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Orange varieties as interstocks increase the salt tolerance of lemon trees

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SUMMARY

We investigated the ability of interstocks to increase salt tolerance in lemon trees. We compared 2-year-old 'Verna' lemon trees [*Citrus limon* (L.) Burm.; VL] grafted on Sour Orange (*C. aurantium* L.; SO) rootstock either without an interstock (VL/SO), or interstocked with 'Valencia' orange (*C. sinensis* Osbeck; VL/V/SO), or with 'Castellano' orange (*C. sinensis* Osbeck; VL/C/SO). Trees were grown under greenhouse conditions and supplied with nutrient solutions containing 0, 30, or 60 mM NaCl. Reductions in leaf growth caused by salt treatment were greatest in non-interstocked (VL/SO) trees, followed by VL/C/SO trees, and were the least in VL/V/SO trees. Although the levels of Cl^- and Na^+ ions in the roots and stems were not affected by either interstock, leaf concentrations of Cl^- and Na^+ were higher in VL/SO trees than in VL/C/SO or VL/V/SO trees, suggesting that an interstock in *Citrus* trees could limit the uptake and transport of such ions to the shoots. Saline-treated VL/SO trees also tended to have the lowest shoot:root (S:R) ratios; so, overall, there was a negative relationship between S:R ratio and leaf Cl^- ion concentration. Leaf transpiration (E_{leaf}) may also be involved in the reduction in leaf Cl^- concentration, as interstocked trees had lower E_{leaf} values at mid-day than non-interstocked trees. Salinity increased leaf concentrations of Ca^{2+} in VL/C/SO trees and increased both leaf K^+ and N concentrations in all trees, regardless of interstock. Salinity reduced leaf water potentials and osmotic potentials, such that leaf turgor was increased in all trees.

Spain is the second-highest lemon fruit–producing country in the World, and the greatest exporter. Approx. 80% of Spanish lemon production is located in the arid southeast, where the irrigation water often has a high salinity due to NaCl. *Citrus* trees are relatively salt-sensitive (Maas, 1993; Storey and Walker, 1999) as saline irrigation water reduces *Citrus* tree growth and fruit yield (Prior *et al.,* 2007; Garcia-Sanchez *et al.,* 2006). Negative effects of salinity on *Citrus* tree growth and physiological processes generally occur because of Cl^- ion toxicity, rather than Na^+ ion toxicity (Romero-Aranda *et al.,* 1998), or because of osmotic stress (Garcia-Sanchez and Syvertsen, 2006) or salt-induced oxidative stress (Arbona *et al.,* 2003). Osmotic adjustments in saline-treated *Citrus* leaves can maintain, or even increase, leaf turgor at low leaf water potentials (Garcia-Sanchez and Syvertsen, 2006). In well-watered, salinised *Citrus* trees, high concentrations of Cl^- and Na^+ ions in the leaves reduced the net assimilation of CO_2 (A_{CO2}) by direct biochemical inhibition of their photosynthetic capacity rather than by a decrease in stomatal conductance (Levy and Syvertsen, 2004; Garcia-Sanchez and Syvertsen, 2006). Biochemical inhibition of A_{CO2} in *Citrus* can be linked to changes in leaf anatomy, interactions with leaf nutrients (Romero-Aranda *et al.,* 1998), and/or reductions in electron transport (López-Climent *et al.,* 2008).

Citrus rootstocks have a substantial influence on the levels of Cl^- and/or Na^+ ions that accumulate in the foliage (Storey and Walker, 1999). Salt-tolerance in *Citrus* has therefore been linked to the exclusion of toxic ions from the shoots (Garcia-Sanchez *et al.,* 2002). Strategies to improve salt tolerance in *Citrus* have included creating new rootstocks and polyploids (Saleh *et al.,* 2008) with an improved ability to restrict the accumulation of Na^+ and/or Cl^- ions in the scion. One interesting method used an interstock, grafted between the rootstock and scion combination, that not only increased tree growth, longevity, fruit production, and quality (Gil-Izquierdo *et al.,* 2004), but also increased salt tolerance in lemon trees by decreasing leaf Cl^- and Na^+ levels in the increased foliar growth (Cámara *et al.,* 2003). These studies used 'Salustiano' or 'Sanguina' orange as interstocks, but other potentially effective interstocks and interstock-related physiological mechanisms that can decrease leaf Cl^- ion concentrations have not been studied.

We have compared tree growth, leaf gas exchange, and water relations, along with leaf and root mineral nutrient responses in 'Verna' lemon trees on Sour Orange ('SO') rootstock, interstocked with 'Valencia' orange or 'Castellano' orange. Since low leaf Cl^- ion concentrations

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have been linked, in part, to low leaf transpiration (Moya *et al.,* 1999; García-Sanchez *et al.,* 2006), we hypothesised that the interstocks would reduce tree water use and so increase water use efficiency.

