J. Amer. Soc. Hort. Sci. 131(1):24–31. 2006.

Salinity Tolerance of Cleopatra Mandarin and Carrizo Citrange Citrus Rootstock Seedlings Is Affected by CO_2 Enrichment during Growth

Francisco García-Sánchez[1] and J.P. Syvertsen[2]
University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

Additional index words. Ca^{2+}, Cl^-, CO_2 assimilation, leaf dry weight per area, N, Na^+, shoot/root ratio, water relations

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. x *Poncirus trifoliata* L.] were fertilized with nutrient solution with or without additional 50 mM NaCl and grown at either ambient CO_2 (360 $\mu L \cdot L^{-1}$) or elevated CO_2 (700 $\mu L \cdot L^{-1}$) in similar controlled environment greenhouses for 8 weeks. Elevated CO_2 increased plant growth, shoot/root ratio, leaf dry weight per area, net assimilation of CO_2, chlorophyll, and water-use efficiency but decreased transpiration rate. Elevated CO_2 decreased leaf Ca^{2+} 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 Ca^{+2} but increased root N in both genotypes regardless of CO_2 level. Neither salinity nor elevated CO_2 affected leaf chlorophyll fluorescence (Fv/Fm). Carrizo had higher Fv/Fm, leaf gas exchange, chlorophyll, N, and Ca^{2+} than Cleopatra. Salinity-induced decreases in leaf osmotic potential increased leaf turgor especially at elevated CO_2. The increase in leaf growth at elevated CO_2 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 CO_2 than at ambient CO_2 in Cleopatra but not in Carrizo. Elevated CO_2 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 CO_2 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 eCO_2 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 CO_2 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 CO_2 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 Ca^{+2} in leaves as high Ca^{+2} 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 Ca^{+2} balances in plants, remain active areas of research in citrus (Levy and Syvertsen, 2004).

High atmospheric CO_2 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 CO_2 (eCO_2) 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 eCO_2 offers a mechanism to separate plant growth from water use, which are normally tightly coupled. Even salt-stressed plants generally grow more at elevated CO_2 than at ambient levels of CO_2 (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

Received for publication 11 Apr. 2005. Accepted for publication 29 June 2005. We gratefully acknowledge Jill Dunlop and Eva Ros for skilled technical assistance. This research was supported by a fellowship from the Ministerio de Educación, Cultura y Deportes of Spain (AGL2003-08502-CO4-02/AGR) and the Florida Agricultural Experiment Station. Approved for publication as Journal Series No. R-10825.

[1]Present address: Centro de Edafologia y Biologia Aplicada del Segura. Consejo Superior de Investigaciones Cientificas. Campus Universitario de Espinardo. Espinardo. 30100. Murcia. Spain.
[2]Corresponding author, Email: jmsn@crec.ifas.ufl.edu

in eCO_2 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 eCO_2. 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 CO_2 monitored with an infrared gas analyzer (S-151; Qubit Systems, Kingtons, Ont., Canada). The eCO_2 concentration was maintained at about twice ambient (700 ± 20 $\mu L \cdot L^{-1}$) while the other greenhouse was maintained at ambient CO_2 concentration [aCO_2 (360 ± 20 $\mu L \cdot L^{-1}$)]. Except for atmospheric CO_2 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, average day/night temperature was ≈36/21 °C and relative humidity varied daily from 40% to 100%.

