Substrate Type and Salinity Affect Growth Allocation, Tissue Ion Concentrations, and Physiological Responses of Carrizo Citrange Seedlings

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Abstract. To gain insight into salinity tolerance of citrus, we studied growth, leaf, and root Cl^- concentrations and physiological responses of 5-month-old seedlings of the citrus rootstock Carrizo citrange [*Citrus sinensis* (L.) Osb. $\times$ *Poncirus trifoliata* L.] grown in a greenhouse in three different substrates: Candler sand soil, Floridana sandy clay soil, or a commercial soilless peat/perlite/vermiculite potting media. Plants were kept well-watered with a complete nutrient solution plus either no salt (control) or 50 mM NaCl for 9 weeks. Without salinity, substrate type did not affect total plant growth although there were differences in shoot/root dry weight ratio and mineral nutrient relationships attributable to substrate. Predawn leaf water potential, midday CO_2 assimilation, and leaf water use efficiency were highest in seedlings grown in the soilless peat. The salt treatment decreased leaf and root growth, reduced leaf Ca^{2+} , and increased leaf K^+ concentration in all the three substrates. Overall, plant growth was negatively related to leaf Cl^- . Leaf growth reductions were least in Candler-grown seedlings and greatest in Floridana soil as Cl^- concentrations were lowest in Candler sand and highest in Floridana soil. Leaf Na^+ was also highest in Floridana seedlings. In contrast to salt ions in leaves, roots of salinized seedlings in Candler sand had the highest Na^+ and Cl^- concentration. Salinity reduced net gas exchange of leaves similarly in all three substrates. Salinity reduced both leaf water potential and osmotic potential such that leaf turgor was increased. Thus, salinity-induced reductions in growth and net gas exchange were not the result of loss of turgor but more likely resulting from toxic ion accumulation in leaves. Based on the relative rankings of leaf growth and leaf Cl^- concentrations, Carrizo seedlings from Candler sand had the highest salt tolerance and those grown in Floridana soil had the lowest salt tolerance. Substrate type should be considered when characterizing plant growth and physiological responses to salinity.

Citrus is one of the most important commercial crops in Florida annually generating a U.S. \$9 billion impact with almost U.S. \$1 billion in tax revenues (Hodges et al., 2001). Citrus tree growth and fruit yield are sensitive to salinity stress (García-Sánchez et al., 2006; Grieve et al., 2007; Prior et al., 2007) and despite an abundance of summer rainfall in Florida, salinity can be an important problem in citrus production, especially during the dry

spring and fall seasons (Syvertsen et al., 1989). In addition, salt stress-related problems are increasing in some coastal areas where salt water intrudes from the ocean (Boman, 2005).

Citrus leaf turgor potential is often higher in salinized than in nonsalinized citrus leaves apparently from the contribution of Na^+ and Cl^- to osmotic adjustment (García-Sánchez and Syvertsen, 2006; Pérez-Pérez et al., 2007). The adverse effects of NaCl on citrus tree growth have been related primarily to high Cl^- in leaves (Bañuls et al., 1997; Romero-Aranda et al., 1998) and secondarily to high leaf Na^+ (Storey and Walker, 1987). Thus, salinity tolerance can be interpreted as the ability of citrus rootstocks to prevent the accumulation of Cl^- in leaves (Levy and Syvertsen, 2004). There is a marked effect of citrus rootstocks on the uptake and transport of Cl^- and/or Na^+ . For example, the rootstock

Cleopatra mandarin has a good capacity to exclude Cl^- , whereas Carrizo citrange is considered to be a Cl^- accumulator but a good Na^+ excluder (Bañuls and Primo-Millo, 1995; Levy et al., 1999). The Cl^- accumulation mechanism in Carrizo could be linked to its relatively high water use, its more efficient root system for water uptake, and/or to its relatively low shoot-to-root ratio (Moya et al., 2003). Salinity tolerance also can be equated to continued growth and high water use efficiency under salinity stress (García-Sánchez and Syvertsen, 2006). High Cl^- and/or Na^+ concentrations in well-watered citrus leaves reduces net assimilation of CO_2 (A_{CO_2}) by a direct biochemical inhibition of photosynthetic capacity rather by decreases in stomatal conductance (g_s) or leaf water potential (García-Sánchez et al., 2006; Levy and Syvertsen, 2004). Using salinity-induced decreases in leaf photosynthetic responses as an index of salt tolerance, however, can be confusing because A_{CO_2} is not always well correlated with plant growth (García-Legaz et al., 1993).

