Salinity Tolerance of Cleopatra Mandarin and Carrizo Citrange Citrus Rootstock Seedlings Is Affected by CO₂ Enrichment during Growth

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ABSTRACT. Three-month-old citrus rootstock seedlings of the Cl⁻ excluder Cleopatra mandarin (*Citrus reticulata* Blanco) and the Cl⁻ accumulator Carrizo citrange [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* L.] were fertilized with nutrient solution with or without additional 50 mM NaCl and grown at either ambient CO₂ (360 μL·L⁻¹) or elevated CO₂ (700 μL·L⁻¹) in similar controlled environment greenhouses for 8 weeks. Elevated CO₂ increased plant growth, shoot/root ratio, leaf dry weight per area, net assimilation of CO₂, chlorophyll, and water-use efficiency but decreased transpiration rate. Elevated CO₂ decreased leaf Ca²⁺ and N concentration in non-salinized Cleopatra. Salinity increased leaf Cl⁻ and Na⁺ in both genotypes. Carrizo had higher concentrations of Cl⁻ but lower Na⁺ in leaves than Cleopatra. Salinity decreased plant growth, shoot/root ratio, net gas exchange, water use, and root Ca²⁺ but increased root N in both genotypes regardless of CO₂ level. Neither salinity nor elevated CO₂ affected leaf chlorophyll fluorescence (Fv/Fm). Carrizo had higher Fv/Fm, leaf gas exchange, chlorophyll, N, and Ca²⁺ than Cleopatra. Salinity-induced decreases in leaf osmotic potential increased leaf turgor especially at elevated CO₂. The increase in leaf growth at elevated CO₂ was greater in salinized than in nonsalinized Carrizo but was similar in Cleopatra seedlings regardless of salt treatment. In addition, salinity decreased water-use efficiency more at elevated CO₂ than at ambient CO₂ in Cleopatra but not in Carrizo. Elevated CO₂ also decreased leaf Cl⁻ and Na⁺ in Carrizo but tended to increase both ions in Cleopatra leaves. Based on leaf growth, water-use efficiency and salt ion accumulation, elevated CO₂ increased salinity tolerance in the relatively salt-sensitive Carrizo more than in the salt-tolerant Cleopatra. In salinized seedlings of both genotypes, Cl⁻ and Na⁺ concentration changes in response to eCO₂ in leaves vs. roots were generally in opposite directions. Thus, the modifications of citrus seedling responses to salinity by the higher growth and lower transpiration at elevated CO₂ were not only species dependent, but also involved whole plant growth and allocations of Na⁺ and Cl⁻.

Based mostly on the accumulation of chlorides in leaves, citrus species are considered salt sensitive (Maas, 1990; Shalhevet and Levy, 1990). There are a variety of mechanisms that contribute to salt tolerance including compartmentation of toxic salt ions in cell vacuoles (Yeo, 1998), accumulation of balancing osmotic ions in the cytoplasm (Hare et al., 1998), the ability to reduce the Cl⁻ and/or Na⁺ uptake by roots (Walker and Douglas, 1983) and the lack of transport of Cl⁻ and/or Na⁺ to the shoot (Bañuls et al., 1990; Chen, 1992; Zekri, 1991). In citrus plants, Cl⁻ influx and accumulation have been shown to be linked to water absorption and transpiration (Castle and Krezdorn, 1975; Syvertsen et al., 1989). Citrus rootstocks differ widely in their tolerance to soil salinity (Levy and Syvertsen, 2004). The relatively chloride-tolerant citrus rootstock Cleopatra mandarin transpires less water per unit leaf area than the chloride-sensitive rootstock Carrizo citrange (Moya et al., 1999, 2003). Since rapidly growing plants usually use more water than slower growing plants, is not clear whether accumulation of Na⁺ and Cl⁻ are related to growth and/or water use.

In many physiological studies, inhibition of plant growth by salinity has been related to a reduction in photosynthesis (Munns,

1993). This reduction could be caused by osmotic stress and/or the accumulation of toxic ions (Walker et al., 1993). Although osmotic stress almost always reduces plant growth, osmotic adjustment can be sufficient to offset the reductions in leaf water potential thereby maintaining or even increasing leaf turgor pressure in salt-treated plants (Bañuls and Primo-Millo, 1992; Lloyd et al., 1987a, 1990). There are good correlations between the reduction in CO₂ assimilation (A_{CO2}) and increased concentration of leaf Cl⁻ (Bañuls and Primo-Millo, 1992; Garcia-Legaz et al., 1993), but others have found such correlations with leaf Na⁺ (Garcia-Legaz et al., 1993; Walker et al., 1993) or with both Cl⁻ and Na⁺ (Garcia-Sanchez et al., 2002; Lloyd et al., 1987b). The relative importance of Cl⁻ and Na⁺ limitations on A_{CO2} may be related to levels of Ca²⁺ in leaves as high Ca²⁺ can mitigate negative effects of Na⁺ (Grattan and Grieve, 1992). Osmotic vs. toxic ion limitations on A_{CO2} and growth as well as NaCl effects on leaf N and Ca²⁺ balances in plants, remain active areas of research in citrus (Levy and Syvertsen, 2004).