The salt treatment was begun at the same time as the supplemental CO_2 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 eCO_2 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 × 2 × 2 factorial of two rootstocks × two CO_2 treatments (360 and 700 $\mu L \cdot L^{-1}$) × 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 CO_2 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 ºC. Leaf osmotic potential (ψ_π) was measured on sap pressed from the thawed tissue at 25 ± 1 ºC 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 CO_2 (A_{CO2}), stomatal conductance (g_s), leaf transpiration (E_{lf}), intercellular CO_2 concentration (C_i), ambient CO_2 concentration (C_a), and water-use efficiency ($WUE = A_{CO2}/E_{lf}$) 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 ≥800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during measurements. This *PAR* is sufficient for sun-acclimated citrus leaves to achieve maximum A_{CO2} (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 ± 2 ºC and leaf-to-air vapor pressure difference (VPD) was 2.4 ± 0.4 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-Fl; 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 (Fv/Fm) of photosystem II were calculated as Fv/Fm = (Fm-Fo)/Fm; where Fm and Fo 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*-Dimethylformamide 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 $mmol \cdot m^{-2} \cdot s^{-1}$.

Growth and leaf nutrient concentration. Eight weeks after initiating the salinity and CO_2 treatments, plants were harvested and separated into leaves, stems, and roots. Tissues were briefly rinsed with deionised water, oven-dried at 60 ºC 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 × two CO_2 levels × 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 CO_2, 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 CO_2 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 ($\mu L \cdot L^{-1}$)	Salt	Dry wt leaves (g)	Dry wt root (g)	TPDW (g)	LDW/a ($g \cdot m^{-2}$)	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
			Analysis of variance				
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

[z]Within 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 eCO_2 had leaf dry weighs similar to those of nonsalinized control plants at aCO_2. The relative increase in leaf dry weight in eCO_2 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 eCO_2-induced increases in Cleo seedlings varied from 27% to 45% and did not differ between salt treatments. Nonsalinized plants at eCO_2 had higher root dry weight than salinized plants at aCO_2 for both genotypes. Root dry weight was only reduced by salinity at eCO_2 in Carr. The shoot-to-root dry weight ratio (S/R) of Carr was significantly higher when grown at eCO_2 than at aCO_2 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 CO_2 level. The concentration of Na^+ was higher in leaves of Cleo than in Carr but roots of both genotypes had similar Na^+ concentrations. Elevated CO_2 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 eCO_2 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 Ca^{+2} and total-N concentration. Leaf Ca^{+2} and N concentrations were higher in Carr than in Cleo (Fig. 2). Elevated CO_2 significantly decreased Ca^{2+} and N and in the nonsalinized Cleo leaves. Salinity decreased leaf Ca^{+2} and increased leaf N in Cleo grown at aCO_2. However, salinity did not affect leaf N and Ca^{2+} at eCO_2. Salinity decreased leaf Ca^{+2} in Carr at eCO_2, but there were no significant effects on leaf N attributable to salinity or CO_2 treatment. Salinity decreased root Ca^{2+} and increased root N in both genotypes regardless of CO_2 level. Root Ca^{+2} and N concentrations were not affected by eCO_2.

Leaf water relations. Elevated CO_2 did not affect ψ_w, ψ_π, and ψ_p in Cleo leaves regardless of salinity treatment (Table 2). In nonsalinized Carr leaves, however, eCO_2 reduced the ψ_w and ψ_p, and increased ψ_π with respect to aCO_2. 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 eCO_2 than at aCO_2 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, eCO_2 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 eCO_2 had higher or similar values of A_{CO2} than their respective nonsalinized plants grown at aCO_2. There was a significant interaction effect between eCO_2 and salinity on g_s in both genotypes as stomatal conductance was reduced by salinity at aCO_2, but the lowered g_s at eCO_2 were unaffected by salinity. Overall, salinity decreased WUE regardless of CO_2 treatment. There was also a significant CO_2 × salinity interaction effect on C_i/C_a in Cleo leaves as salinity decreased C_i/C_a at aCO_2 but increased C_i/C_a at eCO_2. Although trends in C_i/C_a were similar in Carr, there were no significant effects attributable to salinity or CO_2 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 eCO_2 or salinity. Quantum yields (Y) generally increased at eCO_2, but differences were only significant in nonsalinized Cleo. There was a significant CO_2 and salinity interaction effect on total chlorophyll concentration as salinity decreased total chlorophyll at eCO_2 but had no effect at aCO_2.