Poor soil drainage and low available water (Pérez-Pérez et al., 2007) can interact with salinity stress and affect the response of crops to salinity. A high concentration of soil Ca^{2+} improved growth of tomato plants by increasing Ca^{2+} and K^+ absorption and restoring cation balance while restricting Na^+ absorption in relation to soils with low Ca^{2+} (Yamada et al., 2007). Soil ion exchange capacity, mechanical impedance of roots, soil texture, and/or the effect of soil matric potential can influence the availability of Na^+ and Cl^- ions and, consequently, influence salinity tolerance (Villagra and Cavagnaro, 2005). For example, Norway spruce seedlings grown in sandy soil had more severe necrosis and significantly higher concentrations of Na^+ and Cl^- than seedlings grown in loam, silt loam, or peat soils because ion concentrations in the soil solution were generally higher in sandy soils (Fostad and Pedersen, 2000). In citrus, salt tolerance was greater in Etrog citron and Rangpur lime seedlings grown in sandy soil than those grown in solution culture because seedlings grown in sandy soil had a greater ability to exclude Na^+ and Cl^- from leaves (Storey, 1995). Thus, plants grown in different substrates can respond differently to the same amount of salinity and these differences can contribute to conflicting interpretations of relative salt tolerance. The objective of this study was to determine the effect of substrate type on the salt tolerance of seedlings of Carrizo citrange, a common citrus rootstock. We determined leaf Cl^- and Na^+ , the ability to maintain plant growth, and physiological function under salinity stress.

We contrasted three substrates: Candler sand soil, Floridana sandy clay soil, and a soilless peat-based potting media. Candler sand is a typical citrus soil in central Florida and contains very little clay or organic matter (He et al., 2000). Floridana is a dark soil typical of some flatwoods or depressional areas, is very poorly drained, and slowly to

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very slowly permeable (Soil Conservation Service, 1990). Florida citrus trees must be grown on raised beds in this soil to allow for sufficient drainage. The soilless peat-based potting media is typically used in nurseries because it has high organic matter content, is well-drained, but has good ability to retain water and nutrients. Therefore, we hypothesized that plants grown in the soilless peat mix would display greater salt tolerance than those grown in the two soil types.

Materials and Methods

Plant material and growing conditions. This study was carried out at the University of Florida's Citrus Research and Education Center (Lake Alfred, FL; long. 28° N, lat. 82° W). Seeds of Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* L.] were germinated in vermiculite wetted with 0.5 mmol·L⁻¹ CaSO₄. When seedlings were 1 month old, uniform plants were transplanted to 0.15-L containers filled with autoclaved native Candler sand, Floridana soil, or the commercial soilless mixture of peat/perlite/vermiculite at 82.5/12.5/0.5% by volume (Pro-Mix, BX; Premier Horticulture Inc., Red Hill, PA). Candler sand is a well-drained, typic quartzipsamment and Floridana soil is a poorly drained loamy Argiaquoll (Li et al., 2006; Soil Conservation Service, 1990). Characteristics of these substrates are summarized in Table 1. Seedlings were grown in a greenhouse under natural photoperiods during the late summer when maximum photosynthetically active radiation (PAR; LI-170; LICOR, Inc., Lincoln, NE) at plant level was ≈1500 mmol·m⁻²·s⁻¹. Average day/night temperature was 36/21 °C and relative humidity varied diurnally from 40% to 100%.

Plants were well-watered and well-nourished by watering every other day with a dilute solution of a complete fertilizer (8N–0.66P–6.64K) plus 6% iron chelate in a sufficient volume to leach from the bottom of all pots. Plants received ≈21 mg of nitrogen per week. Two months after transplanting, either 0 mM (Control) or 50 mM NaCl (Salt) was added to the nutrient solution. To avoid an osmotic shock, salinity was increased in increments of 10 mM NaCl per day until 50 mM NaCl was achieved. The 50 mM salinity treatments continued for 9 weeks during September to October. The experimental design was a 2 × 3 factorial of two salt treatments (0 mM NaCl or 50 mM NaCl) by three substrates (Candler sand,

Floridana soil, and soilless peat media) with six replicate plants in each treatment.