High atmospheric CO₂ concentrations stimulate photosynthesis by increasing the rate of carboxylation and decreasing the oxygenation rate of Rubisco (Bowes, 1991; Yelle et al., 1989). In addition, elevated CO₂ (eCO₂) increases water-use efficiency by reducing stomatal conductance and water use while increasing growth and A_{CO2} of leaves (Chen and Lenz, 1997; Drake et al., 1997). Thus, growing plants at eCO₂ offers a mechanism to separate plant growth from water use, which are normally tightly coupled. Even salt-stressed plants generally grow more at elevated CO₂ than at ambient levels of CO₂ (Ball and Munns, 1992; Nicolas et al., 1993). If water use and salt uptake are indeed linked (Moya et al., 2003), then increasing water-use efficiency

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in eCO₂ should result in reduced rates of salt accumulation in the leaves. We tested the hypothesis that salinity tolerance of citrus rootstock seedlings would be increased when grown in eCO₂. We compared responses to soil NaCl of seedlings of Carrizo and Cleopatra in order to yield insights into mechanisms of salinity tolerance in citrus.

Materials and Methods

PLANT CULTURE. Two-month-old seedlings of Cleopatra mandarin and Carrizo citrange were grown individually in 1.5-L containers filled with autoclaved Candler fine sand soil. The plants were watered three times per week with a dilute solution of a complete fertilizer (8N–0.88P–6.64K) plus iron-chelate (6%). The volume of nutrient solution was sufficient to leach from the bottom of all pots to avoid any build up of salts. Seedlings received 21 mg of N per week and were grown in an unshaded greenhouse made of clear double-walled polycarbonate under natural photoperiods. When the plants were 3 months old, half were moved to an identical greenhouse which was supplied with additional CO₂ monitored with an infrared gas analyzer (S-151; Qubit Systems, Kingstons, Ont., Canada). The eCO₂ concentration was maintained at about twice ambient ($700 \pm 20 \mu\text{L}\cdot\text{L}^{-1}$) while the other greenhouse was maintained at ambient CO₂ concentration [$a\text{CO}_2$ ($360 \pm 20 \mu\text{L}\cdot\text{L}^{-1}$)]. Except for atmospheric CO₂ concentrations, the two greenhouses had very similar growth conditions. Maximum photosynthetic active radiation [PAR (LI-170; LI-COR, Lincoln, Nebr.)] measured above the plants was $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, average day/night temperature was $\approx 36/21^\circ\text{C}$ and relative humidity varied daily from 40% to 100%.

The salt treatment was begun at the same time as the supplemental CO₂ treatment by adding an additional 10 mM NaCl in the irrigation water each day to give a final NaCl concentration of 50 mM. Salt and eCO₂ treatments continued for 8 weeks. Plants were randomized within the greenhouses every week to avoid any potential positional effects. The experimental design was a $2 \times 2 \times 2$ factorial of two rootstocks $\times$ two CO₂ treatments (360 and $700 \mu\text{L}\cdot\text{L}^{-1}$) $\times$ two salt treatments (0 and 50 mM NaCl) with eight replicate plants in each treatment.

WATER RELATIONS AND GAS EXCHANGE. About 7 weeks after initiating the salt and CO₂ treatments, gas exchange and water relations measurements were made on fully expanded leaves chosen from the mid-shoot area of each seedling in each treatment. All measurement leaves expanded after the beginning of the treatments. Pre-dawn (0600–0800 HR) leaf water potential (ψ_w) was measured using a Scholander-type pressure chamber (PMS instruments, Corvallis, Ore.; Scholander et al., 1965). Following ψ_w , leaves were immediately wrapped in aluminum foil, frozen by immersing in liquid nitrogen and stored at -18°C . Leaf osmotic potential (ψ_π) was measured on sap pressed from the thawed tissue at $25 \pm 1^\circ\text{C}$ with a vapor pressure osmometer (model 5100B, Wescor, Logan, Utah). Turgor potential (ψ_p) was calculated as the difference between the ψ_π and ψ_w .

