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Effect of Root-Zone (RZ) CO₂ on Productivity and Photosynthesis in Aeroponically Grown Lettuce Plants

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Keywords: Leaf area, Photosynthesis, CO₂ assimilation, Shoot and root weight, Stomatal conductance, Transpiration

Abstract

Effects of root-zone (RZ) CO₂ on crisphead-type lettuce (*Lactuca sativa* L. cv. 'Wintergreen') were measured in an aeroponic system under photosynthetic photon flux of 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 12 h photoperiod at 36°C/30°C and 28°C/22°C (day/night), with three enriched RZ CO₂ levels (2000 ppm, 10,000 ppm and 50,000 ppm). Leaf growth was monitored after elevated RZ CO₂ had been supplied for one week. Leaf areas with elevated RZ CO₂ were greater than ambient controls at both temperatures, while shoot and root weights were also higher. Increasing temperature reduced biomass overall, but the relative response to RZ CO₂ was greater. Elevated RZ CO₂ stimulated photosynthetic CO₂ assimilation, with greater increase at higher temperatures. Elevated RZ CO₂ decreased stomatal conductance at both temperatures, reducing transpiration water loss.

INTRODUCTION

Aeroponic systems by their nature are well aerated, with free air supply to the roots. However, it is not unusual for rhizosphere CO₂ concentrations in soil to be up to 10-fold higher than atmospheric CO₂ levels (De Jong and Schappert, 1972; Norstadt and Porter, 1984), due to activity of soil micro-organisms and root respiration. Therefore, in artificial aeroponic systems, RZ CO₂ is often much lower than for plants in soil. This difference could play a key role in photosynthesis and productivity of aeroponically-grown plants.

In this study, effects of RZ CO₂ on photosynthesis and productivity were studied in aeroponically-grown lettuce plants at two temperature regimes of 28°/22°C and 36°/30°C (day/night). Relationships among photosynthetic CO₂ assimilation, stomata conductance, transpiration and midday relative water content for lettuce plant grown under different RZ CO₂ and temperature regimes were investigated.

MATERIALS AND METHODS

Crisphead-type lettuce (*Lactuca sativa* L. cv. 'Wintergreen', South Pacific Seeds Ltd, New Zealand) were germinated on moist paper, and established on Grodan® rockwool in a controlled environment (CE) room (28°/22°C day/night, photosynthetic photon flux $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$) at the New Zealand Controlled Environment Laboratory. Seedlings were then transplanted to 68-litre opaque plastic aeroponic bins (Stowers Ltd, NZ) in two CE rooms. Bin lids held 24 plants, with a root plug size of up to 19 mm. Each bin was supplied with temperature-controlled nutrient solution from a separate reservoir. Micro-sprinklers intermittently misted roots (40 sec / 20 sec on/off) with re-circulated half-strength Hoagland's solution (Brooking, 1976). Solution pH was maintained at ~ 6.5 and it was replaced when the conductivity factor reached 20 (i.e., 2 mS).

Air temperature was controlled in two CE rooms at 28°C/22°C and 36°C/30°C (day/night, $\pm 0.5^\circ\text{C}$) with relative humidity of 70%/65% (day/night, $\pm 5\%$) in both. A 12 h photoperiod was provided by 1 kW high intensity discharge and tungsten halogen lamps

(Warrington et al., 1978), with photosynthetic photon flux of $650 \mu\text{mol m}^{-2} \text{s}^{-1}$ from 1 hr after the diurnal temperature minimum. Shoot-zone (SZ) CO_2 levels were maintained at ambient by automatic supplementation. RZ CO_2 was controlled at ~ 360 (ambient), 2000, 10,000 and 50,000 ppm respectively using pre-mixed CO_2 -air mixtures (BOC Ltd), supplied from compressed air cylinders at $\sim 0.5 \text{ L min}^{-1}$, with separate venting to the CE room exhaust-air duct. CO_2 levels at the shoot base were measured with a LI-COR 6400 photosynthesis system. No significant increase in atmospheric CO_2 was found.

