



Salinity Tolerance of ‘Hamlin’ Orange Trees on the Hybrid Rootstocks US-897 and x639 Is Greater than of Trees on Cleopatra Mandarin

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Tree growth, leaf gas exchange, root and leaf Cl and Na concentrations of salinized 2-year-old ‘Hamlin’ orange trees were evaluated in a greenhouse. We compared trees on the relatively salt tolerant Cleopatra mandarin (Cleo) rootstock with two of its commercial hybrid rootstocks, Cleo × Flying Dragon trifoliolate (TF; US-897) and Cleo × Rubidoux TF (x639). Trees on these rootstock hybrids have some horticultural advantages over the parent type Cleo but their relative tolerance to salinity has not been tested. Well-fertilized and well-watered trees were grown in a high peat, soilless potting mix and treated with 0, 30 or 60 mM NaCl (maximum EC= 7.36 dS·m⁻¹ or TDS = 5,152 ppm) for 4 months. Trees on Cleo were the smallest, had the highest root/shoot (Rt/Sh) dry weight ratio, and used the least water regardless of salinity level. Trees on US-897 grew the most and had the lowest RT/SH ratio. The lower Rt/Sh ratios of trees on both hybrid rootstocks than of trees on Cleo, implied that the hybrids had more efficient root systems that support greater shoot growth than Cleo root systems. Total tree dry weight (TDW) of trees on all three rootstocks was reduced similarly by salinity as Rt/Sh ratio was affected little by salt stress. Rootstock had little effect on leaf Na but leaf Cl concentration and Cl accumulation were lowest in the smallest trees on Cleo. Roots of x639 had some ability to sequester Cl at the intermediate salt level (30 mM) but this ability was overcome at 60 mM as all leaves accumulated more Cl than roots at the high salinity level regardless of rootstock. Net assimilation of CO₂ (ACO₂) was lowest in leaves on Cleo at 0 and 30 mM NaCl but high salinity reduced ACO₂ similarly across rootstocks. Reductions in leaf gas exchange were more strongly related to high concentrations of leaf Cl than to high leaf Na. The greater shoot growth and higher leaf Cl levels of trees on both US-897 and x639, support the idea that both hybrid rootstocks were similarly more salt tolerant than Cleo.

There has been recent interest in two citrus rootstocks, US-897 and x639, that are hybrids between Cleopatra mandarin (Cleo) and Flying Dragon trifoliolate (TF). Trees grafted on US-897 were relatively small with high yield efficiency and are relatively tolerant to *Phytophthora* spp. (Bowman et al., 2002). In addition, trees on US-897 appear to be tolerant or resistant to HLB as they do not develop distinct visible leaf symptoms in the presence of the disease (Albrecht and Bowman, 2011). Trees on x639 were larger, higher yielding trees, with better quality fruit than those on Cleo (Castle et al., 2011). In addition, x639 has been proposed as a potential replacement for sour orange rootstock in calcareous, poorly drained soils (Stover et al., 2004).

Managing salinity in Florida citrus is a continuing problem (Syvertsen et al., 1989; Zekri et al., 2010) especially in flatwoods during periods of low rainfall (Boman and Stover, 2002). There is a broad range of salinity tolerance among commercial citrus rootstocks. Trees on Cleo for example, are considered to be a relatively salt tolerant (Castle et al., 2006). Leaves of trees on Cleo accumulate relatively low concentrations of Cl (Syvertsen et al., 2010), whereas leaves on *Poncirus trifoliata* (L., TF) accumulate high levels of Cl but low levels of Na (Levy and Syvertsen, 2004). There have been some observations regarding the superior salinity tolerance of trees on US-897 and x639 over trees on Cleo growing at the same location with moderately saline (EC ≈ 1.57 dS·m⁻¹ or

1100 ppm TDS) irrigation water (Pete Spyke, Pers. Comm.), but their relative tolerance to salinity has not been tested (Castle et al., 2006). Seedlings of US-897 and x639 were more salt tolerant than Cleo seedlings in a recent greenhouse study (Syvertsen and Bandaranayake, 2011). Based on that work, we hypothesized that ‘Hamlin’ orange trees on US-897 and x639 rootstock would be larger and more salt tolerant than trees on Cleo.