Net assimilation of CO₂ (A_{CO_2}), stomatal conductance (g_s), leaf transpiration (E_{tr}), intercellular CO₂ concentration (C_i), ambient CO₂ concentration (C_a), and water-use efficiency ($\text{WUE} = A_{\text{CO}_2}/E_{\text{tr}}$) were determined with a portable photosynthesis system (LI-6200; LI-COR) using a 0.25-L cuvette. The LICOR-6200 was equipped with an external light source (model QB1205LI-670; Quantum Devices, Barneveld, Wis.) to maintain a constant PAR of $\geq 800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during measurements. This PAR is sufficient for sun-acclimated citrus leaves to achieve maximum A_{CO_2} (Syvertsen,

1984). All measurements were made in the morning from 0800 to 1000 HR to avoid high temperature and low humidity in the afternoon. During all measurements, leaf temperature was $32 \pm 2^\circ\text{C}$ and leaf-to-air vapor pressure difference (VPD) was $2.4 \pm 0.4 \text{ kPa}$ within the cuvette. Measurements were made on a single leaf on each of the eight replicate plants in each treatment.

CHLOROPHYLL A FLUORESCENCE MEASUREMENTS. Chlorophyll *a* fluorescence characteristics were measured with a pulse modulated fluorometer (model OS1-FI; Opti-Sciences, Tyngsboro, Mass.) on similar leaves that were used for gas exchange measurements. Fluorescence measurements were made between 0900 and 1000 HR using a single leaf from each of the eight replicate plants per treatment under ambient light and also after 15 min of dark acclimation under leaf clips (FL-DC; Opti-Sciences). The parameters of maximum quantum efficiency (F_v/F_m) of photosystem II were calculated as $F_v/F_m = (F_m - F_o)/F_m$; where F_m and F_o were maximum and minimum fluorescence of dark acclimated leaves, respectively (Jifon and Syvertsen, 2003; Maxwell and Johnson, 2000). Effective quantum yield (Y) was measured as $Y = (F'_m - F')/F'_m$ where F'_m and F' were the maximum and steady-state fluorescence yield in the light, respectively.

CHLOROPHYLL ANALYSIS. After gas exchange and fluorescence measurements, two leaf disks (0.45 cm^2 each) were sampled from the same leaves avoiding major veins. Chlorophyll was eluted from the discs by submerging them in 2 mL of *N,N*-Dimethyl-formamide in the dark for at least 72 h. Absorbances of extract solutions were read at 647 and 664 nm with a Shimadzu UV-vis spectrophotometer (model UV2401PC; Shimadzu, Columbia, Md.) and used to calculate leaf chlorophyll concentrations using equations in Inskeep and Bloom (1985).

PLANT TRANSPIRATION AND LEAF AREA. Two days before ending of the experiment, whole plant transpiration (E_{wp}) was measured by weight loss from each pot during two 6- to 7-h daytime periods on two selected clear days. Daily weight loss per pot was averaged for the 2 d. Pots were covered with plastic bags sealed at the base of the stem to stop soil evaporation. Total leaf area per plant was measured (LI-3000; LI-COR) 2 d later and used to express E_{wp} in units of $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

GROWTH AND LEAF NUTRIENT CONCENTRATION. Eight weeks after initiating the salinity and CO₂ treatments, plants were harvested and separated into leaves, stems, and roots. Tissues were briefly rinsed with deionised water, oven-dried at 60°C for at least 48 h, weighed and ground to a fine powder. Leaf and root tissue samples were extracted with 0.1 N solution of nitric acid and 10% acetic acid. Tissue chloride concentration was measured using a silver ion titration chlorodimeter (HBI Chlorodimeter; Haake Buchler, Saddle Brook, N.J.). Tissue N, Na, and Ca^{+2} concentrations were determined by a commercial laboratory (Waters Agricultural Lab, Camilla, Ga.).

STATISTICAL ANALYSIS. Data were subjected to analysis of variance using two rootstocks $\times$ two CO₂ levels $\times$ two salinity levels as main effects and eight replicate plants per treatment. Treatment means were compared using Duncan's multiple range test at $P < 0.05$ using SPSS statistical package (SPSS, Chicago).