MATERIALS AND METHODS

Plant material and growth conditions

Two-year-old 'Verna' lemon trees (*Citrus limon* (L.) Burm.; VL) grafted on Sour Orange (*C. aurantium* L.; SO) without an interstock (VL/SO), or interstocked with 'Valencia' orange (*C. sinensis* Osbeck; VL/V/SO) or 'Castellano' orange (*C. sinensis;* VL/C/SO) were compared in this experiment. Trees were grown in 12 l pots filled with a native clay-loam soil in a partially shaded greenhouse under a maximum photosynthetically active radiation level of 1,000 µmol m^{-2} s^{-1}, with day/night temperatures of 30º/18ºC ± 3ºC, and day/night relative humidities of 60/75% ± 5%, with a natural 16 h photoperiod.

All plants were well-watered and well-nourished by watering daily with a complete nutrient solution containing 7.75 mM NO_3^-, 0.70 mM $H_2PO_4^-$, 4.05 mM K^+, 2.20 mM Ca^{2+}, 0.50 mM Mg^{2+}, 0.50 mM SO_4^{2-} and 0.60 mM Fe, with 0 mM NaCl (S0 treatment), 30 mM NaCl (S1 treatment), or 60 mM NaCl (S2 treatment). To avoid osmotic shock in the salt treatments, the salinity level was increased in increments of 10 mM NaCl d^{-1} until the 30 mM NaCl or 60 mM NaCl level was achieved. Irrigation was carried out using a drip system at 4 l h^{-1} per tree, with sufficient volume to leach from the bottom of all pots. The experimental design was a 3 × 3 factorial, of the three interstock combinations × three salt treatments (control, 30 mM, or 60 mM NaCl), with six replicate trees in each treatment. The salinity treatments were continued for 8 weeks.

Plant water relations

All leaf measurements were done using a single mature leaf in the mid-stem region of each of the six replicate trees. Pre-dawn leaf water potential (Ψ_w) was measured after 8 weeks of saline treatment using a Scholander-type pressure chamber (PMS Instruments; Corvallis, OR, USA). After Ψ_w had been measured, the leaves were immediately wrapped tightly in aluminium foil, frozen by immersing in liquid nitrogen, and subsequently stored in airtight plastic bags at –18°C until analysis. After thawing, the osmotic potential (Ψ_π) of the expressed sap was measured at 25° ± 1°C using an osmometer (Digital Osmometer; Wescor, Logan, UT, USA). The turgor potential (Ψ_P) was calculated as the difference between Ψ_w and Ψ_π. At mid-day (11.00 – 12.00 h), similar leaves to those used for Ψ_w, were harvested and weighed immediately to obtain a leaf fresh weight (FW) prior to measuring their relative water content (RWC). Leaves were placed in a beaker with their petioles submerged in water, in the dark, overnight, so that the leaves could become fully hydrated. The leaves were then reweighed to obtain their turgid weight (TW) and dried at 60ºC for 48 h to obtain their dry weight (DW). The RWC was then calculated as:

$$RWC = [(FW - DW) \times (TW - DW)^{-1}] \times 100$$

(Morgan, 1984).

Leaf osmotic potential at full turgor (Ψ_π^{100}) was also measured on one leaf per plant after full hydration overnight, as above. Fully turgid leaves were then frozen in liquid nitrogen, and their Ψ_π^{100} was measured as above.

Proline and quaternary ammonium compound concentrations

At the end of the experiment, proline was extracted from fresh leaf and root tissues using 3% (w/v) sulphosalicylic acid and quantified according to the