Plant water relations. Leaf water relations and all subsequent leaf measurements were done on 5-month-old seedlings using fully expanded mature leaves from the mid stem area of each plant. Near the end of the experiment (October), predawn (0500 to 0600 HR) leaf water potential (Ψ_w) was measured in one leaf for each replicate plant with a pressure chamber (PMS Instrument, Corvallis, OR). After Ψ_w , measurement leaves were tightly enclosed in aluminum foil, frozen by immersing in liquid nitrogen, and stored in a freezer at –18 °C until analysis. After thawing at 25 ± 1 °C, leaf Ψ_s (Ψ_π) was quickly measured in the expressed cell sap collected in a syringe and placed in an osmometer (Digital Osmometer; Wescor, Logan, UT). Leaf pressure potential (Ψ_p) was calculated as the difference between the Ψ_π and Ψ_w expressed in MPa. At midday (1100 to 1200 HR), similar leaves to those leaves used for Ψ_w were harvested and immediately weighed to obtain a leaf fresh weight (f. wt) to measure the relative water content (RWC). Leaves were placed in a beaker with petioles submerged in water overnight in the dark so that leaves could become fully hydrated. Leaves were reweighed to obtain turgid weight (t. wt) and dried at 60 °C for 48 h to obtain leaf dry weight (dr wt). RWC was calculated as [(f. wt – dr wt) × (t. wt – dr wt)⁻¹] × 100 (Morgan, 1984).

Gas exchange and chlorophyll fluorescence measurements. Near the end of the experiment, net assimilation of CO₂ (A_{CO_2}) and leaf transpiration (E_{lf}) were measured using one similar leaf from each replicate plant using a portable photosynthesis system (LI-6200; LI-COR Inc.) with a 0.25-L cuvette. Photosynthetic water use efficiency ($WUE = A_{CO_2} E_{lf}^{-1}$), g_s , and intercellular CO₂ concentration (C_i) were calculated by the system. The cuvette was equipped with an external light source (Model QB1205LI-670; Quantum Devices Inc., Barneveld, WI) to maintain a constant PAR of 800 mmol·m⁻²·s⁻¹ during measurements, which is sufficient to saturate citrus leaves grown in full sun (Syvertsen, 1984). All measurements were made in the morning from 0800 to 1000 HR to avoid high afternoon temperatures and low humidity. During all measurements, the leaf temperature was 32 ± 2 °C and leaf-to-air vapor pressure difference was 2.4 ± 0.4 kPa within the cuvette.

Leaf chlorophyll *a* fluorescence was measured with a pulse-modulated fluorometer (Model OS1-FI; Opti-Sciences, Tyngsboro, MA) using similar leaves on each plant. Fluorescence measurements were made between 0900 and 1000 HR under ambient light and also after 20 min of acclimation to dark under leaf clips (FL-DC; Opti-Sciences). The maximum quantum efficiency (F_v/F_m) of photosystem II was calculated as $F_v/F_m = (F_m - F_o)/F_m$, in which F_m and F_o were maximal and minimal fluorescence of dark-adapted leaves, respectively (Jifon and Syvertsen, 2003; Maxwell and Johnson, 2000). Quantum yield (*Y*) was measured as $Y = (F'_m - F)/F'_m$ in which F'_m and *F* were the maximal and steady-state fluorescence yield in the light, respectively. Light pulse intensity for achievement of F'_m and *F* was of 600 nm.

Plant transpiration and leaf area. Two days before the end of the experiment, whole plant transpiration was measured gravimetrically. Individual pots were covered with plastic bags sealed at the base of the stem to stop evaporation. Weight loss from each pot was measured during two 6- to 7-h daytime periods on 2 selected clear days. Daily weight loss per pot was averaged for the 2 d. At harvest, total leaf area per plant was destructively measured (LI-3000; LI-COR) and used to calculate E_{leaf} in mol·m⁻²·h⁻¹.

Electrical conductivity and Cl⁻ concentration in the drainage solution. Electrical conductivity and Cl⁻ concentrations in the drainage solution leached from each pot were measured at the end of the experiment. The leachate was collected after watering with 20 mL of distilled water. EC was measured with an Orion EC meter and Cl⁻ concentration in solution was measured using a silver ion titration chloridometer (HBI Chloridometer; Haake Buchler, Saddle Brook, NJ).