Results

GROWTH. Leaf dry weight and total plant dry weight (TPDW) of Cleopatra (Cleo) were higher than that of Carrizo (Carr; Table 1). After 8 weeks in elevated CO₂, TPDW was increased by increasing leaf dry weight and LDW/a of both genotypes. Salinity reduced leaf dry weights and TPDW of both genotypes. Despite

Table 1. Effects of CO₂ concentration and soil NaCl (0 or 50 mM NaCl) on mean (n = 8) dry weight of leaves, roots, total plant dry weight (TPDW), leaf dry weight per area (LDW/a), and shoot-to-root ratio (S/R) of Cleopatra mandarin and Carrizo citrange seedlings.

Rootstock	CO ₂ (μL·L ⁻¹)	Salt	Dry wt leaves (g)	Dry wt root (g)	TPDW (g)	LDW/a (g·m ⁻²)	S/R
Cleopatra	360	No salt	4.22 b ^z	2.87 abc	11.68 b	93.3 bc	3.33 ab
		Salt	2.80 c	2.19 c	8.28 cd	87.7 c	2.78 bc
	700	No salt	6.12 a	3.62 a	16.33 a	105.7 a	3.62 a
		Salt	3.57 b	2.87 abc	10.54 bc	93.4 bc	2.75 bc
Carrizo	360	No salt	1.66 d	3.22 ab	9.01 c	80.8 d	1.80 d
		Salt	0.92 e	2.42 bc	5.99 d	77.3 d	1.74 d
	700	No salt	2.38 c	3.83 a	12.90 b	91.6 bc	2.44 c
		Salt	1.66 d	2.61 bc	8.94 c	95.4 b	2.45 c
<i>Analysis of variance</i>							
Rootstock			***	NS	***	***	***
CO ₂			***	*	***	***	**
Salinity			***	***	***	*	*
Rootstock × CO ₂			NS	NS	NS	NS	NS
CO ₂ × salinity			NS	NS	NS	NS	NS
Rootstock × CO ₂ × salinity			NS	NS	NS	*	NS

^zWithin each column, means followed by the same letters are not significantly different at 5%.

NS, *, **, ***Nonsignificant differences or significant differences at $P < 0.05$, 0.01, or 0.001, respectively.

the salinity reduced leaf dry weights, salinized plants of both genotypes grown at eCO₂ had leaf dry weights similar to those of nonsalinized control plants at aCO₂. The relative increase in leaf dry weight in eCO₂ was significantly greater in salinized Carr (from 0.92 to 1.66 g = 80%) than in nonsalinized Carr (1.66 to 2.38 g = 43%). The analogous eCO₂-induced increases in Cleo seedlings varied from 27% to 45% and did not differ between salt treatments. Nonsalinized plants at eCO₂ had higher root dry weight than salinized plants at aCO₂ for both genotypes. Root dry weight was only reduced by salinity at eCO₂ in Carr. The shoot-to-root dry weight ratio (S/R) of Carr was significantly higher when grown at eCO₂ than at aCO₂ for both salinity treatments but not in Cleo.

LEAF CL⁻ AND NA⁺ CONCENTRATION. Salinity increased the concentrations of Cl⁻ and Na⁺ in leaves and roots of both genotypes (Fig. 1). The Cl⁻ concentration in leaves and roots was higher for Carr than for Cleo regardless of CO₂ level. The concentration of Na⁺ was higher in leaves of Cleo than in Carr but roots of both genotypes had similar Na⁺ concentrations. Elevated CO₂ decreased Cl⁻ and Na⁺ concentrations in leaves of salinized Carr and also decreased both Cl⁻ and Na⁺ in roots of salinized Cleo. In salinized seedlings of both genotypes, Cl⁻ and Na⁺ concentration changes in response to eCO₂ in leaves vs. roots were generally in opposite directions. Visible Cl⁻ toxicity symptoms such as leaf tip burn were observed on a few leaves of both Cleo and Carr but there was no leaf abscission during the period of this study.

LEAF CA²⁺ AND TOTAL-N CONCENTRATION. Leaf Ca²⁺ and N concentrations were higher in Carr than in Cleo (Fig. 2). Elevated CO₂ significantly decreased Ca²⁺ and N and in the nonsalinized Cleo leaves. Salinity decreased leaf Ca²⁺ and increased leaf N in Cleo grown at aCO₂. However, salinity did not affect leaf N and Ca²⁺ at eCO₂. Salinity decreased leaf Ca²⁺ in Carr at eCO₂, but there were no significant effects on leaf N attributable to salinity or CO₂ treatment. Salinity decreased root Ca²⁺ and increased root N in both genotypes regardless of CO₂ level. Root Ca²⁺ and N concentrations were not affected by eCO₂.

LEAF WATER RELATIONS. Elevated CO₂ did not affect ψ_w , ψ_π , and ψ_p in Cleo leaves regardless of salinity treatment (Table 2).