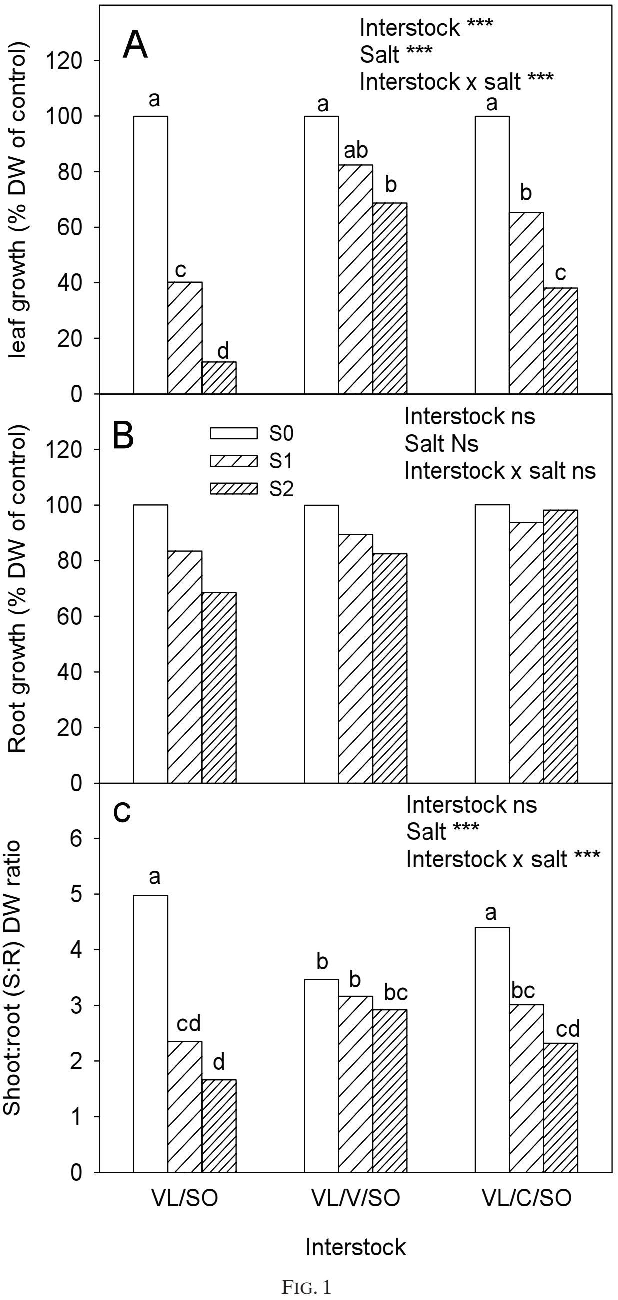

FIG. 1

Effects of salt treatment (S0, S1, or S2 = 0, 30, or 60 mM NaCl) on leaf growth (% DW relative to 0 mM NaCl control trees; Panel A), root growth (% DW relative to 0 mM NaCl control trees; Panel B), and the shoot:root (S:R) DW ratio (Panel C) in 'Verna' lemon (VL) trees grafted on Sour Orange (SO) rootstock, either without an interstock (VL/SO) or interstocked with 'Valencia' orange (VL/V/SO) or interstocked with 'Castellano' Orange (VL/C/SO). Each value is the mean of six plants. ns and *** indicate non-significant, or significant differences at $P < 0.001$, respectively, for the two-way interaction salt × interstock treatment. Different lower-case letters indicate significant differences at $P < 0.05$ by both Duncan's multiple range tests.

protocol described by Bates *et al.* (1973). Quaternary ammonium compounds (QAC) were also extracted from fresh leaf and root tissues using 1 M H_2SO_4 and quantified using the method of Grieve and Grattan (1983).

Leaf gas exchange parameters

At the end of the experiment, the diurnal patterns of net assimilation of CO_2 (A_{CO2}) and leaf transpiration (E_{leaf}) were monitored from 09.00 – 19.00 h, over two consecutive days using a portable photosynthesis system (Model LCA-4; ADC Bioscientific Ltd., Hoddesdon, UK) with an 11.35 cm^2 leaf chamber (PLC-4N), configured in an open system. During these measurements, the air temperature, relative humidity, and vapour pressure deficit (VPD) ranged from 28° – 31°C, 52 – 68%, and 11.0 – 15.3 kPa, respectively. These gas exchange parameters were measured in a single mature leaf from the mid-stem region of three replicate trees from the control and S2 treatments.

Growth and mineral nutrient concentrations

At the end of the experiment, plants were harvested and separated into leaves, stems, and roots. Tissues were rinsed briefly with deionised water, oven-dried at 60ºC for at least 48 h, weighed, then ground to a fine powder. Sub-samples of leaf, stem, and root tissues were extracted with deionised water, and tissue Cl^- ion concentrations were measured using a Corning 926 chloridometer (Sherwood, Cambridge, UK). Tissue N concentrations were measured using a Thermo-Finnigan 1112 EA elemental analyser (Thermo-Finnigan, Milan, Italy). Tissue Na^+, K^+, Mg^{2+}, Ca^{2+}, and P concentrations were determined by inductively-coupled plasma spectrometry (Iris Intrepid II; Thermo-Electron Corporation, Franklin, NJ, USA) after acid digestion in 5:3 (v/v) HNO_3:H_2O_2 in a microwave, reaching 200ºC in 20 min, and holding at this temperature for 2 h (CEM Mars Xpress, Matthews, NC, USA).

Statistical analysis

Data were subjected to analysis of variance using a two-way ANOVA (SPSS Statistical Package; SPSS Inc., Chicago, IL, USA) with three interstocks × three salt treatments and six replicate plants per treatment. When the interaction term was significant ($P < 0.05$), treatment means were separated using Duncan's multiple range test. When the interaction term was not significant, and the main factors (interstock and/or saline treatment) were significant, Duncan's multiple range test was run to compare the main factors alone (Little and Hills, 1987).