Growth and nutrient concentrations. Nine weeks after initiating the salinity treatments, plants were harvested and separated into leaves, stems, and washed roots. Tissues were briefly rinsed with deionized water, oven-dried at 60 °C for at least 48 h, weighed, and ground to a fine powder. Subsamples of leaf and root tissues were extracted with a 0.1 N solution of nitric acid and 10% acetic acid. Tissue chloride concentration was measured with the chloridometer as stated previously. Tissue Na⁺, K⁺, Mg²⁺, Ca²⁺, and phosphorus concentrations were determined by inductively coupled plasma spectrometry (Iris Intrepid II; Thermo Electron Corporation, Franklin, TN) after previous acid digestion in HNO₃:H₂O₂ (5:3 by volume) in a microwave reaching 200 °C in 20 min and holding at this temperature for 2 h (CEM Mars Xpress, Matthews, NC). Tissue nitrogen concentration was measured using a Thermo-Finnigan 1112 EA elemental analyzer (Thermo-Finnigan, Milan, Italy). All tissue nutrient concentrations were expressed in units of mmol·kg⁻¹ dr wt.

Statistical analysis. Data were analyzed using a two-way analysis of variance (SPSS Statistical Package, Chicago, IL) with three substrate and two salinity treatments. When

Table 1. Moist bulk density (MBD), cation exchange capacity (CEC), percentages of sand, silt, clay, and organic matter (OM), and available water capacity (AWC) of Candler sand, Floridana soil,² and soilless peat mix.

Substrate	MBD (g·cm ⁻³)	CEC (meq/100 g)	Sand (%)	Silt (%)	Clay (%)	OM (%)	AWC (%)	pH
Candler	1.45	5	97	1	1	1	5.5	5.2
Floridana	1.60	7	54	15	30	1	15	6.4
Soilless peat	0.35–0.50 ³	58	—	—	—	81	60–75	5.2–6.2

²Li et al., 2006; Soil Conservation Service, 1990.

³Pro-Mix; Premier Horticulture Inc., Red Hill, PA.

the substrate treatments, salinity treatments, or their interactions were significant ($P < 0.05$), means were separated using Duncan's multiple range test. Linear regression and Pearson product moment correlation coefficient were used to estimate relationships between selected variables.

Results

Substrate characteristics. Although Candler sand and Floridana soil differed in percentages of sand, silt, and clay, their moist bulk density (MBD) and cation exchange capacity (CEC) were similar (Table 1). The soilless peat mix had a much lower MBD along with the higher CEC, organic matter, and available water than the soils.

Plant growth. Despite substrate differences in available water content, total plant growth of the nonsalinized control seedlings was unaffected by substrate type because leaf, root, and total plant dry weight averaged 1.17 g, 5.0 g, and 10.5 g, respectively, regardless of substrate (Fig. 1A–C). Growth allocation was affected by substrate, however, because Floridana seedlings had a higher shoot-to-root dr wt ratio (Sh/Rt) than those from soilless peat (Fig. 1D). The salt treatment reduced leaf, root, and total plant growth in plants from all the three substrates. Salinized plants from Candler sand had the highest leaf dry weight and plants from Floridana soil had the lowest leaf dry weight, whereas plants from soilless peat were intermediate. Salinized plants from Floridana soil also had the lowest root and total plant growth. Salinity did not affect Sh/Rt ratio in plants from all the three substrates.

Chloride and sodium concentrations in leaves and roots. There were no differences in the relatively low Cl^- and Na^+ concentrations in leaves and roots of plants grown with 0 mM NaCl (Fig. 2). Salinity increased the concentration of Cl^- and Na^+ in leaves and roots of all seedlings regardless of substrate. Overall, leaf Cl^- increased significantly more than leaf Na^+ as tested by linear regression and a t test of slopes at $P < 0.05$ (not shown but see Fig. 2A–B). Overall, leaf growth was negatively related to leaf Cl^- ($P < 0.001$, $r = -0.80$) because seedlings in the Candler sand had the lowest leaf Cl^- , whereas seedlings in Floridana soil had the highest leaf Cl^- . The highest leaf Na^+ concentration occurred in seedlings grown in Floridana soil, whereas seedlings grown in Candler sand and soilless peat had similar leaf Na^+ . The concentration of Cl^- and Na^+ in roots, however, was highest in seedlings from Candler sand, whereas roots from Floridana soil had the lowest Na^+ concentration (Fig. 2C–D).

Mineral nutrient concentrations. Leaves from seedlings grown in Candler sand had the highest Ca^{2+} , whereas seedlings from soilless peat had the highest leaf Mg^{2+} concentration (Table 2). Seedlings from Floridana soil had the highest leaf K^+ concentration and seedlings from Candler sand had a higher leaf phosphorus concentration than those grown in Floridana soil. The salinity treat-

Fig. 1. Effects of growth substrate (Candler sand, Floridana soil, or soilless peat) and salt treatment (control = 0 NaCl, open bars or salt = 50 mM NaCl, shaded bars) on mean ($n = 6$) leaf dry weight (dw, **A**), root dw (**B**), total plant dw (**C**), and shoot-to-root dw ratio (Shoot/Root; **D**) of Carrizo citrange rootstock seedlings. NS, **, ***, ****Nonsignificant or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the main effects and two-way interaction of substrate $\times$ salt treatments. Different upper case letters within each figure indicate significant differences at $P < 0.05$ between substrates; different lower case letters within each figure indicate significant differences at $P < 0.05$ among substrate $\times$ salt treatments.