Transpiration. Carr leaves had a higher E_{wp} than Cleo leaves (Fig. 3). Salinity and eCO_2 reduced E_{wp} in both genotypes except in Carr plants at eCO_2 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 eCO_2 was either equal to or higher than that of nonsalinized

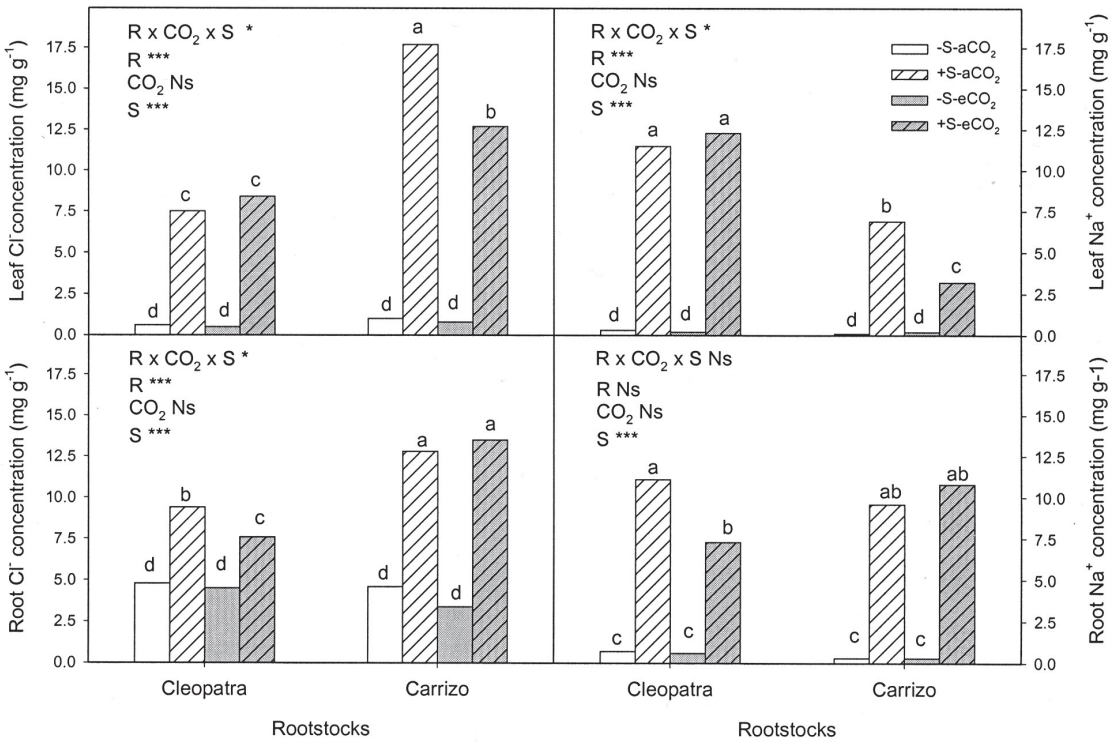

Fig. 1. Effects of soil NaCl (-S = 0 mM, +S = 50 mM) and growing at ambient CO_2 (aCO_2 = 360 $\mu L \cdot L^{-1}$) or at elevated CO_2 (eCO_2 = 700 $\mu L \cdot L^{-1}$) on mean (n = 8) Cl^- and Na^+ concentration ($mg \cdot g^{-1}$ dry weight) in leaf and root tissue of Cleopatra mandarin and Carrizo 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 CO_2 levels (R × C × S) and for the rootstock effect.