RESULTS

Growth parameters

Both saline treatments (S1 and S2) reduced leaf growth in VL/SO and VL/C/SO trees, but only the S2 treatment reduced leaf growth significantly in VL/V/SO trees (Figure 1). The highest reduction in leaf growth occurred in VL/SO trees, while VL/V/SO trees had the lowest reduction in leaf growth. Root growth was not significantly affected by salt concentration or by interstock treatment. The S:R DW ratio decreased similarly in the S1 and S2 treatments, on both VL/SO trees and VL/C/SO trees; but the S:R DW ratio in VL/V/SO trees was not affected by saline treatment.

Leaf and root Cl^- and Na^+ ion concentrations

Chloride and Na^+ ion concentrations in leaves, stems, and roots increased with increasing NaCl concentration in the nutrient solution in all trees (Figure 2). Leaf Cl^- and Na^+ concentrations were similar in VL/V/SO trees and VL/C/SO trees, but the levels of both ions were significantly lower than in VL/SO trees in both saline treatments. Interstocked trees in the S2 treatment had similar leaf Cl^- and Na^+ ion concentrations to those in the S0 and S1 treatments. Stem and root Cl^- and Na^+ levels were not different between the three interstock treatments.

Leaf water relations and leaf proline and QAC concentrations

Saline treatment decreased the leaf water and osmotic potentials in all three interstock treatments (Table I). Leaf turgor potential was similar in trees in the S1 and S2 treatments, but Ψ_P was significantly higher in the saline treatments than in the control treatment, regardless of interstock. Relative water content and Ψ_π^{100} decreased with increasing NaCl concentration in all trees. Leaf proline concentrations also increased with saline

TABLE I

Effects of salt treatment (S0, S1, or S2 = 0, 30 or 60 mM NaCl) on pre-dawn water potential (Ψ_w), osmotic potential (Ψ_π), turgor potential (Ψ_P), relative water content (RWC), osmotic potential at full turgor (Ψ_π^{100}), and proline and quaternary ammonium compound (QAC) concentrations in leaves of 'Verna' lemon (VL) trees grafted on Sour Orange (SO) rootstock, either without an interstock (VL/SO) or interstocked with 'Valencia' orange (VL/C/SO), or with 'Castellano' orange (VL/C/SO)

Rootstock	Salt treatment	Ψ_w (MPa)	Ψ_π (MPa)	Ψ_P (MPa)	RWC (%)	Ψ_π^{100} (MPa)	Proline (µg mg^{-1} DW)	QAC (µg mg^{-1} DW)
VL/SO (Control)	S0	–0.51 a[†]	–1.85 a	1.34 b	95.3 a	–2.03 a	14.2 b	13.5 b
	S1	–0.63 b	–2.19 b	1.56 a	93.8 b	–2.18 b	16.7 a	13.6 b
	S2	–0.85 c	–2.67 c	1.82 a	93.1 c	–2.47 c	17.9 a	18.7 a
VL/V/SO	S0	–0.52 a	–1.85 a	1.34 b	94.5 a	–2.02 a	13.7 b	13.9 b
	S1	–0.72 b	–2.32 b	1.60 a	93.4 b	–2.10 b	15.9 a	18.8 a
	S2	–0.96 c	–2.46 c	1.50 a	92.6 c	–2.36 c	16.2 a	14.2 ab
VL/C/SO	S0	–0.50 a	–2.02 a	1.52 b	95.0 a	–1.89 a	14.2 b	12.2 b
	S1	–0.76 b	–2.36 b	1.60 a	92.9 b	–2.22 b	15.2 a	14.4 ab
	S2	–0.93 c	–2.64 c	1.71 a	92.2 c	–2.48 c	16.2 a	15.5 ab
Interstock		ns	ns	ns	ns	ns	ns	ns
Salt		***	***	***	***	***	***	*
Interstock × Salt		ns	ns	ns	ns	ns	ns	*

ns, *, *** indicate non-significant, or significant differences at $P < 0.05$, or $P < 0.001$ respectively, for the two-way interaction rootstock × salt treatment.

[†] Values are the means of six replicate plants. Different lower-case letters in each column indicate significant differences at $P < 0.05$ by Duncan's multiple range test.