Fig. 2. Effects of growth substrate (Candler sand, Floridana soil, or soilless peat) and salt treatment (control = 0 NaCl, open bars or salt = 50 mM NaCl, shaded bars) on mean ($n = 6$) leaf Cl^- (**A**), leaf Na^+ (**B**), root Cl^- (**C**), root Na^+ (**D**) concentrations of Carrizo citrange seedlings. NS, **, ***, ****Nonsignificant or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the main effects and two-way interaction of substrate $\times$ salt treatments. Different lower case letters within each figure indicate significant differences at $P < 0.05$ among substrate $\times$ salt treatments.

ment decreased leaf Ca^{2+} and increased K^+ concentration regardless of substrate type because there were no significant interactions between substrate and salinity. Leaf Mg^{2+} and phosphorus concentration were unaffected by the salt treatment. Leaf N con-

centrations of nonsalinized control leaves averaged 3087 mmol·kg⁻¹ dr wt (= 4.3% dr wt). Leaf nitrogen was unaffected by substrate, but salinity reduced average leaf nitrogen to 2382 mmol·kg⁻¹ dr wt (= 3.3%).

Table 2. Effects of growth substrate (Candler sand, Floridana soil, soilless peat) and salt treatment (control = no NaCl or salt = 50 mM NaCl) on mean (n = 6) leaf and root Ca^{2+} , K^+ , Mg^{2+} , phosphorus (P), and nitrogen (N) concentrations ($\text{mmol}\cdot\text{kg}^{-1}$ dr wt) of Carrizo citrange seedlings.

Substrate	Salt treatment	Leaf				
		Ca^{2+}	K^+	Mg^{2+}	P	N
Candler	Control	699 A ^z	686 B	123 B	81 A	3,150
	Salt	616	737	119	71	2,421
Floridana	Control	666 B	767 A	136 B	65 B	3,114
	Salt	544	829	128	65	2,218
Soilless peat	Control	626 B	701 B	169 A	68 AB	2,997
	Salt	549	775	160	68	2,506
Substrate		***y	**	***	*	NS
Salt		***	**	NS	NS	***
Substrate $\times$ salt		NS	NS	NS	NS	NS

Substrate	Salt treatment	Root				
		Ca^{2+}	K^+	Mg^{2+}	P	N
Candler	Control	262 B	583 A	169 A	155 a	1,612
	Salt	162	468	156	90 bc	1,605
Floridana	Control	165 C	512 B	128 C	71 cd	1,640
	Salt	130	317	78	52 d	1,403
Soilless peat	Control	269 A	507 B	140 B	129 a	1,571
	Salt	215	417	115	103 b	1,599
Substrate		***y	**	***	***	NS
Salt		***	***	***	***	NS
Substrate $\times$ salt		NS	NS	NS	*	NS

^zDifferent upper case letters within a column indicate significant differences at $P < 0.05$ between substrates. Substrate $\times$ salt means within a column followed by different lower case letters are significantly different at $P < 0.05$.

^yNS, *, **, ***Nonsignificant or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the main effects and two-way interactions between substrate $\times$ salt treatments.

Root Ca^{2+} concentration was lowest in Floridana soil and highest in roots from soilless peat (Table 2). Root Mg^{2+} concentration was also lowest in Floridana soil but highest in Candler sand in which root K^+ concentration was also highest. In the control treatment, root phosphorus concentration was lowest in seedlings from Floridana soil. Salt treatment decreased root Ca^{2+} , K^+ , and Mg^{2+} concentration in all substrates. Salinity also decreased root phosphorus concentration in seedlings from the soilless peat and Candler sand but not significantly so in the already lowest phosphorus in seedlings from Floridana soil. Root nitrogen concentrations varied from 1403 to 1640 $\text{mmol}\cdot\text{kg}^{-1}$ dr wt (2.0% to 2.3% dr wt) and were unaffected by substrate or salinity.