After elevated RZ CO_2 had been supplied for one week, the area of the second leaf (5 plants) were measured (Area Measurement System, Delta T-Devices Ltd., England). After one, two and three weeks, plants were divided into shoot and roots to determine fresh and dry weights. Leaf CO_2 uptake, stomatal conductance and transpiration were measured on newly matured leaves with a LI-6400 Photosynthesis System (LI-COR Biosciences, U.S.) two weeks after RZ CO_2 treatments began. Measurements were made at 1000 to 1100 h, with $650 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, and leaf chamber temperature of 25°C and 33°C respectively at $28^\circ/22^\circ\text{C}$ and $36^\circ/30^\circ\text{C}$. To estimate relative water content ($\text{RWC} = (\text{fresh} - \text{dry weight})/(\text{turgid weight} - \text{dry weight}) \times 100$), leaf discs were immediately weighed for field fresh weight, floated on distilled water for 24 hr in darkness to measure turgid (or saturated) weight, then weighed again after drying at 80°C for 48 hours. All data were analysed by single factor analysis of variance.

RESULTS

Plants with elevated RZ CO_2 had faster leaf expansion (Fig. 1) and more leaf area than ambient controls at both growth temperatures. Increases in leaf areas at $28^\circ/22^\circ\text{C}$ (Fig. 1 a) were greater than at $36^\circ/30^\circ\text{C}$ (Fig. 1 b) at the same RZ CO_2 at any given time. Responses of leaf growth to elevated RZ CO_2 were similar at both temperatures- i.e., leaf areas were similar when roots were exposed to 10,000 and 50,000 ppm RZ CO_2 and they were greater than at 2000 ppm RZ CO_2 .

Increases in fresh weight of shoot and root at $28^\circ/22^\circ\text{C}$ were greater than those at $36^\circ/30^\circ\text{C}$ at the same RZ CO_2 . Responses of fresh shoot weight to RZ CO_2 were similar at both temperatures. There were no differences in fresh shoot weight between plants at 10,000 and 50,000 ppm RZ CO_2 at both temperatures, and they were both greater than those at 2000 ppm. However, root fresh weight at 50,000 ppm RZ CO_2 was greater than at 10,000 ppm: i.e., more roots were produced at higher RZ CO_2 . Effects on shoot and root dry weight (data not shown) were very similar to those on fresh weight (Figs. 2 & 3). At $28^\circ/22^\circ\text{C}$, fresh shoot/root ratios increased with RZ CO_2 from ambient, 2000 to 10,000 ppm, with highest shoot/root ratio at 10,000 ppm RZ CO_2 (Fig. 4a). Higher shoot/root ratios were due to higher relative increases in shoot weight compared to the roots. However, there were no changes in fresh shoot/root ratio at 50,000 ppm of RZ CO_2 due to relatively higher root production (Fig. 3). Fresh shoot/root ratios at $36^\circ/30^\circ\text{C}$ (Fig. 4b) were lower than those at $28^\circ/22^\circ\text{C}$ (Fig. 4a) at the same RZ CO_2 . At $36^\circ/30^\circ\text{C}$, increases in shoot/root ratio slowed down from 2 weeks in all treatments. This indicated that high growth temperature resulted in relatively higher root production.

Photosynthetic CO_2 assimilation (A) measured under ambient SZ CO_2 changed at different levels of elevated RZ CO_2 as compared to ambient RZ CO_2 (Table 1). Elevated RZ CO_2 stimulated A at both temperatures, with a greater relative increase at the higher temperature for the same RZ CO_2 . For instance, A at 50,000 ppm RZ CO_2 was 156% higher than at ambient RZ CO_2 under $36^\circ\text{C}/30^\circ\text{C}$. However, at $28^\circ/22^\circ\text{C}$, values of A at 50,000 ppm RZ CO_2 were 135% higher than at ambient RZ CO_2 . At the same temperature, A was the same at RZ CO_2 of 10,000 and 50,000 ppm but it was much higher than 2000 ppm or ambient RZ CO_2 .

Although higher RZ CO_2 increased A at both temperatures, stomatal conductance (g_s) was lower than at ambient RZ CO_2 at both temperatures, reducing transpiration. Decreases in g_s and transpiration at the highest RZ CO_2 (50,000 ppm) were greater at $36^\circ/30^\circ\text{C}$ than at $28^\circ/22^\circ\text{C}$ (Table 1). Midday RWC was also much lower at $36^\circ/30^\circ\text{C}$ compared to $28^\circ/22^\circ\text{C}$ (Table 1). However, elevated RZ CO_2 increased the midday RWC

of leaves at both temperatures. The percentage increase under elevated RZ CO₂ was greater at high temperature than at low temperature.