Materials and Methods

This experiment was carried out in a greenhouse from March through Aug. 2011 at the UF/IFAS Citrus Research and Education Center, Lake Alfred (lat. 28°N, long. 82°W; elevation 51 m or 168 ft). Eighteen 2-year-old ‘Hamlin’ (*Citrus sinensis* L. Osbeck) orange trees grafted on either Cleopatra mandarin (Cleo, *C. reticulata*), on US-897 (Cleo × Flying Dragon trifoliolate, *Poncirus trifoliata*) or on x639 (Cleo × Rubidoux trifoliolate) rootstocks, were purchased from a local nursery. Trees were grown in 2-L (0.5 gal) 25 cm (10 inch) deep × 12-cm (5 inch) diameter pots of well-drained commercial soilless media containing a mixture of peat/perlite/vermiculite at 3:1:1 by volume and were about 1 m (39 inches) tall. At the end of March, all trees were topped at about 0.8 m (31 inches) to encourage new growth and grown in an unshaded greenhouse under natural photoperiods during the summer when maximum photosynthetically active radiation (PAR; LI-170; LICOR, Inc., Lincoln, NE) at plant level was about 1200 μmol·m⁻²·s⁻¹. Average day/night temperature was 38/25 °C (100/77 °F) and the relative humidity varied diurnally from 40% to 100%.

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Trees were irrigated thoroughly with a dilute 7:2:7 fertilizer solutions of 120 mg·L⁻¹ (= ppm) N and K and 34 mg·L⁻¹ P plus 5 mg·L⁻¹ iron. After 4 weeks, the three salinity treatments, 0, 30, or 60 mM NaCl, were introduced gradually to the fertilizer solution in increasing increments of 10–20 mM per day to avoid osmotic shock. This resulted in three final irrigation solutions, beginning 4 May, with average ECs of 1.29, 4.21, and 7.36 dS·m⁻¹ (TDS = 903, 2947, and 5152 ppm). Irrigation treatments were applied every second day beginning with 350 mL (12 oz) per pot [=42 mg (6.4 × 10⁻⁴ oz) of N per plant per week] and volumes were increased gradually as plants grew, to a maximum of 430 mL (15 oz) per plant or 44 mg N (1.5 × 10⁻³ oz) per tree per week. Thus, all trees were well-fertilized and watered; these volumes were sufficient to leach about 15 to 20 mL (0.5 to 0.7 oz) after each irrigation. Salt treatments were maintained for 4 months (May through August). The experimental design was a 3 × 3 factorial design with 3 rootstocks, 3 salinity treatments, and 6 replicate trees in each treatment. One week prior to beginning salinity treatments and again 1 week after treatments began, plants were sprayed for mites and thereafter, trees were sprayed every other week with alternating sprays of 1% oil or soap solution. About every 3 weeks during the course of the experiment, the three treatment blocks were randomized within the greenhouse.

Whole plant transpiration (EWP) was measured 3.5 months after treatments began after pots were watered, drained, and covered with a plastic bag sealed at the base of the stem. Pots were weighed for 2 consecutive days, and their 24-h weight loss averaged. Total leaf areas were measured with a leaf area meter (LI-3000; LI-COR) at harvest and the results used to calculate EWP in mg·m⁻²·s⁻¹. Net assimilation of CO₂ (A_{CO₂}), leaf transpiration (E_{lf}), stomatal conductance (g_s), intercellular CO₂ concentration (C_i) and leaf water use efficiency (WUE = A_{CO₂}/E_{lf}) were determined with a portable photosynthesis system (LI-6400; LI-COR). All measurements were made during the morning from 0800 to 1000 HR to avoid high temperatures and low humidity in the afternoon. During measurements, leaf temperature was 32 ± 2 °C (≈89 °F) and leaf-to-air vapor pressure difference (VPD) was 2.4 ± 0.4 kPa (≈0.02 atm) within the cuvette. Measurements were made on a single leaf on each of the 6 replicate trees in each treatment. Using the same leaves used for net gas exchange measurements, relative leaf greenness was measured with a SPAD meter (SPAD-502) meter (Minolta Corp., Ramsey, NJ) and leaf chlorophyll concentration was estimated (Jifon et al., 2005).

