

A Comparison of the Effects of Carbon Dioxide Concentration and Temperature on Respiration, Translocation and Nitrate Reduction in Darkened Soybean Leaves

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• **Background and aims** Respiration of autotrophs is an important component of their carbon balance as well as the global carbon dioxide budget. How autotrophic respiration may respond to increasing carbon dioxide concentrations, [CO₂], in the atmosphere remains uncertain. The existence of short-term responses of respiration rates of plant leaves to [CO₂] is controversial. Short-term responses of respiration to temperature are not disputed. This work compared responses of dark respiration and two processes dependent on the energy and reductant supplied by dark respiration, translocation and nitrate reduction, to changes in [CO₂] and temperature.

• **Methods** Mature soybean leaves were exposed for a single 8-h dark period to one of five combinations of air temperature and [CO₂], and rates of respiration, translocation and nitrate reduction were determined for each treatment.

• **Key results** Low temperature and elevated [CO₂] reduced rates of respiration, translocation and nitrate reduction, while increased temperature and low [CO₂] increased rates of all three processes. A given change in the rate of respiration was accompanied by the same change in the rate of translocation or nitrate reduction, regardless of whether the altered respiration was caused by a change in temperature or by a change in [CO₂].

• **Conclusions** These results make it highly unlikely that the observed responses of respiration rate to [CO₂] were artefacts due to errors in the measurement of carbon dioxide exchange rates in this case, and indicate that elevated [CO₂] at night can affect translocation and nitrate reduction through its effect on respiration.

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Key words: carbon dioxide, temperature, respiration, translocation, nitrate reduction, *Glycine max*.

INTRODUCTION

Carbon dioxide in the atmosphere is rapidly cycled through the biota by photosynthesis and respiration, and changes in the rates of photosynthesis or respiration by autotrophs could strongly affect the concentration of carbon dioxide in the atmosphere (e.g. Gifford, 1994). While there is much information on how plant photosynthesis may change if atmospheric carbon dioxide concentration continues to rise, less is known about possible changes in respiration. Several reviews of the literature have concluded that elevated concentrations of carbon dioxide, [CO₂], generally reduce rates of respiration, primarily by a direct effect (c.f. Drake *et al.*, 1999). However, some recent studies have found little or no direct response of respiration to [CO₂] and have suggested that direct effects of [CO₂] on respiration are measurement artefacts attributable primarily to leaks (Amthor, 2000a; Jahnke, 2001; Tjoelker *et al.*, 2001a; Bruhn *et al.*, 2002; Jahnke and Krewitt, 2002). While some studies that have measured leakage and taken it into account have still found effects of [CO₂] on respiration (e.g. Hilbert *et al.*, 1987; Baker *et al.*, 2000; Hamilton *et al.*, 2001), it is difficult to be sure that CO₂ exchange rates are completely without systematic error. Another approach to resolve the controversy would be to determine whether [CO₂] affects processes directly dependent on respiration.

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Photosynthetically active non-growing leaves generally accumulate carbohydrates during the day, which are mainly translocated to other parts of the plant during the night. Translocation, at least in darkness, requires energy, supplied by respiration, for the active transport of carbohydrates and other materials into the phloem and sometimes also for inter-conversions among forms of carbohydrates (Amthor 2000b). While carbohydrates are the major type of material translocated in the phloem, a decision was made here to estimate translocation from changes in total dry mass rather than from changes in non-structural carbohydrates, because non-structural carbohydrates as usually measured may be a variable fraction of the total dry mass accumulated and translocated diurnally (e.g. Bunce, 1982).

Earlier work (Bunce, 2002) indicated that translocation from soybean leaves was affected by [CO₂] at night. However, in that work, translocation was determined from changes in dry mass in relation to photosynthesis and respiration rate over three day/night cycles. In the present work, the fact that plants exposed to long days have rapid rates of translocation at night (e.g. Chatterton and Silvius, 1979) was utilized, which allowed translocation to be measured with sufficient accuracy over a single 8-h dark period. The estimate of translocation was therefore independent of photosynthetic measurements, and depended only to a minor extent on estimates of respiration.

