a significant enhancement of CO₂ assimilation for both CO₂ growth concentrations (Fig. 3). However, the relative stimulation of photosynthesis at the higher soil temperature was still reduced with time, becoming zero for the elevated CO₂ treatment by 21 DAS (Fig. 3). These results are consistent with photosynthetic acclimation (or downregulation) to elevated CO₂ over time, irrespective of soil temperature. It is unlikely that the observed increase in acclimation was a result of pot size *per se* (e.g. Arp, 1991; Thomas and Strain,

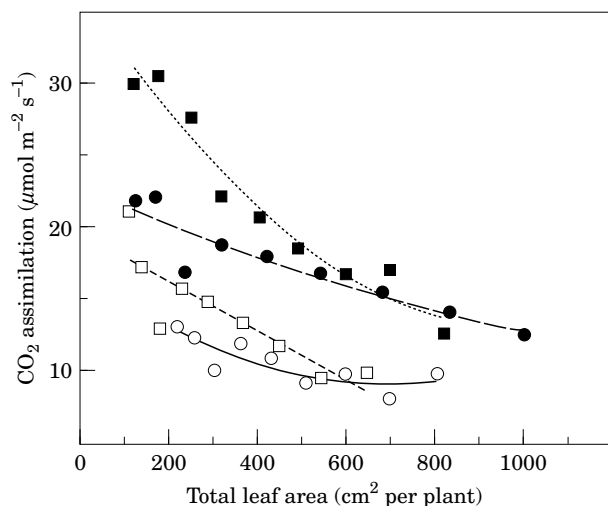


FIG. 4. Changes in CO_2 assimilation for ambient (\circ , \square) and elevated (\bullet , \blacksquare) CO_2 grown soybean seedlings as a function of total leaf area at either 25 °C (\circ , \bullet) or 30 °C (\square , \blacksquare) soil temperatures.

1991) since by 21 DAS visual observation indicated that the plants were not obviously root-bound.

It has been previously suggested that self-shading could limit the relative stimulation of photosynthesis at the whole plant level in soybean. Sicher *et al.* (1995) noted that long-term growth at elevated CO_2 reduced the quantum yield of photosynthesis and Bunce (1997) observed that the amount of photosynthetic acclimation to elevated CO_2 varied with the PPFD used to measure photosynthesis. In the current experiment, CO_2 assimilation as a function of total leaf area demonstrated photosynthetic insensitivity to a soil temperature of 30 °C at leaf areas of approx. 500 cm^2 per plant. However at a soil temperature of 25 °C, elevated CO_2 continued to demonstrate a significantly higher CO_2 assimilation rate up to leaf areas of 800 cm^2 per plant compared to the ambient CO_2 grown treatment. This suggests that self-shading *per se* did not cause the insensitivity of CO_2 assimilation to increased soil temperature (Fig. 4).

Total dry weight of soybean was significantly enhanced at the elevated CO_2 treatment after 21 DAS at either soil temperature (Table 1). Overall, elevated CO_2 stimulated

vegetative (top) growth to a greater extent than root growth with a subsequent reduction in root:shoot ratio when averaged for both soil temperatures (0.42 and 0.36 for the ambient and elevated CO_2 treatments, respectively). Increased soil temperature had no effect on either total dry weight or leaf area at a given CO_2 concentration. However, increased soil temperature significantly increased the root to shoot ratio for a given CO_2 treatment, indicating a shift in assimilate partitioning (Table 1).

The relative growth rate of roots (RGRr) at a soil temperature of 30 °C compared to 25 °C for the initial 48 h evaluation was significantly stimulated for both CO_2 concentrations in the first 2 d (i.e. 0.201 and 0.130 *vs.* 0.330 and 0.260 $\text{g g}^{-1} \text{d}^{-1}$ for the 30 and 25 °C soil temperatures at ambient and elevated CO_2 , respectively). In contrast, no change in RGRr with increased soil temperature was observed at the end of the 8 d experiment for a given CO_2 treatment. This indicated that the initial stimulation at the higher soil temperature (30 °C) was not maintained over time at either CO_2 concentration.

Any factor which inhibits root growth has been thought to decrease the relative stimulation of photosynthesis (determined as CO_2 assimilation) with increasing CO_2 concentrations (see Arp, 1991 for a discussion). This may be due, to part, to the ratio of available sinks to sources of assimilate in an enriched carbon dioxide atmosphere and the nature of feedback inhibition. Clearly, in the current experiment a change in the environment which promotes root growth or root/shoot ratio (i.e. soil temperature) is consistent with an enhancement in photosynthetic rate at both ambient and elevated CO_2 concentrations.

However, the degree of photosynthetic stimulation in the current experiment was short-term. That is, by 20–21 DAS the photosynthetic enhancement (and change in RGRr) induced by increased soil temperature disappeared. This suggests that increasing the root to shoot ratio or enhancing root growth *per se* does not necessarily result in long-term (weeks, months) photosynthetic enhancement under an enriched CO_2 condition for soybean seedlings. Presumably, continuous enhanced root (relative to shoot) growth over several weeks could provide longer-term photosynthetic stimulation by increasing the ratio of non-photosynthesizing sinks to carbon source, but it is unreasonable to assume that such growth takes place for plants *in situ* where limitations

TABLE 1. Change in harvest parameters (d. wt basis) for soybean seedling ('Clark') grown at ambient and elevated carbon dioxide at root temperatures of 25 and 30 °C at 21 DAS

[CO_2] ($\mu\text{mol mol}^{-1}$)	Root temp. (°C)	Leaf area (cm^2)	Leaf	Stem	Root	Total	R/S
			wt.	wt.	wt.	wt.	
			g per plant				
368	25	738 ^b	3.21 ^c	0.98 ^b	1.62 ^b	5.80 ^b	0.38 ^b
	30	676 ^b	2.64 ^c	0.80 ^c	1.55 ^b	5.05 ^b	0.45 ^a
729	25	1033 ^a	4.50 ^a	1.47 ^a	1.92 ^a	7.88 ^a	0.32 ^c
	30	870 ^a	3.93 ^b	1.27 ^b	1.98 ^a	7.18 ^a	0.40 ^b

R/S is the ratio of root to total above ground dry weight. Results were analysed using a two-way ANOVA to test for effects of CO_2 concentration and soil temperature and their interaction on harvest parameters. Superscripts indicate significant differences for a measured parameter as determined by least square means.

of water, nutrients or light may restrict potential root growth over time. Even in environmental growth chamber conditions where light, water and nutrients are readily available, root growth and root to shoot ratios may be limited genetically.

The idea therefore, that long-term photosynthetic enhancement with elevated CO₂ will automatically occur if root growth is not limited by a specific biotic or abiotic parameter (i.e. nutrients, physical restrictions etc.) may be an over-simplistic explanation of photosynthetic acclimation. Inter- and intra-specific variation with respect to signalling of assimilate transfer and permanent alterations of sink growth in response to carbon dioxide enrichment may be additional factors in selecting for soybean plants which demonstrate long-term continuous photosynthetic stimulation as atmospheric carbon dioxide continues to rise.

ACKNOWLEDGEMENTS

I thank Frances Caulfield, Sam Benson and Lois Barb for their valuable technical assistance. I also thank Jim Bunce, Dave Patterson and Joe Sullivan for their insight and suggestions for improving the manuscript.

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