After 4 months of treatments, plants were gently uprooted, roots were washed, and total leaf area was measured. Leaves, stems, and roots were oven dried at 60 °C (140 °F), weighed and ground to a powder. Fibrous root and leaf tissue N, Na, and Cl concentrations (percent dry weight) were determined by a commercial laboratory (Waters Agricultural Lab, Camilla, GA). Total Na and Cl contents in roots and leaves were estimated by multiplying tissue dry weights by their determined concentrations and expressed in mg. Salinity treatment effects were compared using regression analysis (SAS Institute Inc., Cary, NC).

Results and Discussion

Leaf greenness readings only varied from 69–74 units, which corresponded to an average of about 3.1 to 3.3 g·m⁻² of chlorophyll (Jifon et al., 2005). Although leaves on trees grown with 60 mM NaCl tended to have lower chlorophyll concentrations than those on trees grown at 0 mM NaCl, there was no significant effect of

salt treatment or rootstock on leaf chlorophyll (data not shown). Average leaf N concentration of these well-fertilized trees varied from 3.3% to 3.8% (well above sufficient; Obreza and Morgan, 2008) with no effect of salt treatment or rootstock on leaf N. Average fibrous root N concentration varied from 2.5% to 2.8% with again, no effect of salt treatment or rootstock on root N.

Under these growth conditions, all trees appeared to grow well; towards the end of the experiment, there was some visible tip burn in 60 mM treated trees on Cleo but no leaf drop was observed. Based on whole tree dry weight (TDW; Fig. 1A) and