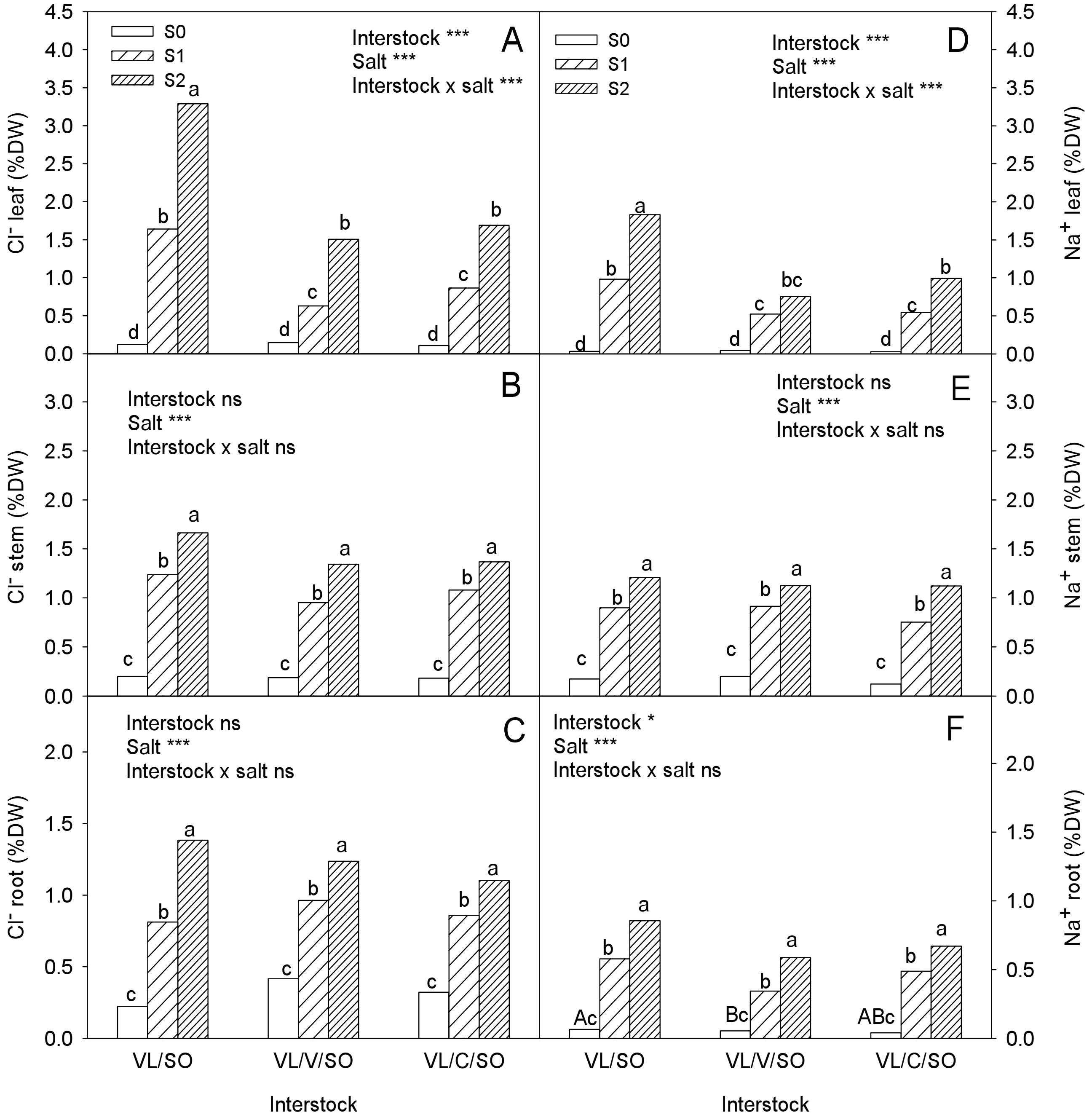

FIG. 2

Effects of salt treatment (S0, S1, or S2 = 0, 30, or 60 mM NaCl) on Cl^- (Panels A–C) and Na^+ (Panels D–F) ion concentrations in leaves, stems, and roots of 'Verna' lemon (VL) trees grafted on Sour Orange (SO) rootstock, either without an interstock (VL/SO) or interstocked with 'Valencia' orange (VL/V/SO) or interstocked with 'Castellano' orange (VL/C/SO). Each value is the mean of six plants. ns, *,*** indicate non-significant or significant differences at $P < 0.05$, or $P < 0.001$, respectively, for the two-way interaction interstock × salt treatment. Different lower-case letters indicate significant differences at $P < 0.05$, and differences between interstocks are indicated by different upper-case letters by both Duncan's multiple range tests.

treatment, but leaf proline levels in the S1 and S2 treatments did not differ. The concentration of leaf quaternary ammonium compounds (QAC) increased significantly in VL/SO trees in the S2 treatment and in VL/V/SO trees in the S1 treatment, but QAC was not affected by any salt treatment in the VL/C/SO trees.