Water relations. In the nonsaline control treatment, predawn Ψ_w was lower in seedlings from Floridana and Candler soil than those from peat, but Ψ_{π} , Ψ_p , and midday

RWC were unaffected by substrate (Table 3). The salt treatment reduced Ψ_w and Ψ_{π} in all three substrates and these reductions were greater in seedlings from Floridana and soilless peat than for those from Candler soil. Salinity increased Ψ_p in seedling grown in Floridana soils and soilless peat but not in Candler sand. Leaf RWC was unaffected by salt treatment.

Leaf gas exchange and chlorophyll a fluorescence parameters. Leaves from seedlings in soilless peat had highest A_{CO_2} and WUE in both control and salt treatments (Table 4). Salt treatment reduced A_{CO_2} , g_s , WUE, and E_{leaf} relative to control leaves in all three substrates. Internal concentration of CO_2 (C_i) varied from 321 to 348 $\mu\text{mol}\cdot\text{mol}^{-1}$ and was unaffected by substrate type or salinity (data not shown). Leaves from non-salinized seedlings grown in soilless peat had higher steady-state fluorescence yield (F) than those from Floridana soil but not differ-

ent from those in Candler sand. Salt treatment increased F_o , F , and F'_m in leaves from seedlings in all three substrates. Photosystem II F_v/F_m and Y varied between 0.79 to 0.81 and 0.58 to 0.71, respectively, but there were no effects of substrate type or salinity on these parameters (data not shown).

Electrical conductivity and Cl^- concentration in the drainage solution. In the salt treatment, EC and Cl^- concentration in the leached drainage solution was highest from Floridana soil (Fig. 3), whereas there were no significant differences between EC and Cl^- in the leachate from Candler sand and soilless peat. In the nonsaline control treatment, there was no effect of substrate on the low EC and Cl^- concentrations in the leachate.

Discussion

Under salinity stress, leaf Cl^- concentrations were higher than leaf Na^+ supporting the well-known idea that Carrizo citrange is considered a Cl^- accumulator but also a good Na^+ excluder (Levy and Syvertsen, 2004; Storey and Walker, 1999). Salinity had a negative effect on leaf growth in all three substrates because seedlings in the Candler sand grew the most leaf dry weight, whereas seedlings in Floridana soil grew the least. So, leaf growth was negatively related to leaf Cl^- because seedlings in the Candler sand had the lowest leaf Cl^- , whereas seedlings in Floridana soil had the highest leaf Cl^- . Based on leaf growth and Cl^- rankings, the salinity tolerance of seedlings grown in Candler sand was highest and that of seedlings grown in Floridana soil was lowest. These data, therefore, did not support our original hypothesis that responses to salinity would be least in seedlings grown in the soilless peat mix.

Although substrates with high nutrient availability can support greater vegetative growth than low nutrient substrates (Fochesato et al., 2007), the higher waterholding capacity and CEC of the soilless peat mix did not affect whole plant growth in the well-watered and fertilized nonsalinized control seedlings. It was possible that the frequent watering and fertilization every other day minimized any substrate differences in available water or nutrients. Leaf Ca^{2+} , K^+ , phosphorus, and Mg^{2+} concentrations were within optimum range and leaf nitrogen was above the optimum range for bearing citrus (Embleton et al., 1973) in all three substrates reflecting the fact that seedlings were well-fertilized. Although leaf nitrogen was unaffected by substrate, other leaf mineral concentrations were affected by substrate type because leaf Ca^{2+} and phosphorus concentrations were highest in Candler, K^+ concentration was highest in Floridana soil, and Mg^{2+} concentration was highest in soilless peat. Thus, different leaf mineral concentrations among the seedlings from the three substrates could have played a role in their salt tolerance.

In citrus, negative effects of salinity have been attributed primarily to Cl^- but also to Na^+ accumulation (Storey and Walker,

Table 3. Effects of growth substrate (Candler sand, Floridana soil, soilless peat) and salt treatment (control = no NaCl or salt = 50 mM NaCl) on mean (n = 6) leaf water potential (Ψ_w), leaf Ψ_s (Ψ_{π}), leaf turgor potential (Ψ_p), and leaf relative water content (RWC) of Carrizo citrange seedlings.