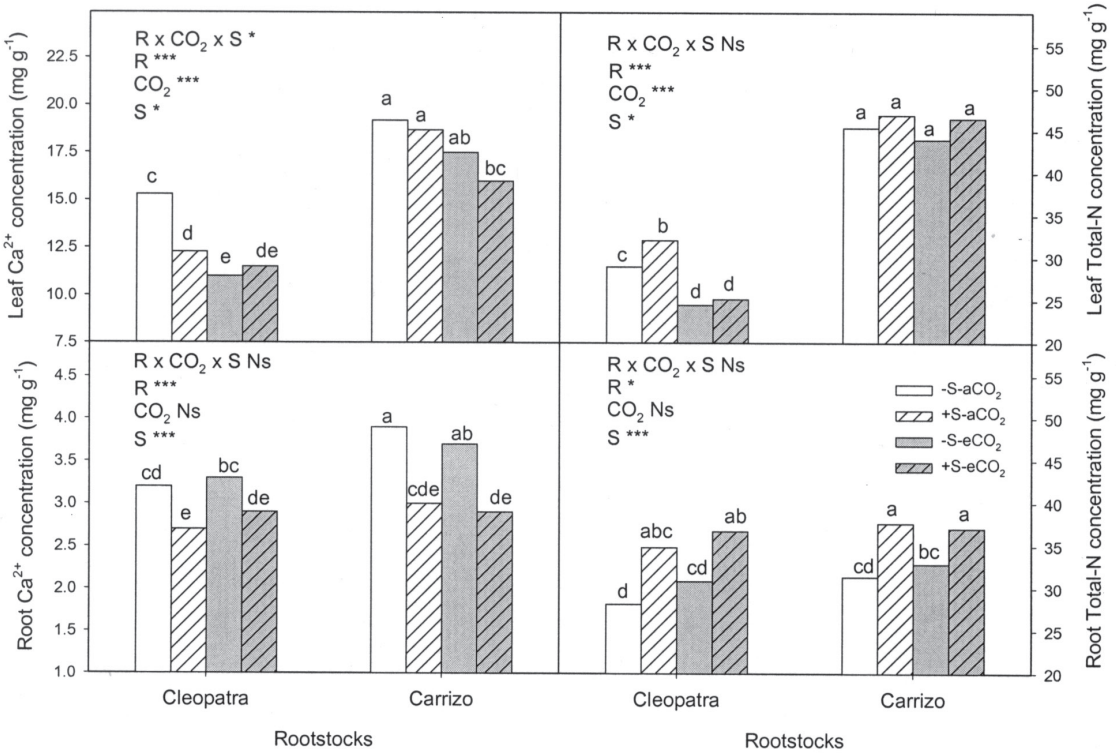

Fig. 2. Effects of soil NaCl (-S = 0 mM, +S = 50 mM) and growing at ambient CO_2 (aCO_2 = 360 $\mu L \cdot L^{-1}$) or at elevated CO_2 (eCO_2 = 700 $\mu L \cdot L^{-1}$) on mean (n = 8) Ca^{2+} and N concentration ($mg \cdot g^{-1}$ dry weight) in leaf and root tissue of Cleopatra mandarin and Carrizo 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 CO_2 levels (R × C × S) and for the rootstock effect.

Table 2. Effects of CO_2 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 ($\mu L \cdot L^{-1}$)	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 × CO_2			**	NS	*
Rootstock × salinity			***	NS	NS
CO_2 × salinity			*	***	*
Rootstock × CO_2 × salinity			NS	NS	NS