In nonsalinized Carr leaves, however, eCO₂ reduced the ψ_w and ψ_p , and increased ψ_π with respect to aCO₂. Salinity decreased ψ_w and ψ_π such that ψ_p was increased in both Cleo and Carr leaves. In salinized Carr, ψ_w was lower for plants grown at eCO₂ than at aCO₂ but ψ_p was not reduced significantly.

LEAF GAS EXCHANGE. Net A_{CO₂} and g_s of Carr leaves was generally greater than that of Cleo regardless of treatment (Table 3). Under nonsaline conditions, eCO₂ increased A_{CO₂} and WUE but decreased g_s in both genotypes. Salinity reduced net gas exchange in both genotypes. Salinized Carr and Cleo plants grown at eCO₂ had higher or similar values of A_{CO₂} than their respective nonsalinized plants grown at aCO₂. There was a significant interaction effect between eCO₂ and salinity on g_s in both genotypes as stomatal conductance was reduced by salinity at aCO₂, but the lowered g_s at eCO₂ were unaffected by salinity. Overall, salinity decreased WUE regardless of CO₂ treatment. There was also a significant CO₂ $\times$ salinity interaction effect on C_i/C_a in Cleo leaves as salinity decreased C_i/C_a at aCO₂ but increased C_i/C_a at eCO₂. Although trends in C_i/C_a were similar in Carr, there were no significant effects attributable to salinity or CO₂ treatments.

CHLOROPHYLL FLUORESCENCE AND LEAF CHLOROPHYLL CONTENT. Fv/Fm, Y, and leaf chlorophyll concentration were higher in Carr than in Cleo leaves (Table 4). There were no significant differences in Fv/Fm attributed to eCO₂ or salinity. Quantum yields (Y) generally increased at eCO₂, but differences were only significant in nonsalinized Cleo. There was a significant CO₂ and salinity interaction effect on total chlorophyll concentration as salinity decreased total chlorophyll at eCO₂ but had no effect at aCO₂.

TRANSPIRATION. Carr leaves had a higher E_{wp} than Cleo leaves (Fig. 3). Salinity and eCO₂ reduced E_{wp} in both genotypes except in Carr plants at eCO₂ where the already lowered E_{wp} was not decreased additionally by the salt treatment.

Discussion

Although salt-stress generally reduces nutrient uptake, leaf growth and the rate of A_{CO₂}, the A_{CO₂} of salinized plants grown at eCO₂ was either equal to or higher than that of nonsalinized

Fig. 1. Effects of soil NaCl (-S = 0 mM, +S = 50 mM) and growing at ambient CO₂ (aCO₂ = 360 μL·L⁻¹) or at elevated CO₂ (eCO₂ = 700 μL·L⁻¹) on mean (n = 8) Cl⁻ and Na⁺ concentration (mg·g⁻¹ dry weight) in leaf and root tissue of Cleopatra mandarin and Carrizo citrange seedlings. Different letters within each figure indicate significant differences at $P < 0.05$ (Duncan's test). NS, *, **, ***Nonsignificant differences or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the three-way interaction of two rootstocks × two salt treatments × two CO₂ levels (R × C × S) and for the rootstock effect.

Fig. 2. Effects of soil NaCl (-S = 0 mM, +S = 50 mM) and growing at ambient CO₂ (aCO₂ = 360 μL·L⁻¹) or at elevated CO₂ (eCO₂ = 700 μL·L⁻¹) on mean (n = 8) Ca²⁺ and N concentration (mg·g⁻¹ dry weight) in leaf and root tissue of Cleopatra mandarin and Carrizo citrange seedlings. Different letters within each figure indicate significant differences at $P < 0.05$ (Duncan's test). NS, *, **, ***Nonsignificant differences or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the three-way interaction of two rootstocks × two salt treatments × two CO₂ levels (R × C × S) and for the rootstock effect.

Table 2. Effects of CO₂ concentration and NaCl (0 or 50 mM NaCl) on mean (n = 8) leaf water potential (ψ_w), leaf osmotic potential (ψ_π), and leaf turgor potential (ψ_p) of Cleopatra mandarin and Carrizo citrange leaves.