The chemical reduction of nitrate to nitrite requires metabolic energy. During the light period, some of this energy may be supplied photochemically, but during the dark period it must come from respiration (Amthor, 2000*b*). In the present work, the rate of reduction of nitrate in the dark was also examined for responsiveness to [CO₂]. The rate of nitrate reduction was measured by the rate of disappearance of nitrate. This was necessarily determined with excised leaves, because in intact leaves the amount of nitrate entering a leaf via the transpiration stream would be unknown and might vary with [CO₂] treatment.

While responses of respiration to [CO₂] are controversial, responses of respiration to temperature are not disputed. Comparisons were made of the responses of respiration, translocation and nitrate reduction to [CO₂] and to temperature, to test whether a given change in respiration produced the same change in translocation or nitrate reduction regardless of whether respiration was changed by altered [CO₂] or temperature. Similar responses would strongly suggest that the apparent response of respiration to [CO₂] was real.

MATERIALS AND METHODS

Soybean (*Glycine max* L. Merr. 'Clark') was grown in multiple batches in a single controlled-environment chamber. Chamber air temperature was 25 ± 0.2 °C, the dew point temperature was 18 ± 2 °C, the [CO₂] was 370 ± 5 $\mu\text{mol mol}^{-1}$ in the daytime and 390 ± 5 $\mu\text{mol mol}^{-1}$ at night, and there were 16 h per day of light from a mixture of high pressure sodium and metal halide lamps with a PPFD of $1.0 \text{ mmol m}^{-2} \text{ s}^{-1}$. Plants were grown one per pot in 15 cm diameter plastic pots filled with 1.8 L of vermiculite and flushed daily with a complete nutrient solution containing 14.5 mol m^{-3} nitrogen, with a nitrate to ammonium ratio of 4.8 : 1.

Change in leaf mass per area

There were 16 plants in each batch. At the end of the photoperiod on day 17 from planting, half of the plants were selected at random for measurement of area and dry mass of the terminal leaflet of the first trifoliolate leaf. These leaves had finished area expansion a few days before these measurements. The remaining plants were placed in a second controlled-environment chamber at one of five combinations of air temperature and [CO₂] for a single 8-h dark period. At the end of the 8-h dark period, terminal leaflets of first trifoliolate leaves were harvested for determination of area and dry mass. A mean value for the change in leaf dry mass per unit of area over the 8-h dark period was determined separately for each batch of plants. There were three replicate batches of plants for each temperature and [CO₂] condition.

Experimental conditions

The five conditions were: at a [CO₂] of $370 \mu\text{mol mol}^{-1}$, air temperatures were 20, 25, or 30 ± 0.2 °C, and at 25 °C air temperature, the [CO₂] was 40, 370, or $1400 \pm 5 \mu\text{mol mol}^{-1}$.

Respiration

Respiration, measured as net CO₂ efflux in darkness, was determined for one plant of each batch of plants in the dark treatment chamber. The terminal leaflet of the first trifoliolate leaf was enclosed in a water-jacketed cuvette kept at the same air temperature and [CO₂] conditions as the rest of the plant. To avoid problems with gaskets sealing against leaf surfaces, discussed by Pons and Welscher (2002) and Jahnke and Krewitt (2002), the whole leaflet was enclosed in the cuvette, with the petiole inserted through a groove and sealed with caulk. The air streams were dried before entering the differential CO₂ analyser, and the other precautions in the determination of gas exchange rates detailed in Bunce (2001) were also taken. Rates of respiration were determined every 10 min throughout the dark period, and a mean value for the whole dark period was obtained. The three replicate batches of plants provided three replicate measurements of dark respiration for each temperature and [CO₂] treatment.

Translocation

Because fully expanded soybean leaves do not import carbon from other parts of the plant (Thrower, 1962), the decrease in leaf dry mass over the dark period represented translocation out of the leaf plus respiration. The decrease in mass due to respiration was calculated from the measured rate of net dark CO₂ efflux by assuming that the materials respired contained 40 % carbon (Bunce, 2002). For each [CO₂] and temperature treatment, estimates of translocation from measurements of the change in mass and respiration rate were made on three independent batches of plants, as described earlier.