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

Rootstock	CO_2 ($\mu L \cdot L^{-1}$)	Salt	A_{CO2} ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	g_s ($mol \cdot m^{-2} \cdot s^{-1}$)	WUE ($\mu mol \cdot mmol^{-1}$)	C_i/C_a
Cleopatra	360	No salt	12.02 d[z]	0.35 bc	2[illegible].10	0.82 ab
		Salt	8.93 e	0.18 f	1[illegible].95	0.74 c
	700	No salt	19.97 b	0.28 de	3[illegible].57	0.79 b
		Salt	12.30 d	0.23 ef	2[illegible].69	0.84 a
Carrizo	360	No salt	15.68 c	0.50 a	2[illegible].48	0.82 ab
		Salt	9.91 de	0.26 de	1[illegible].82	0.80 a
	700	No salt	26.80 a	0.40 b	4[illegible].14	0.80 a
		Salt	20.07 b	0.33 bc	3[illegible].63	0.82 ab
Analysis of variance						
Rootstock			***	***	***	NS
CO_2			***	NS	***	**
Salinity			***	***	***	NS
Rootstock × CO_2			***	NS	**	*
Rootstock × salinity			NS	NS	NS	NS
CO_2 × salinity			*	***	NS	***
Rootstock × CO_2 × salinity			NS	NS	*	**

[z]Within 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 aCO_2. In addition, salinized plants at eCO_2 had similar leaf growth and TPDW as nonsalinized plants at aCO_2. Thus, eCO_2 mitigated both the A_{CO2} and growth reduction response to salinity in both Cleo and Carr seedlings. In Cleo, the increase of leaf growth in response to eCO_2 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 CO_2 also reduced leaf Cl^- and Na^+ concentration in Carr leaves allowing photosynthetic rate to double at eCO_2 compared to salinized plants at aCO_2. Thus, based on leaf growth, net gas exchange and salt ion concentration, eCO_2 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 eCO_2 and eCO_2 increased A_{CO2} by only ≈1.3 fold.

Elevated CO_2 apparently increased the sensitivity of leaf chlorophyll to salinity but this was not related to the measured fluorescence parameters or ACO_2 where there were no significant interacting effects between CO_2 and salinity. Salinity stress tended to have similar effects on leaf water relations, fluorescence parameters, and net gas exchange characteristics regardless of the CO_2 level. The decrease in ACO_2 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_{CO2} assimilation (also

Table 4. Effects of CO_2 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 ($g \cdot cm^{-2}$) of Cleopatra mandarin and Carrizo citrange leaves.

Rootstock	CO_2 ($\mu L \cdot L^{-1}$)	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

[z]Within 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.

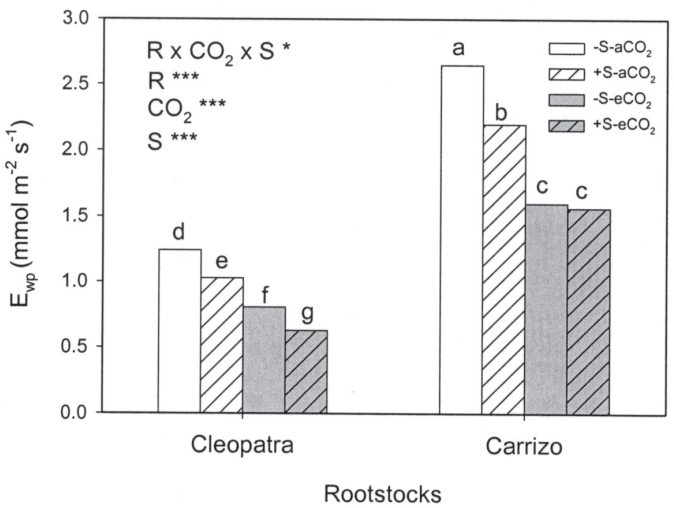

Fig. 3. Effects of soil NaCl (-S = 0 mM, +S = 50 mM) and growing at ambient CO_2 (aCO_2= 360 $\mu L \cdot L^{-1}$) or at elevated CO_2 (eCO_2 = 700 $\mu L \cdot L^{-1}$) on mean (n = 8) whole plant transpiration (E_{wp}) of Cleopatra mandarin and Carrizo citrange seedlings. Different letters within each 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 CO_2 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 aCO_2, C_i/C_a also decreased in salinized Cleo at aCO_2 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 eCO_2, A_{CO2} and g_s both decreased as result of salt stress in both genotypes, but the g_s limitation was removed by eCO_2 and C_i/Ca increased. This underscored the fact that at eCO_2, internal limitations on A_{CO2} from salinity stress were more important than the stomatal limitations on A_{CO2}.