Rootstock	CO ₂ ($\mu\text{L}\cdot\text{L}^{-1}$)	Salt	ψ_w (MPa)	ψ_π (Mpa)	ψ_p (Mpa)
Cleopatra	360	No salt	-0.34 a ^z	-2.54 ab	2.19 de
		Salt	-0.46 bc	-3.00 c	2.55 ab
	700	No salt	-0.35 a	-2.34 a	1.99 ef
		Salt	-0.52 c	-3.23 c	2.71 a
Carrizo	360	No salt	-0.42 ab	-2.67 b	2.25 cd
		Salt	-0.69 d	-3.15 c	2.47 abc
	700	No salt	-0.51 c	-2.32 a	1.81 f
		Salt	-0.91 e	-3.21 c	2.30 bcd

Analysis of variance

Rootstock	***	NS	*
CO ₂	***	NS	*
Salinity	***	***	***
Rootstock $\times$ CO ₂	**	NS	*
Rootstock $\times$ salinity	***	NS	NS
CO ₂ $\times$ salinity	*	***	*
Rootstock $\times$ CO ₂ $\times$ salinity	NS	NS	NS

^zWithin each column, means followed by the same letters are not significantly different at 5%.

NS, *, **, ***Nonsignificant differences or significant differences at $P < 0.05$, 0.01, or 0.001, respectively.

Table 3. Effects of CO₂ concentration and soil NaCl (0 or 50 mM NaCl) on mean (n = 8) net CO₂ assimilation rate (A_{CO_2}), stomatal conductance (g_s), water-use efficiency (WUE), and CO₂ internal/external [Ci/Ca (dimensionless)] of Cleopatra mandarin and Carrizo citrange leaves.

Rootstock	CO ₂ ($\mu\text{L}\cdot\text{L}^{-1}$)	Salt	A_{CO_2} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	g_s ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	WUE ($\mu\text{mol}\cdot\text{mmol}^{-1}$)	Ci/Ca
Cleopatra	360	No salt	12.02 d ^z	0.35 bc	2.10	0.82 ab
		Salt	8.93 e	0.18 f	1.95	0.74 c
	700	No salt	19.97 b	0.28 de	3.57	0.79 b
		Salt	12.30 d	0.23 ef	2.69	0.84 a
Carrizo	360	No salt	15.68 c	0.50 a	2.48	0.82 ab
		Salt	9.91 de	0.26 de	1.82	0.80 a
	700	No salt	26.80 a	0.40 b	4.14	0.80 a
		Salt	20.07 b	0.33 bc	3.63	0.82 ab

Analysis of variance

Rootstock	***	***	***	NS
CO ₂	***	NS	***	**
Salinity	***	***	***	NS
Rootstock $\times$ CO ₂	***	NS	**	*
Rootstock $\times$ salinity	NS	NS	NS	NS
CO ₂ $\times$ salinity	*	***	NS	***
Rootstock $\times$ CO ₂ $\times$ salinity	NS	NS	*	**

^zWithin each column, means followed by the same letters are not significantly different at 5%.

NS, *, **, ***Nonsignificant differences or significant differences at $P < 0.05$, 0.01, or 0.001, respectively.

plants at aCO₂. In addition, salinized plants at eCO₂ had similar leaf growth and TPDW as nonsalinized plants at aCO₂. Thus, eCO₂ mitigated both the A_{CO_2} and growth reduction response to salinity in both Cleo and Carr seedlings. In Cleo, the increase of leaf growth in response to eCO₂ was similar regardless of salt treatment whereas in Carr, the leaf growth increase was higher for the salinized than in nonsalinized seedlings (Table 1). Elevated CO₂ also reduced leaf Cl⁻ and Na⁺ concentration in Carr leaves allowing photosynthetic rate to double at eCO₂ compared to salinized plants at aCO₂. Thus, based on leaf growth, net gas exchange and salt ion concentration, eCO₂ increased salinity tolerance in the relative salt sensitive Carr which supports our

original hypothesis. In contrast, leaf Cl⁻ and Na⁺ concentrations in the more salt tolerant Cleo were not affected by eCO₂ and eCO₂ increased A_{CO_2} by only ≈ 1.3 fold.

Elevated CO₂ apparently increased the sensitivity of leaf chlorophyll to salinity but this was not related to the measured fluorescence parameters or ACO₂ where there were no significant interacting effects between CO₂ and salinity. Salinity stress tended to have similar effects on leaf water relations, fluorescence parameters, and net gas exchange characteristics regardless of the CO₂ level. The decrease in ACO₂ by salinity was probably due to Na⁺ and/or Cl⁻ ion accumulation as changes in leaf turgor were not responsible for the decline in A_{CO_2} assimilation (also

Table 4. Effects of CO₂ concentration and soil NaCl (0 or 50 mM NaCl) on mean (n = 8) fluorescence of maximum quantum yield of dark acclimated leaves (Fv/Fm), effective quantum yield of light acclimated leaves (Y) and total chlorophyll concentration (g·cm⁻²) of Cleopatra mandarin and Carrizo citrange leaves.