Nitrate reduction

The nitrate concentration of extracts of leaf discs was determined by HPLC as previously described (Sicher and Bunce, 2001), and expressed per unit of area. Leaf discs were taken from leaves with a cork borer, then immediately frozen in liquid nitrogen and stored at -80 °C until extraction and analysis. Preliminary experiments indicated that the nitrate content of detached leaves with petioles placed in water decreased measurably during an 8-h dark period. Tests also showed that no nitrate or dry mass accumulated in the water in which the cut ends of the petioles were placed, indicating that translocation was stopped. The decrease in the leaf nitrate content over the 8-h dark period was therefore assumed to represent metabolic reduction of nitrate.

For each of the five temperature and [CO₂] treatments detailed previously, terminal leaflets of first trifoliolate leaves were excised at the end of the photoperiod, with the petioles recut under water. Paired leaf discs from a single leaflet were taken at the beginning and end of the 8-h dark period. There were three leaflets per temperature and [CO₂] environment, and a mean change in nitrate content was determined for each environment. Respiration rates of intact excised leaves in which petioles were recut and kept under

TABLE 1. Decrease in dry mass per unit of area, the dry mass equivalent of measured rates of respiration, and the calculated dry mass translocated out of soybean first trifoliolate leaves over single 8-h dark periods, for plants exposed to different [CO₂] and temperatures during the dark period

[CO ₂] (μmol mol ⁻¹)	Temperature (°C)	Decrease in Mass (g m ⁻² 8 h ⁻¹)	Respiration (g m ⁻² 8 h ⁻¹)	Translocation (g m ⁻² 8 h ⁻¹)
370	20	1.0 ± 1.3 ^d	1.0 ± 0.1 ^c	0.0 ± 1.3 ^d
370	25	9.2 ± 0.6 ^b	1.6 ± 0.1 ^b	7.6 ± 0.7 ^b
370	30	12.6 ± 1.0 ^a	2.2 ± 0.2 ^a	10.4 ± 1.0 ^a
40	25	12.8 ± 0.7 ^a	2.2 ± 0.1 ^a	10.6 ± 0.6 ^a
370	25	9.2 ± 0.6 ^b	1.6 ± 0.1 ^b	7.6 ± 0.7 ^b
1400	25	4.2 ± 0.9 ^c	1.2 ± 0.1 ^c	3.0 ± 0.9 ^c

Each value is a mean ± s.e. for three replicates. Within columns, means followed by different letters are significantly different at $P = 0.05$, using the Tukey HSD test

water were also determined at the same temperature and [CO₂] applied to the leaves in which nitrate loss was measured (one leaf per run), as previously described. There were three replicate runs for each air temperature and [CO₂] treatment.

Statistics

For each variable, analysis of variance was conducted using three true replicate samples, from the three separate batches of plants per treatment. Mean values for decreases in mass, respiration, and translocation were compared among treatments using the Tukey HSD test at $P = 0.05$. Linear regressions were calculated for relationships between respiration and translocation and between respiration and the decrease in nitrate.

RESULTS

The effects of the temperature and [CO₂] treatments on the decreases in mass over the dark period were much larger in absolute value than the treatment effects on the loss of mass estimated from the measured respiration rates (Table 1). Respiration and translocation (Table 1) and nitrate reduction (Fig. 1) all increased with increasing temperature at constant [CO₂] and all three processes decreased with increasing [CO₂] at constant temperature. The temperature and [CO₂] treatment effects were significant for all three processes, as assessed by analysis of variance. Both translocation and nitrate reduction changed relatively more with the temperature and [CO₂] treatments than did respiration. However, a given change in respiration was accompanied by the same change in translocation and nitrate reduction, whether the change in respiration was caused by altered temperature or altered [CO₂]. This was indicated by both the temperature and [CO₂] treatments having the same linear regression with respiration rate (Fig. 2).