Leaf gas exchange parameters

At the end of the experimental period, A_{CO2} tended to decrease progressively from 09.00 – 17.00 h in all trees, as the atmospheric VPD remained high throughout the day (see inset, Figure 3). The S2 treatment had lower A_{CO2} values than the S0 (non-saline) treatment until late in the afternoon. In interstocked trees, gas exchange declined more rapidly than in VL/SO trees such that, from 11.00 – 12.00 h, the VL/SO trees had higher A_{CO2} and leaf transpiration (E_{leaf}) values than the interstocked trees. Although the saline treatments also reduced E_{leaf} throughout the day, the VL/SO trees appeared to have a different diurnal pattern from the interstocked trees.

Mineral nutrient concentrations

Leaf Ca^{2+} concentrations in S2-treated VL/C/SO trees increased relative to those in the S0 treatment, and leaf

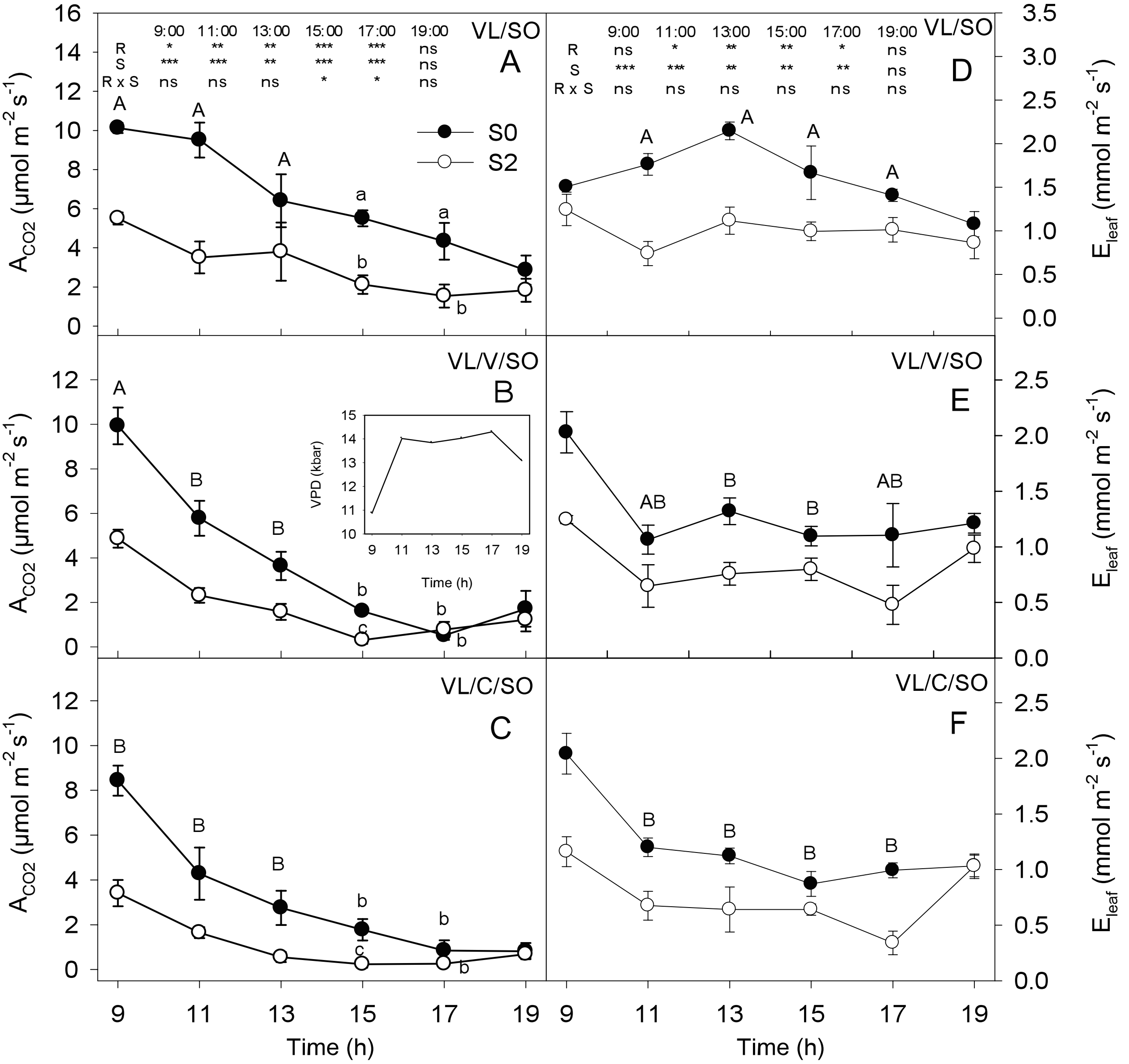

FIG. 3

Effects of salt treatment (S0 or S2 = 30 or 60 mM NaCl) on the diurnal pattern of net assimilation of CO_2 (A_{CO2}; Panels A–C) and on leaf transpiration (E_{leaf}; Panels D–F) in 'Verna' lemon (VL) trees grafted on Sour Orange (SO) rootstock, either without an interstock (VL/SO) or interstocked with 'Valencia' orange (VL/V/SO) or interstocked with 'Castellano' orange (VL/C/SO). The inset in Panel B depicts the daytime pattern of vapour pressure deficit (VPD). ns, *,**,*** indicate non-significant, or significant differences at $P < 0.05$, $P < 0.01$, or $P < 0.001$ respectively, for the two-way interaction interstock × salt treatment. Different lower-case letters indicate significant differences at $P < 0.05$, and differences between interstocks are indicated by different upper-case letters by both Duncan's multiple range tests. Each datum point is a mean (n = 3) ± SE.

P concentrations in VL/SO trees increased with saline treatment (Table II). Saline treatment increased leaf K^+ and N concentrations in all trees, but leaf K^+ was highest in the VL/V/SO trees. Leaf Ca^{2+}, Mg^{2+}, P, and N concentrations were not affected by interstock treatment. Likewise, none of the nutrients measured in roots were affected by interstock treatment. In contrast to leaves, all saline-treated roots had lower K^+ and N concentrations than roots from non-saline treated trees. Root Ca^{2+}, Mg^{2+}, and P concentrations were not affected by saline treatment.

DISCUSSION