Rootstock	CO ₂ (μL·L ⁻¹)	Salt	Fv/Fm	Y	Total chlorophyll
Cleopatra	360	No salt	0.713 bcd ^z	0.38 c	0.307 d
		Salt	0.705 bcd	0.42 c	0.315 d
	700	No salt	0.674 d	0.51 ab	0.425 c
		Salt	0.693 cd	0.44 bc	0.274 d
Carrizo	360	No salt	0.783 a	0.56 a	0.513 b
		Salt	0.748 abc	0.51 ab	0.475 bc
	700	No salt	0.775 a	0.58 a	0.585 a
		Salt	0.760 ab	0.53 a	0.485 b

Analysis of variance

Rootstock	***	***	***
CO ₂	NS	*	**
Salinity	NS	NS	***
Rootstock × CO ₂	NS	NS	NS
Rootstock × salinity	NS	NS	NS
CO ₂ × salinity	NS	NS	***
Rootstock × CO ₂ × salinity	NS	NS	NS

^zWithin each column, means followed by the same letters are not significantly different at 5%.

NS, *, **, ***Nonsignificant differences or significant differences at *P* < 0.05, 0.01, or 0.001, respectively.

Fig. 3. Effects of soil NaCl (-S = 0 mM, +S = 50 mM) and growing at ambient CO₂ (aCO₂ = 360 μL·L⁻¹) or at elevated CO₂ (eCO₂ = 700 μL·L⁻¹) on mean (n = 8) whole plant transpiration (E_{wp}) of Cleopatra mandarin and Carrizo citrange seedlings. Different letters within each figure indicate significant differences at *P* < 0.05 (Duncan's test). NS, *, **, ***Nonsignificant differences or significant differences at *P* < 0.05, 0.01 or 0.001, respectively, for the three-way interaction of two rootstocks × two salt treatments × two CO₂ levels (R × C × S) and for the rootstock effect.

see Bañuls and Primo-Millo, 1992). The absence of a significant decrease in fluorescence parameters (Fv/Fm and Y) also negated the possibility of photoinhibition as a cause of reduced photosynthetic rates. Lloyd et al. (1987b) also observed an insensitivity of fluorescence to high foliar salt levels in citrus leaves when turgor was maintained.

Although A_{CO2} and g_s were decreased by salinity in both genotypes at aCO₂, C_i/C_a also decreased in salinized Cleo at aCO₂ but not in Carr. Thus, g_s could have limited A_{CO2} (Farquhar and Sharkey, 1982) in salinized Cleo leaves where low g_s may have been related to high leaf Na⁺. In salinized Carr leaves with higher

g_s and leaf Cl⁻ than Cleo leaves, however, C_i/C_a did not decrease with decreasing A_{CO2}. Thus, g_s was not as important in limiting A_{CO2} as internal limitations (biochemical) in the mesophyll of salinized Carr. At eCO₂, A_{CO2} and g_s both decreased as result of salt stress in both genotypes, but the g_s limitation was removed by eCO₂ and C_i/C_a increased. This underscored the fact that at eCO₂, internal limitations on A_{CO2} from salinity stress were more important than the stomatal limitations on A_{CO2}.

Elevated CO₂ decreased leaf Cl⁻ and Na⁺ concentration in Carr but not in Cleo. This may have been attributable to decreased Ewp in Carr or to the increased S/R ratio and LDW/area. In previous studies, increasing shoot-to-root ratio by allowing part of the root system to dry out, decreased the amount of Cl⁻ transported from roots to shoots in both Cleo and Carr under salinity stress (Moya et al., 2003). This supported their assumption that water and salt uptake were coupled. In our study, the lower leaf Na⁺ and Cl⁻ concentrations in salinized Carr at eCO₂ than at aCO₂, were also related to the lower leaf transpiration rate. If transpiration is not essential for ion transport (Tanner and Beevers, 1990), however, it is unlikely that lower transpiration rates at eCO₂ were solely responsible for the reduced ion uptake. The increased S/R ratio for Carr plants could have resulted in a dilution of leaf Cl⁻ and Na⁺ concentrations relative to plants with low S/R ratio. Our results were similar to those in tomato plants grown at eCO₂, where limitations of root temperature and photon flux density interacted with salinity (Maggio et al., 2002). In all cases, the plant environment that produced the highest tolerance to root zone salinity also produced the highest S/R. In addition, growth in eCO₂ increased LDW/a (also see Syvertsen et al., 2000) such that Cl⁻ and Na⁺ concentrations also could have been decreased by dilution in the higher LDW/area.