Substrate	Salt treatment	Ψ_w (MPa)	Ψ_{π} (MPa)	Ψ_p (MPa)	RWC (%)
Candler	Control	-0.44 b ^z	-2.57 a	2.13 bc	92.0
	Salt	-0.64 c	-3.18 b	2.54 b	88.2
Floridana	Control	-0.48 b	-2.35 a	1.88 c	90.9
	Salt	-0.72 d	-3.74 c	3.02 a	91.2
Soilless peat	Control	-0.34 a	-2.44 a	2.10 c	92.2
	Salt	-0.70 cd	-3.68 c	2.98 a	84.3
Substrate		***y	NS	NS	NS
Salt		***	***	***	NS
Substrate $\times$ salt		**	*	*	NS

^zSubstrate $\times$ salt means within a column followed by different lower case letter are significantly different at $P < 0.05$.

^yNS, *, **, ***Nonsignificant or significant differences at $P < 0.05$, 0.01, or 0.001, respectively, for the main effects and two-way interactions between substrate $\times$ salt treatments.

Table 4. Effects of growth substrate (Candler sand, Floridana soil, soilless peat) and salt treatment (control = no NaCl or salt = 50 mM NaCl) on mean (n = 6) net assimilation of CO₂ (A_{CO2}), stomatal conductance (g_s), photosynthetic water use efficiency (WUE = A_{CO2} E_{leaf}⁻¹), leaf transpiration (E_{leaf}), minimal fluorescence of dark-adapted leaves (F₀), steady-state fluorescence yield (F), and maximal (F'_m) of Carrizo citrange seedlings.

Substrate	Salt treatment	A _{CO2} (μmol·m ⁻² ·s ⁻¹)	g _s (mol·m ⁻² ·s ⁻¹)	WUE (μmol·mmol ⁻¹)	E _{leaf} (mol·m ⁻² ·h ⁻¹)	F ₀	F	F' _m
Candler	Control	10.77 B ^z	0.372	1.58 B	4.41	141	141 AB	413
	Salt	6.63	0.249	1.23	3.43	156	155	453
Floridana	Control	9.73 B	0.325	1.45 B	4.29	147	134 B	336
	Salt	7.11	0.293	1.23	3.86	163	147	513
Soilless peat	Control	13.61 A	0.357	1.99 A	4.47	148	150 A	445
	Salt	7.58	0.243	1.51	4.03	152	161	471
Substrate		*y	NS	*	NS	NS	*	NS
Salt		***	***	*	***	**	**	*
Substrate × salt		NS	NS	NS	NS	NS	NS	NS

^zDifferent upper case letters within a column indicate significant differences at *P* < 0.05 between substrates.

^yNS, *, **, ***Nonsignificant or significant differences at *P* < 0.05, 0.01, or 0.001, respectively, for the main effects and two-way interactions between substrate × salt treatments.

Fig. 3. Effects of growth substrate (Candler sand, Floridana soil, or soilless peat) and salt treatment (control = 0 NaCl, open bars or salt = 50 mM NaCl, shaded bars) on mean (n = 6) electrical conductivity (EC; A) and Cl⁻ concentration (B) in the drainage solution from Carrizo citrange seedlings. *, **, ***Significant differences at *P* < 0.05, 0.01, or 0.001, respectively, for the main effects and two-way interaction of substrate × salt treatments. Different lower case letters within each figure indicate significant differences at *P* < 0.05 among substrate × salt treatments.

1987), but the severity of the effect of Cl⁻ accumulation on growth can also depend on the quantities of other nutrient ions in leaves and their interactions with salt ions (Bañuls et al., 1997). In our experiment, the higher leaf Ca²⁺ concentration in seedlings from Candler soil relative to leaf Ca²⁺ in soilless peat and Floridana soil could have diminished the negative effect of Cl⁻ toxicity. Calcium has an intrinsic role in displacing Na⁺ (Tattini and Traversi, 2009) and in improving growth of salinized citrus (Romero-Aranda, et al., 1998) in which high leaf Ca²⁺

concentration ameliorated salt-induced leaf abscission and maintained membrane permeability (Bolat et al., 2006). Salinized Floridana seedlings also had the low values of Mg²⁺ and lowest phosphorus, which could have also contributed to their lowest salt tolerance. Salinity stress may increase plant phosphorus requirement to continue normal function (Awad et al., 1990) and the maintenance of leaf Mg²⁺ also might be especially important under salinity stress because Mg²⁺ is a cofactor of tonoplast ATPase (Donovan et al., 1997) and contributes to protein translation in the presence of high Na⁺ (Marschner, 1995).