Elevated CO_2 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 eCO_2 than at aCO_2, 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 eCO_2 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 eCO_2, 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 eCO_2 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 CO_2 treatments despite the lower leaf transpiration at eCO_2. 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 aCO_2 and eCO_2 but in Cleo, root Cl^- and Na^+ were lower in eCO_2 than in aCO_2. 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 eCO_2 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 eCO_2, 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 CO_2 levels.

In nonsalinized Cleo plants, leaf N and Ca^{2+} concentrations also could have been reduced by a growth dilution effect eCO_2. 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 eCO_2 for 2 months. Elevated CO_2 also can inhibit the assimilation of NO_3^- in shoots of C_3 plants because this assimilation is strongly dependent on photorespiration which can be inhibited by eCO_2 (Rachmilevitch et al., 2004). Of all the macronutrients (N, P, K, Ca, Mg) studied in calamondin grown at eCO_2 (Keutgen and Chen, 2001), only leaf N was decreased significantly by elevated CO_2. In our experiment, a dilution of N due to enhanced plant growth at eCO_2 cannot be ruled out because leaf Ca^{2+} was also reduced.

Under saline conditions, competition between Cl^- and NO_3-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 aCO_2 could have been due to a greater inhibition of shoot growth than of NO_3^- uptake by salinity. This is supported by the fact that salinity increased N concentrations in roots of both genotypes regardless of the CO_2 level. Cleo leaves had lower Ca^{2+} than Carr and salinity reduced Ca^{2+} further in Cleo at aCO_2. This low Ca^{2+} could have been related to the high accumulation of leaf Na^+ in Cleo (Cámara et al., 2003). The concentrations of Ca^{2+} in roots were reduced by salinity in both genotypes regardless of the CO_2 level. Thus, Ca^{2+} 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 Ca^{2+}.

In conclusion, NaCl salinity in the soil reduced plant growth of these citrus rootstock seedlings at both elevated and ambient levels of atmospheric CO_2 concentrations. Elevated CO_2 increased leaf growth in both nonsalinized plants and salinized plants. The increase in leaf dry weight at eCO_2 in Carr was higher for salinized plants than for nonsalinized plants. In Cleo, however, this eCO_2-induced increase was similar at both salt levels. In Carr, eCO_2 also reduced leaf Na^+ and Cl^- concentration and leaf transpiration rates while increasing shoot-to-root ratio. Similar effects of eCO_2 on S/R, leaf Na^+ and Cl^- did not occur Cleo despite similar decreases in whole plant transpiration. Thus, eCO_2 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 eCO_2 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 eCO_2, not only depended on species specific salinity tolerance but also involved whole plant growth and allocation of Na^+ and Cl^-.

Literature Cited

Ball, M.C. and R. Munns. 1992. Plant responses to salinity under elevated atmospheric concentrations of CO_2. Austral. J. Bot. 40:515–525.

Bañuls, J. and E. Primo-Millo. 1992. Effects of chloride and sodium on gas exchange parameters and water relations of *Citrus* plants. Physiol. Plant. 86:115–123.

Bañuls, J., F. Legaz, and E. Primo-Millo. 1990. Effect of salinity on uptake and distribution of chloride and sodium in some *Citrus* scion–rootstock combinations. J. Hort. Sci. 65:715–724.

Bowes, G. 1991. Growth at elevated CO_2: Photosynthesis responses mediated through Rubisco. Plant Cell Environ. 14:795–806.

Cámara, J.M., F. García-Sánchez, M. Nieves, and A. Cerdá. 2003. Effect of interstock ("Salustiano" orange) on growth, leaf mineral composition, and water relations of one-year-old citrus under saline condition. J. Hort. Sci. Biotechnol. 78:161–167.