For many species other than *Citrus,* Na^+ appears to reach toxic concentrations before Cl^- becomes toxic. In *Citrus,* however, Cl^- is considered to be more toxic than Na^+ (Moya *et al.,* 2003). In our experiments, leaf Cl^- concentrations were higher than leaf Na^+ concentrations, so it is likely that leaf Cl^- ions were responsible for the reductions observed in leaf growth. Overall, leaf growth and leaf Cl^- concentrations were strongly correlated ($r^2 = 0.843$; $P < 0.01$). For example, VL/SO trees had the greatest reductions in leaf growth and the highest leaf Cl^- concentrations. Thus, trees with either interstock had higher salt tolerance due to reduced Cl^- accumulation in their leaves. In previous studies with *Citrus* rootstock seedlings, or with grafted *Citrus* trees, low leaf Cl^- concentrations could be linked to low leaf transpiration, high S:R DW ratios (Syvertsen and Garcia-Sanchez,

TABLE II

Effects of salt treatment (S0, S1, or S2 = 0, 30, or 60 mM NaCl) on mineral (Ca^{2+}, K^+, Mg^{2+}, P and N) concentrations (in % DW) in the leaves and roots of 'Verna' lemon (VL) trees grafted on Sour Orange (SO) rootstock, either without an interstock (VL/SO) or interstocked with 'Valencia' orange (VL/V/SO), or with 'Castellano' orange (VL/C/SO)

Rootstock	Salt (mM)	Ca^{2+}	K^+	Mg^{2+}	P	N
Leaves						
VL/SO	S0	1.66 bc[†]	2.35 Bc	0.20	0.11 d	2.06 b
	S1	1.95 abc	2.77 b	0.21	0.15 bc	2.44 a
	S2	2.24 ab	2.96 a	0.20	0.20 a	2.49 a
VL/V/SO	S0	1.98 abc	2.76 Ac	0.22	0.14 bcd	219 b
	S1	1.75 bc	3.08 b	0.18	0.16 b	2.41 a
	S2	1.69 bc	3.41 a	0.18	0.15 bc	2.30 a
VL/C/SO	S0	1.49 c	2.27 Bc	0.19	0.12 cd	2.14 b
	S1	1.71 bc	2.70 b	0.19	0.14 bcd	2.22 a
	S2	2.33 a	3.29 a	0.21	0.14 bcd	2.28 a
Interstock		ns	*	ns	ns	ns
Salt		*	***	ns	*	*
Interstock × Salt		*	ns	ns	*	ns
Roots						
VL/SO	S0	4.80	2.24 a	0.34	0.25	2.56 a
	S1	4.70	1.84 b	0.26	0.22	2.33 b
	S2	4.96	1.62 b	0.32	0.19	2.06 b
VL/V/SO	S0	4.67	2.20 a	0.30	0.26	2.41 a
	S1	4.75	1.59 b	0.24	0.42	2.22 b
	S2	4.06	1.53 b	0.25	0.21	2.11 b
VL/C/SO	S0	5.26	2.11 a	0.32	0.25	2.31 a
	S1	5.31	1.53 b	0.32	0.22	1.96 b
	S2	3.82	1.61 b	0.23	0.22	2.19 b
Interstock		ns	ns	ns	ns	ns
Salt		ns	***	ns	ns	**
Interstock × Salt		ns	ns	ns	ns	ns

ns, *,**,*** indicate non-significant, or significant differences at $P < 0.05$, $P < 0.01$, or $P < 0.001$ respectively, for the two-way interaction rootstock × salt treatments.