DISCUSSION

Our results show that higher RZ CO₂ increased shoot and root growth (Figs. 2, 3) and that higher productivity reflected increased leaf areas (Fig.1) and *A* (Table 1), despite a decrease in *g_s* and transpiration (Table 1). Our results also showed increases of 117 to 135% and 119 to 156% in *A* at elevated RZ CO₂ at 28°/22°C and 36°/30°C, relative to plants grown at ambient RZ CO₂ (Table 1).

Previously, we reported that decreased productivity, *A* and *g_s* of lettuce at high temperatures resulted from water stress (He and Lee, 1998; He et al., 2001). This study prompts questions as to how RZ CO₂ stimulated higher net assimilation and growth rate. Most obvious is whether the results are artefacts of elevated SZ CO₂ (i.e., due to RZ CO₂ escape), given that only roots were exposed to elevated CO₂, while aerial parts were at ambient CO₂. However, no increase in SZ CO₂ was found. Hence, did lower *g_s* not so much limit atmospheric CO₂ uptake as enhance assimilation by restricting loss of CO₂ from leaves? Are non-atmospheric pathways for CO₂ uptake significant, or do other effects of RZ CO₂ mediate impacts on growth, such as on root nutrient uptake?

Elevated RZ CO₂ could have enhanced growth and *A* by increasing nutrient uptake. In this study, stimulation could simply reflect a larger root system (Fig. 3) and higher nitrate uptake (unpublished data) at elevated RZ CO₂. Increased NO₃⁻ uptake at elevated RZ CO₂ has been reported in hydroponic tomatoes (van der Merwe and Cramer, 2000) and NO₃⁻ uptake could be associated with exchange for HCO₃⁻ (Barneix et al., 1984). Elevated DIC also stimulated respiratory electron transport and increased incorporation of NO₃⁻ into amino acids (van der Westhuizen and Cramer, 1998).

Photosynthesis and transpiration have long been known to respond to atmospheric CO₂ level, and respiration is also affected. Thus, reduced stomatal aperture and *g_s* explain the reduction in leaf transpiration observed in plants grown in elevated atmospheric CO₂ (Morrison, 1987), and in plants at elevated RZ CO₂ in this experiment. But does reduced *g_s* in elevated atmospheric CO₂ limit photosynthesis in plants adapted to high atmospheric CO₂? This does not appear so. *C_i/C_a* (intercellular / atmospheric CO₂ concentration) ratio can be used as an index of photosynthetic limitation. Drake et al. (1997) found that mean and range of *C_i/C_a* were nearly identical for both ambient and elevated *C_a* even if *g_s* was lower in under elevated *C_a*. Hence, low *g_s* does not appear to limit photosynthesis at elevated atmospheric CO₂ more than it does at normal ambient CO₂.

In this study, leaves used to measure *A* developed under different RZ CO₂. Because *g_s* is mediated by changes in photosynthesis, high *g_s* in plants having higher photosynthetic capacity is to be expected. However, in the present study, values of *g_s* were *lower* at elevated RZ CO₂ (Table 1). This could reflect acclimation to elevated RZ CO₂; i.e., similar to the physiological changes that occur in plants grown at elevated atmospheric CO₂ (Drake et al., 1997). For instance, leaf stomatal density varies inversely with CO₂ concentration in air around leaves (Retallack, 2001; Lake et al, 2001), and so plants at elevated CO₂ have fewer stomata. Mature leaves appear to detect conditions around them and send a signal (still unknown) that reduces stomata number on developing leaves (Lake et al., 2001). Hence, lower *g_s* might be due to fewer stomata in the leaves developed at elevated RZ CO₂. This merits further investigation.

Elevated RZ CO₂ also stimulated *A* regardless of temperature, with greater relative increase at higher temperatures. Higher *A* at elevated RZ CO₂ may result from higher internal CO₂ and thus lower dark respiration, since elevated ambient CO₂ usually results in a lower rate of dark respiration of leaves (Drake et al. 1997). Higher internal CO₂ may also favour Rubisco carboxylation over oxygenation, increasing *A* with concomitant reduction in photorespiration. Higher *A* at elevated RZ CO₂ could reflect higher internal CO₂ transport from roots, even though *A* was measured at ambient SZ CO₂. In natural soils, respiration by roots and micro-organisms produce CO₂ which can reach 200,000 ppm (Norstadt and Porter, 1984), and positive effects of rhizosphere DIC on plant growth

have been reported (Vapaavuori and Pelkonen, 1985; Bialczyk et al., 1996). By contrast, in an aeroponics system, roots are suspended in air and there are no organic materials. Although root respiration produces CO₂, dissolved inorganic carbon (DIC) concentrations would normally be much lower than in soil. Hence, when elevated CO₂ is supplied, inorganic carbon could enter roots either as CO₂ or HCO₃⁻ depending on pH (Cramer et al., 1996).