Cámara-Zapata J.M., F. García-Sánchez, V. Martinez, M. Nieves, and A. Cerdá. 2004. Effect of NaCl on citrus cultivars. Agronomie 24:155–160.

Castle, W.S. and A.H. Krezdorn. 1975. Effect of citrus rootstocks on root distribution and leaf mineral content of Orlando tangelo trees. J. Amer. Soc. Hort. Sci. 100:1–4.

Chen, K. and F. Lenz. 1997. Responses of strawberry to doubled CO_2 concentration and phosphorus deficiency II. Gas exchange and water consumption. Gartenbauwissenchaft 62:90–96.

Chen, Z.S. 1992. Identification of salt-tolerance of citrus germplasm. Acta Hort. Sinica 19:289–295.

Drake, A.M., M. Gonzalez-Meler, and S.P. Long. 1997. More efficient plants: A consequence of rising atmospheric CO_2. Annu. Rev. Plant Physiol. Mol. Biol. 48: 607–637.

Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 33:317–345.

Fernández-Ballester, G., F. García-Sánchez, A. Cerdá, and V. Martínez. 2003. Tolerance of citrus rootstock seedlings to saline stress based on their ability to regulate ion uptake and transport. Tree Physiol. 23:265–271.

García-Legaz, M.F., J.M. Ortiz, A.G. García-Lidon, and A. Cerdá. 1993. Effect of salinity on growth, ion content and CO_2 assimilation rate in lemon varieties on different rootstocks. Physiol. Plant. 89:427–432.

García-Sánchez, F., J. Jifon, M. Carvajal, and J.P. Syvertsen. 2002. Gas exchange, chlorophyll, and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstock. Plant Sci. 162:705–712.

Grattan, S.R. and C.M. Grieve. 1992. Mineral element acquisition and growth response of plants grown in saline environments. Agr. Ecosystem Environ. 38:275–300.

Hare P.D., W.A. Cress, and J. Van Staden. 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21:535–553.

Inskeep, W.P. and P.R. Bloom. 1985. Extinction coefficients of chlorophyll a and b in *N,N*-dimethylformamide and 80% acetone. Plant Physiol. 77:483–485.

Jifon, J.L. and J.P. Syvertsen. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. Tree Physiol. 23:119–127.

Keutgen, N. and K. Chen. 2001. Response of citrus photosynthesis, chlorophyll fluorescence, macronutrient, and carbohydrate contents to elevated CO_2. J. Plant Physiol. 158:1307–1316.

Lea-Cox, J.D. and J.P. Syversten. 1993. Salinity reduces water use and nitrate-N-use efficiency of citrus. Ann. Bot. 72:47–54.

Levy, Y. and J. Syvertsen. 2004. Irrigation water quality and salinity effects in citrus trees. Hort. Rev. 30:37–82.

Li, F., S. Kang, J. Zhang, and S. Cohen. 2003. Effects of atmospheric CO_2 enrichment, water status and applied nitrogen on water-and-nitrogen-use efficiencies of wheat. Plant Soil 254:279–289.

Lloyd, J., J.P. Syvertsen, and P.E. Kriedemann. 1987b. Salinity effects on leaf water relations and gas exchange of 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] on rootstocks with different salt exclusion characteristics. Austral. J. Plant Physiol. 14:605–617.