In salinized Cleo, leaf Cl⁻, and Na⁺ concentrations were similar in both CO₂ treatments despite the lower leaf transpiration at eCO₂. This may be related the opposite patterns of Cl⁻ and Na⁺ accumulation in roots relative to their respective concentrations in leaves. In Carr, there were no significant differences in root

Cl⁻ and Na⁺ between aCO₂ and eCO₂ but in Cleo, root Cl⁻ and Na⁺ were lower in eCO₂ than in aCO₂. There is an upper limit to the extent of Cl⁻ loading in citrus roots (Cámara-Zapata et al., 2004; Walker and Douglas, 1983). This limit can be reached as soon as several days after commencing the salt treatment (Fernandez-Ballester et al., 2003). In our experiment, the decrease in Cl⁻ and Na⁺ in roots of Cleo at eCO₂ suggested that roots could lose their ability to regulate Cl⁻ and Na⁺ uptake even in the relatively Cl⁻-tolerant Cleo. Although leaf transpiration was lower at eCO₂, the Cl⁻ and Na⁺ concentration in the xylem sap could have become more concentrated thereby achieving a similar balance of leaf Cl⁻ and Na⁺ concentration at both CO₂ levels.

In nonsalinized Cleo plants, leaf N and Ca²⁺ concentrations also could have been reduced by a growth dilution effect eCO₂. A similar decrease in leaf N occurred in both calomondin (*Citrus madurensis* Loureiro; Keutgen and Chen, 2001) and wheat (*Triticum aestivum* L.; Li et al., 2003) grown at eCO₂ for 2 months. Elevated CO₂ also can inhibit the assimilation of NO₃⁻ in shoots of C₃ plants because this assimilation is strongly dependent on photorespiration which can be inhibited by eCO₂ (Rachmilevitch et al., 2004). Of all the macronutrients (N, P, K, Ca, Mg) studied in calamondin grown at eCO₂ (Keutgen and Chen, 2001), only leaf N was decreased significantly by elevated CO₂. In our experiment, a dilution of N due to enhanced plant growth at eCO₂ cannot be ruled out because leaf Ca²⁺ was also reduced.

Under saline conditions, competition between Cl⁻ and NO₃⁻ N uptake by roots may occur, which can decrease the N concentration in citrus leaves (Lea-Cox and Syvertsen, 1993). In our experiment, however, the increase in leaf N concentration of Cleo at aCO₂ could have been due to a greater inhibition of shoot growth than of NO₃⁻ uptake by salinity. This is supported by the fact that salinity increased N concentrations in roots of both genotypes regardless of the CO₂ level. Cleo leaves had lower Ca²⁺ than Carr and salinity reduced Ca²⁺ further in Cleo at aCO₂. This low Ca²⁺ could have been related to the high accumulation of leaf Na⁺ in Cleo (Cámara et al., 2003). The concentrations of Ca²⁺ in roots were reduced by salinity in both genotypes regardless of the CO₂ level. Thus, Ca²⁺ uptake was reduced by the NaCl treatment. Similar findings have been reported for salinized Cleo (Cámara et al., 2004; García-Sánchez et al., 2002; Syvertsen and Yelenosky, 1988) as high Na⁺ in the soil solution can displace Ca²⁺.

In conclusion, NaCl salinity in the soil reduced plant growth of these citrus rootstock seedlings at both elevated and ambient levels of atmospheric CO₂ concentrations. Elevated CO₂ increased leaf growth in both nonsalinized plants and salinized plants. The increase in leaf dry weight at eCO₂ in Carr was higher for salinized plants than for nonsalinized plants. In Cleo, however, this eCO₂-induced increase was similar at both salt levels. In Carr, eCO₂ also reduced leaf Na⁺ and Cl⁻ concentration and leaf transpiration rates while increasing shoot-to-root ratio. Similar effects of eCO₂ on S/R, leaf Na⁺ and Cl⁻ did not occur Cleo despite similar decreases in whole plant transpiration. Thus, eCO₂ increased the salt tolerance of the relatively salt sensitive Carr more than that of in the more salt tolerant Cleo. In salinized seedlings of both genotypes, Cl⁻ and Na⁺ concentration changes in response to eCO₂ in leaves vs. roots were generally in opposite directions. Thus, the modifications of citrus seedlings responses to salinity by the higher growth and lower water use at eCO₂, not only depended on species specific salinity tolerance but also involved whole plant growth and allocation of Na⁺ and Cl⁻.

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