In previous studies with Carrizo citrange seedlings, physiological mechanisms responsible for Cl⁻ uptake and transport have been linked to plant water use (Moya et al., 2003). A reduction of leaf Cl⁻ can occur when the Sh/Rt ratio is decreased by removing leaves and when leaf transpiration is decreased by root pruning (Moya et al., 1999) or by increasing atmospheric CO₂ concentration (García-Sánchez et al., 2006). Here, however, leaf transpiration rates of salinized plants were unaffected by substrate (Table 4) so variations in water use could not have caused the high leaf Cl⁻ concentrations in leaves from Floridana soil and low leaf Cl⁻ from Candler sand. The high leaf Cl⁻ and Na⁺ concentrations in Floridana seedlings could have been attributable in part to higher levels of salt ions in the soil solution than those growing in Candler sand and soilless peat, supported by the high EC and Cl⁻ concentration from salinized Floridana soil. The high clay content (30%) in Floridana soil could have reduced the leaching fraction and increased the salt ion accumulation (see Fig. 3). Substrate type also influenced Cl⁻ and Na⁺ distribution within seedlings because the highest root Cl⁻ and Na⁺ concentrations occurred in salinized seedlings from Candler sand while they maintained the highest leaf dry weight and lowest leaf Cl concentrations. Thus, growing Carrizo seedlings in Candler sand increased the ability of roots to accumulate salt ions avoiding their accumulation in leaves and allowing greater growth under

salt stress. We did not evaluate root morphology, but it is possible that substrate-induced differences in root characteristics affected Na⁺ and Cl⁻ distribution by changes in Na⁺ and Cl⁻ uptake and transport (Storey, 1995). When grown in aerated solution culture, rates of net Na⁺ and Cl⁻ uptake and transport were two- to eightfold higher than ion uptake by roots in sand culture. Clearly, roots grow and behave differently in different growth media.

In salinized citrus, decreases in A_{CO2} are a consequence of high leaf Cl⁻ concentration because increasing leaf Cl⁻ concentration progressively decreases A_{CO2} (Bañuls and Primo-Millo, 1992; García-Sánchez et al., 2002). Despite the higher leaf Cl⁻ concentration in seedlings from Floridana soil, however, salinity-induced reductions in A_{CO2}, g_s, and E_{leaf} were similar for seedlings in all three substrates. At least under these conditions, therefore, leaf gas exchange was not a good indicator of growth and leaf Cl⁻ accumulation under salinity stress. This underscores the potential problem of describing relative salinity tolerance based solely on leaf gas exchange responses. The high leaf K⁺ concentration in Floridana-grown seedlings and the high leaf Mg²⁺ in peat-grown could have mitigated the negative leaf Cl⁻ effects on leaf gas exchange (Cakmak, 2005). Salinity-induced reductions in A_{CO2} were not accompanied by any decrease in C_i implying that reductions in A_{CO2} were the result of internal limitations (biochemical) rather stomatal limitations (García-Sánchez and Syvertsen, 2006). Despite the lack of salinity effects on F_v/F_m and Y, the increase in F₀ by the salt treatment supported the idea that decreased A_{CO2} was related to biophysical damage of photosystem II (PSII; Paranychniak and Chartzoulakis, 2005). An increase of F₀ can be interpreted as a reduction of the rate constant of energy trapping by PSII centers resulting from a physical dissociation of the light harvesting complex in the PSII core (Havaux and Lannoye, 1983).

Although all seedlings were well-watered, seedlings in soilless peat had higher predawn leaf water potentials reflecting the higher available water content of soilless peat than of the two soil types. However, substrate type had no effect on leaf turgor or RWC. Salinity reduced both Ψ_w and Ψ_π such that leaf turgor was increased in seedlings grown in Floridana soils and soilless peat. As was shown previously (García-Sánchez and Syvertsen, 2006; Melgar et al., 2008), salinity-induced reductions in growth and net gas exchange were not the result of loss of turgor, but more likely the result of toxic ion accumulation in leaves.

In summary, based on the rankings of leaf growth and leaf Cl⁻ in well-irrigated salinized Carrizo seedlings, those grown in Candler soil had the highest salinity tolerance, whereas those grown in Floridana soil had the lowest salt tolerance. This could have been related to the high clay content in the Floridana soil resulting in higher salt stress than in the other substrates. The high leaf Ca²⁺ concentration in seedlings from Candler

sand could have mitigated the negative effects of Cl^- and Na^+ toxicity. Substrate type also changed plant allocation patterns of Cl^- and Na^+ under salinity stress because seedlings grown in Candler sand accumulated high salt concentrations in roots while limiting salt ion transport to shoots. Comparative studies of crop responses to salinity and relative salt tolerance should consider the potential contributions of growth substrate.

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