[†] Values are the mean of six replicate plants. Different lower-case letters in each column indicate significant differences at $P < 0.05$ by Duncan's multiple range test. Differences between interstocks are indicated by different upper-case letters.

2008), and/or the ability of roots to retain a high Cl^- concentrations (Garcia-Sanchez and Syvertsen, 2006; Prior *et al.,* 2007). In this experiment, there were no significant differences in root Cl^- concentrations between interstocked and non-interstocked trees, but saline-treated VL/V/SO trees did have higher S:R DW ratios than VL/SO trees. This could have resulted in a dilution of leaf Cl^- concentrations in the interstocked trees. In addition to the lower S:R DW ratios, mid-day E_{leaf} was higher for salt-treated VL/SO trees than for interstocked trees, indicating that Cl^- uptake could have been reduced by the lower level of leaf transpiration in interstocked trees.

Citrus trees are characterised by having a large canopy with leaf area indices as high as 11 (Jahn, 1979), and a correspondingly large evaporative surface. Yet their stem and root hydraulic conductivities are relatively low. Thus, when the VPD is high, these characteristics can result in low transpiration (Alarcon *et al.,* 2006). In our experiment, the fact that interstocked trees had a lower E_{leaf} than non-interstocked trees suggests that an interstock between the scion and the rootstock could have limited water transport to the scion. In *Citrus,* both the size and the number of xylem elements can differ, depending on the variety. For example, 'Cleopatra' mandarin had relatively low rates of water use and a lower number of smaller xylem elements than 'Carrizo' citrange with higher water use rates (Moya *et al.,* 2003). Thus, xylem vessel capacity may restrict water transport. Since interstocked trees have two graft unions (rootstock × interstock and interstock × scion), any restriction of water transport could be greater in interstocked trees than in non-interstocked trees with only one graft union.

Net assimilation of CO_2 (A_{CO2}) was reduced by saline treatment of trees, but lowered Ψ_w values were not responsible for this decline since Ψ_P was increased by salinity. This implies that the salt-induced decreases in A_{CO2} were not related to an osmotic effect on leaf water relations, but rather to effects of toxic Cl^- ions. Leaf Cl^- ion concentrations in salt-treated trees ranged from 0.6 – 3.4% DW; however, reductions in A_{CO_2}, relative to the control treatment, were similar across all S2 treatments regardless of interstock. Thus, the photosynthetic machinery of leaves from 'Verna' lemon trees was sensitive to high Cl^- ion levels whenever the leaf Cl^- ion concentration exceeded 0.6% DW.

Based on lower reductions in leaf growth and lower accumulations of Cl^- in the leaves of salt-treated interstocked trees, interstocked trees were considered to be more salt-tolerant than standard non-interstocked trees. Moreover, VL/V/SO trees were more salt-tolerant than VL/C/SO trees, as salinity-induced reductions in leaf growth in VL/V/SO trees were approx. 17% and 31% for the 30 mM and 60 mM NaCl treatments, while leaf growth reductions in VL/C/SO trees were approx. twice that, at 35% and 62%, respectively. Thus, as an interstock, 'Valencia' imparted greater salt-tolerance to VL trees than 'Castellano', even though leaf Cl^- and Na^+ levels were similar in the two interstocked trees. The underlying mechanism supporting greater growth in 'Valencia' than in 'Castellano' interstocked trees was not apparently related to different growth allocation, or to salt ion partitioning between shoots and roots.

Concentrations of K^+ and N increased in salt-treated leaves, but decreased in roots in all trees, regardless of interstock. This contrast between leaves and roots could have been a response to the high Na^+/K^+ and Cl^-/NO_3^- ratios in the nutrient solution inhibiting the uptake of K^+

and N by an antagonism between these pairs of ions (Cerezo *et al.,* 1999; Leacox and Syvertsen, 1993; Tyerman *et al.,* 1998). The reductions in leaf growth due to salt treatement may have offset the decreases in K^+ and N absorption (Figure 1). Salt treatment decreased K^+ and N levels in roots, even though root growth was not affected.

In conclusion, this work has shown that interstocked *Citrus* trees are more salt-tolerant than non-interstocked trees. The mechanism involved in the reduction of leaf Cl^- ions by an interstock could be linked to its low E_{leaf} values during the day and/or to their high S:R DW ratios. In addition, the variety of interstock used in the scion × interstock × rootstock combination also influenced salt tolerance, as trees interstocked with 'Valencia' orange showed less salt-induced reductions in leaf growth than trees interstocked with 'Castellano' orange.

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