DISCUSSION

The 16-h day, 8-h night regime resulted in rapid loss of leaf dry mass during the night, allowing effects of the temperature and [CO₂] treatments on rates of loss of mass to be

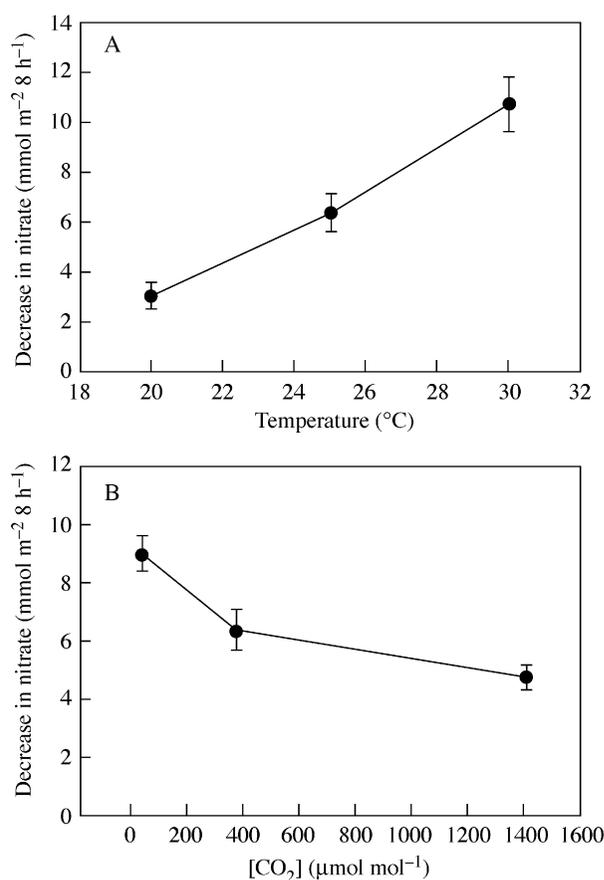


FIG. 1. The decrease in the nitrate content of excised soybean leaves over an 8-h dark period with (A) different temperatures and (B) different [CO₂]. The [CO₂] in the three temperature treatments was 370 μmol mol⁻¹, and in the three [CO₂] treatments the temperature was 25 °C. The bars represent s.e. for $n = 3$ batches of plants. The effects of temperature and [CO₂] on nitrate loss were both significant at $P = 0.05$, using analysis of variance.

detected in a single dark period. Because the treatments consisted of only a single dark period, estimates of translocation did not depend on estimates of photosynthesis, and were not confounded by after-effects of the treatments