It is conceivable DIC translocation could contribute significantly to a plants carbon budget, especially if carbonic anhydrase, an enzyme present in roots (Coba de la Peña et al. 1997), catalyses conversion of dissolved CO₂ to carbonic acid (H₂CO₃). In this study, pH was ~6.5 and so inorganic carbon could enter roots as CO₂ and HCO₃⁻. Thus, given a stable equilibrium between CO₂, H₂CO₃ and HCO₃⁻, at pH 6.5 and 5% CO₂, the aqueous medium entering roots is likely to contain ~20 µmol ml⁻¹ DIC. Given transpiration of ~10 mmol m⁻² s⁻¹ (Table 1: ≈0.18 ml m⁻² s⁻¹), this suggests an effective DIC translocation rate of 3.6 µmol m⁻² s⁻¹ is possible. This compares with the net atmospheric CO₂ assimilation rate of 8 to 16 µmol m⁻² s⁻¹. Hence, greater growth of plants at elevated RZ CO₂ could be due to contribution of internal CO₂ as DIC from the RZ to photosynthesis despite lower g_s (Roden, and Ball, 1996). Thus, carbon derived from root DIC could be translocated to the shoot and utilized in photosynthesis (Cramer and Richards, 1999). This has application where aeroponic technique is used for crop production, and suggests higher RZ CO₂ could increase water-use efficiency, providing growers of temperate crops in tropical countries with savings in cooling costs (He and Lee, 1998).

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Tables

Table 1 Photosynthetic CO₂ assimilation (*A*), stomatal conductance (*g_s*), transpiration and midday relative water content (RWC) of lettuce at 28^o/22^oC and 36^o/30^oC (day/night regimes).

Temperature (day/night)	RZ CO ₂ (ppm)	<i>A</i> (μmol m ⁻² s ⁻¹) ^z	<i>g_s</i> (mmol H ₂ O m ⁻² s ⁻¹)	Transpiration (mmol H ₂ O m ⁻² s ⁻¹)	RWC (%)
28 ^o /22 ^o C	360	12.1 ± 0.3	Aa 787 ± 27	Da 13.1 ± 0.6	86.6 ± 0.4
	2000	14.1 ± 0.3 (117%)	Ba 696 ± 22 (88%)	Ea 11.3 ± 0.4 (83%) ^{Ha}	88.3 ± 0.4 (102%) ^{La}
	10,000	16.1 ± 0.4 (133%)	Ca 658 ± 14 (84%)	Fa 9.9 ± 0.4 (76%) ^{Ia}	91.5 ± 0.3 (106%) ^{Ma}
	50,000	16.4 ± 0.5 (135%)	Ca 649 ± 21 (82%)	Fa 9.4 ± 0.4 (72%) ^{Ia}	91.1 ± 0.6 (106%) ^{Ma}
36 ^o /30 ^o C	360	7.7 ± 0.2	Ab 673 ± 31	Db 10.2 ± 0.7	Hb 64.3 ± 0.3
	2000	9.2 ± 0.3 (119%)	Bb 596 ± 30 (89%)	Eb 8.2 ± 0.4 (80%) ^{Ib}	70.4 ± 0.5 (109%) ^{Lb}
	10,000	11.9 ± 0.3 (155%)	Cb 561 ± 22 (83%)	Fb 7.0 ± 0.3 (69%) ^{Jb}	77.6 ± 0.4 (121%) ^{Mb}
	50,000	12.0 ± 0.7 (156%)	Cb 485 ± 18 (72%)	Gb 6.4 ± 0.3 (63%) ^{Kb}	80.7 ± 0.5 (126%) ^{Nb}

^z Means of 5 measurements on same-age leaves (4th from base), 5 plants. Percentages (parentheses) relative to ambient RZ CO₂ at same temperature. Comparisons: A, B etc.= RZ CO₂; a, b etc.= temperature. Means with common letters do not differ: *P* = 0.05.