Lloyd, J., P. Kriedemann, and D. Aspinall. 1990. Contrasts between Citrus species in response to salinization: An analysis of photosynthesis and water relations for different rootstock–scion combinations. Physiol. Plant. 78:236–246.
Lloyd, J., P.E. Kriedemann, and J.P. Syvertsen. 1987a. Gas exchange, water relations, and ion concentrations of leaves on salt-stressed 'Valencia' orange *Citrus sinensis* (L.) Osbeck. Austral. J. Plant Physiol. 14:387–396.
Maas, E.V. 1990. Crop salt tolerance, p. 262–304. In: K.K. Tanji (ed.). Agricultural salinity assessment and management. Amer. Soc. Civil Eng. Man. Rpts. Eng. No. 71, ASCE, New York.
Maggio, A., F.N. Dalton, and G. Piccinni. 2002. The effect of elevated carbon dioxide on static and dynamic indices for tomato salt tolerance. Eur. J. Agron. 16:197–206.
Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence—A practical guide. J. Expt. Bot. 51:659–668.
Moya, J.L., A. Gomez-Cadenas, E. Primo-Millo, and M. Talon. 2003. Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant Cleopatra mandarin citrus rootstocks is linked to water use. J. Expt. Bot. 54:825–833.
Moya, J.L., E. Primo-Millo, and M. Talon. 1999. Morphological factors determining salt tolerant in citrus seedlings: The shoot to root ratio modulates passive root uptake of chloride ions and their accumulation in leaves. Plant, Cell Environ. 22:1425–1433.
Munns, R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. Plant Cell Environ. 16:15–24.
Nicolas, M.E., R. Munns, A.B, Samarakoon, and R.M. Gifford. 1993. Elevated CO_2 improves the growth of wheat under salinity. Austral. J. Plant Physiol. 20:349–360.
Rachmilevitch, S., A.B. Cousins, and A.J. Bloom. 2004. Nitrate assimilation in plants shoots depends on photorespiration. Proc. Natl. Acad. Sci. 101:11506–11510.
Scholander, P., H. Hammel, E. Bradstreet, and E. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339–345.
Shalhevet, J. and Y. Levy. 1990. Citrus trees, p. 951–986. In: B.A. Stewart and D.R. Nielsen (eds.). Irrigation of agricultural crops. Amer. Soc. Agron. Madison, Wis.
Syvertsen, J.P. and G. Yelenosky. 1988. Salinity can enhance freeze tolerance of citrus rootstock seedlings by modifying growth, water relations, and mineral nutrition. J. Amer. Soc. Hort. Sci. 113:889–893.
Syvertsen, J.P. 1984. Light acclimation in citrus leaves. II. CO_2 assimilation and light, water and nitrogen use efficiency. J. Amer. Soc. Hort. Sci. 109:812–817.
Syvertsen, J.P., B. Boman, and D.P.H. Tucker. 1989. Salinity in Florida citrus production. Proc. Fla. State Hort. Soc. 102:61–64.
Syvertsen. J.P., L.S. Lee, and J.W. Grosser. 2000. Limitations on growth and net gas exchange of diploid and tetraploid citrus rootstock cultivars grown at elevated CO_2. J. Amer. Soc. Hort. Sci. 125:228–234.
Tanner, W. and H. Beevers. 1990. Does transpiration have an essential function in long-distance transport in plants? Plant Cell Environ. 13:745–750.
Walker, R.R. and T.J. Douglas. 1983. Effect of salinity level on uptake and distribution of chloride, sodium and potassium ions in citrus plants. Austral. J. Agr. Res. 34:145–153.
Walker, R.R., D.H. Blackmore, and Qing Sun. 1993. Carbon dioxide assimilation and foliar ion concentrations in leaves of lemon (*Citrus limon* L.) trees irrigated with NaCl or Na_2SO_4. Austral. J. Plant. Physiol. 20:173–185.
Yelle, S., R.C. Beeson Jr., M.J. Trudel, and A. Gosselin. 1989. Acclimation of the tomato species to high atmospheric CO_2. Ribulose-1,5-biphosphate carboxylase/oxygenase and phosphoenol-pyruvate carboxylase. Plant Physiol. 90:1473–1477.
Yeo A.R. 1998. Molecular biology of salt tolerance in the context of whole plant physiology. J. Expt. Bot. 49:915–929.
Zekri, M. 1991. Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. Sci. Hort. 47:305–315.