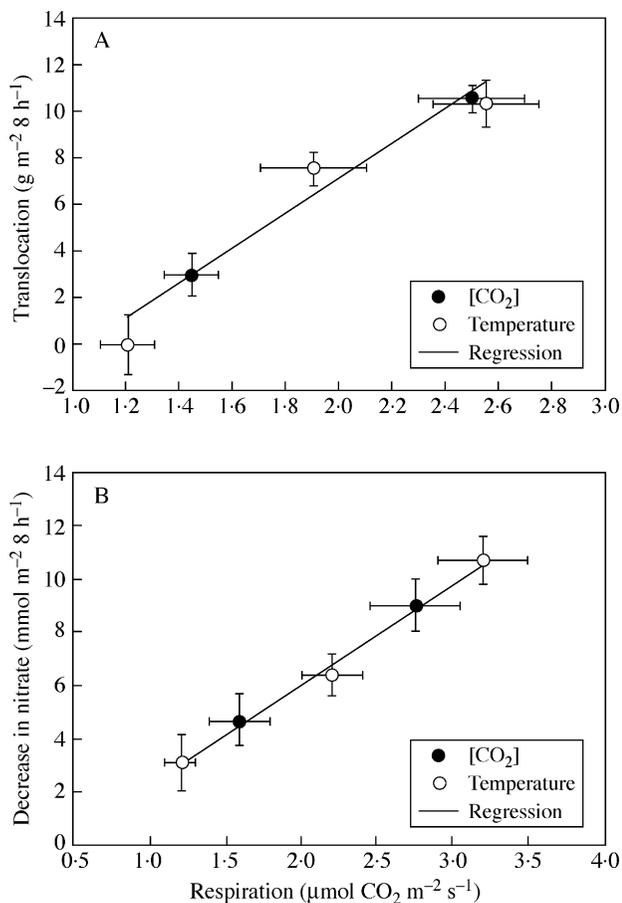


FIG. 2. (A) Translocation and (B) the decrease in nitrate content of soybean leaves over an 8-h dark period as a function of respiration rate, for different temperature and $[\text{CO}_2]$ treatments. Intact, attached leaves were used for the measurements of translocation, and excised leaves were used for the measurements of nitrate loss. The $[\text{CO}_2]$ in the three temperature treatments was $370 \mu\text{mol mol}^{-1}$, and in the three $[\text{CO}_2]$ treatments the temperature was 25°C . The bars represent s.e. for $n = 3$ batches of plants. The lines represent overall linear regressions, with $r^2 = 0.943$ for translocation and $r^2 = 0.989$ for the decrease in nitrate, which are both significant at $P = 0.05$.

imposed in the dark period on subsequent photosynthesis, as was the case in a prior study indicating $[\text{CO}_2]$ effects on translocation (Bunce, 2002). Because the changes in respiration in response to the treatments were small compared with the changes in the rates of loss of dry mass, the treatment effects on rates of loss of mass reflect treatment effects on translocation. Since the loss of nitrate was measured in the absence of translocation, the only clear link between these processes is their dependence on energy and reductant supplied by respiration. The observation that the three distinct processes of respiration, translocation and nitrate reduction all responded to $[\text{CO}_2]$ makes it highly unlikely that the apparent response of respiration to $[\text{CO}_2]$ was an artefact. Possible mechanisms of direct effects of $[\text{CO}_2]$ on respiration were reviewed in Drake *et al.* (1999).

Respiration of attached leaves increased by a factor of 2.2 for the 10°C range in temperature, similar to innumerable other studies (reviewed in Tjolkner *et al.*, 2001b). The increase in respiration with short-term increases in temperature has been envisioned as a direct effect of temperature on biochemical reaction rates in the respiratory pathway, and not the result of an increase in the demand for respiration by processes utilizing the energy supplied by respiration (Amthor, 2000b). While it seems possible that temperature could affect translocation and nitrate reduction by mechanisms unrelated to respiratory energy supply, it seems less likely that $[\text{CO}_2]$ directly affects both translocation and nitrate reduction. The similarity of the changes in translocation relative to respiration and of the changes in nitrate reduction relative to respiration for both the temperature and $[\text{CO}_2]$ treatments therefore suggests that both the changes in translocation and nitrate reduction were mediated by the changes in respiration. This suggests that translocation and nitrate reduction were limited by the energy supplied by respiration in this case. However, regardless of the mechanisms involved, the parallel responses of translocation and nitrate reduction for both the temperature and $[\text{CO}_2]$ treatments make it unlikely that the response of respiration to one variable was an artefact while the response to the other was real, particularly since respiration was measured by the same method and in the same apparatus in both cases. Recently, Pinelli and Loreto (2003) measured dark respiration using measurements of $^{12}\text{CO}_2$ emission and also found that it decreased as $[\text{CO}_2]$ increased.

While there is considerable information about the amount of respiratory energy used in the many processes dependent on it during normal growth, little is known about how the energy is partitioned among the processes competing for it when the respiration is limited (Amthor, 2000b). In these results, translocation was relatively more sensitive to the inhibition of respiration by the low temperature and elevated $[\text{CO}_2]$ treatments than was nitrate reduction, which suggests nitrate reduction had higher priority for respiratory energy supply. While complete cessation of translocation by the 20°C treatment seems a drastic response, subsequent experiments have shown that respiration and translocation both recovered to their initial rates after a few days of acclimation to 20°C .

These new observations of linked responses of rates of respiration, translocation and nitrate reduction to $[\text{CO}_2]$ during the dark period may provide mechanisms to explain observations that whole-plant growth rates sometimes respond to $[\text{CO}_2]$ at night (e.g. Bunce, 2003; Reuveni *et al.*, 1997; Ziska and Bunce, 1999). The results also indicate that $[\text{CO}_2]$ effects on respiration may have an unrecognized impact on plants in many elevated $[\text{CO}_2]$ experiments, through effects on translocation, nitrate reduction, and possibly other processes whose rate is dependent on respiration.

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