Figures

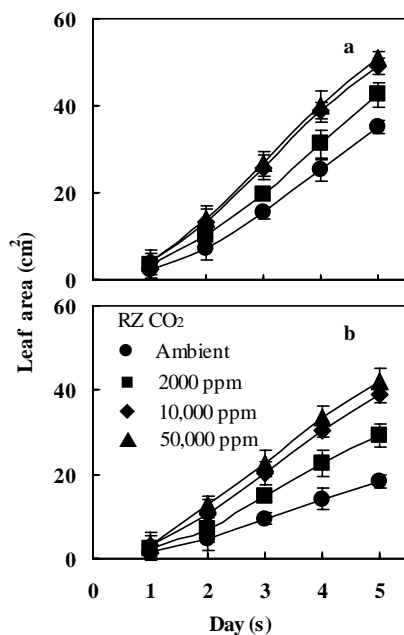


Fig. 1. Lettuce leaf growth at different RZ CO₂ and temperature on 5 consecutive days and 1 wk of RZ CO₂ treatment: a) 25°/22° C; b) 36°/30° C. (Means of leaves of 5 plants. Bars=std err.)

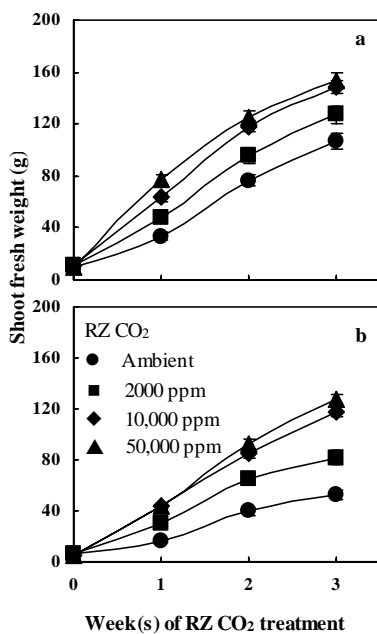


Fig. 2. Lettuce leaf growth at different RZ CO₂ and temperature a) 28°/22° C; b) 36°/30° C. Bars= std err., n=5

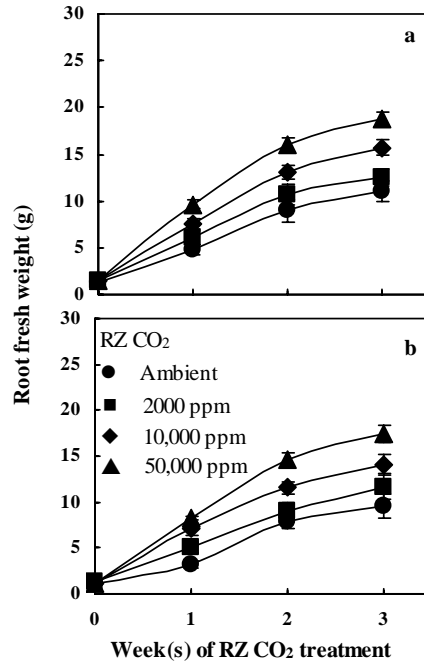


Fig. 3. Lettuce leaf growth at different RZ CO₂ and temperature a) 28°/22°C; b) 36°/30°C. Bars= std err., n=5

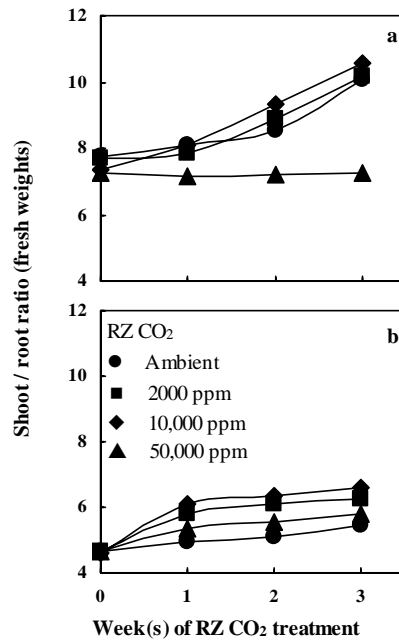


Fig. 4. Lettuce shoot/root ratio for fresh weight (Fig. 2&3) at different RZ CO₂: a) 28°/22° C; b) 36°/30°C.