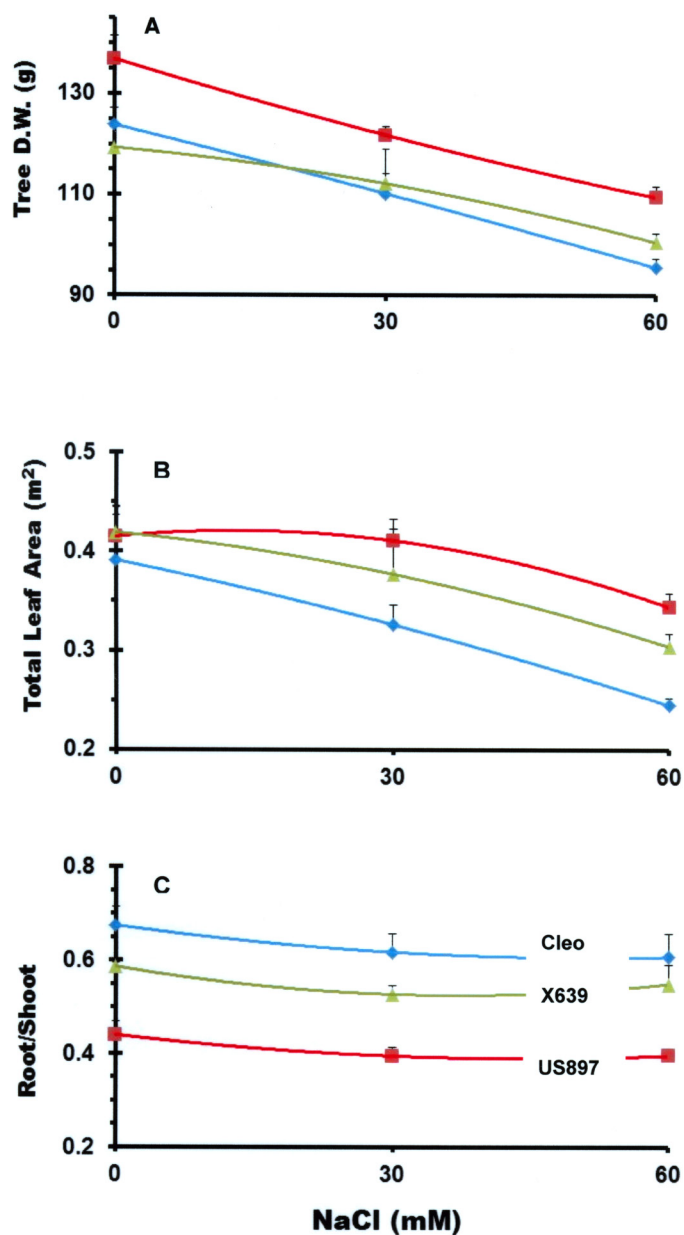


Fig 1. Effects of 0, 30, or 60 mM NaCl added to the irrigation water on (A) whole tree dry weight (TDW), (B) total leaf area, and (C) root / shoot dry weight ratio of 2-year-old 'Hamlin' orange trees on US-897 (squares), x639 (triangles), or Cleopatra mandarin (diamonds) rootstocks. Each symbol is the mean of n=6 for trees on each rootstock; vertical bars = ±1SE. Best-fit linear or polynomial regression lines were fit to the n=18 data for each rootstock.

total leaf area (Fig. 1B), trees on Cleo were the smallest and those on US897 were the largest. Canopy growth was similarly reduced by salinity regardless of rootstock. The root/shoot (Rt/Sh) dry weight ratio was decreased slightly by salinity in trees on all three rootstocks (Fig. 1C); the Rt/Sh of Cleo was highest and that of US-897 was lowest across all salinity treatments. Although root and shoot DW were both decreased by salinity, total Sh DW of US-897 was greatest but its root DW was lowest (data not shown), which resulted in the lowest Rt/Sh for trees on US-897. The lower Rt/Sh ratios of trees on both hybrid rootstocks than of trees on Cleo, implied that the hybrids had more efficient root systems that can support greater shoots growth than Cleo root systems. Roots of Cleo seedlings are known to have a lower hydraulic conductivity than roots of *Poncirus trifoliata* or its hybrid Carrizo citrange (Syvertsen and Graham, 1985).

Leaf dry weight/area ratio (LDW/A), an estimate of leaf thickness or tissue density, was increased by salinity 79 to 87 g·m⁻² at 0 mM to 93 to 101 g·m⁻² at 60 mM and was unaffected by rootstock (data not shown). Such increases in LDW/A are common responses to reduced growth from salinity stress (García-Sánchez and Syvertsen, 2006).

All leaf Na concentrations in salinized treatments exceeded levels considered to be toxic for bearing trees (0.25%; Obreza and Morgan, 2008) even at the moderate salinity (30 mM NaCl; Fig. 2A). Root and leaf Na concentrations of trees on US-897 and x639 were comparable but leaf Na concentrations of leaves

on Cleo exceeded root Na concentrations (Fig. 2A–B). The moderate salinity treatment (30 mM) resulted in leaf Cl levels that exceeded toxic levels (0.7%; Obreza and Morgan, 2008) in leaves on US-897 and x639 but not in leaves on Cleo (Fig. 2C). Leaf Cl concentrations at 60 mM were about 1% regardless of rootstock. Root Cl concentrations of x639 at 30 mM were relatively high but this Cl was apparently not transported to the ‘Hamlin’ leaves (Fig. 2D). Root Cl concentrations at 60 mM were similar among rootstocks and were also similar to leaf Cl concentrations.

Despite salinity induced decreases in tree growth, total leaf and root Na content (in grams) was generally increased by salinity (Fig. 3A–B). Leaf Na content was higher than root Na content but total Na accumulation in leaves and roots was not affected by rootstock. Total leaf Cl content was increased by salinity in all trees; leaves on US-897 and x639 had higher Cl content than leaves on the smaller Cleo trees (Fig. 3C). Thus, US-897 and x639 not only took up higher amounts of Cl than Cleo, but they also transported higher amounts of Cl to leaves than Cleo. Although leaf Cl accumulation has been negatively related to growth under salinity stress (Syvertsen et al., 2010), it can be difficult to establish if Cl uptake limits growth or if low growth limits Cl uptake. In this case, however, leaf Cl concentrations (Fig. 2C) and leaf Cl contents (Fig. 3C) followed similar patterns underscoring the greater Cl tolerance of trees on US-897 and x639 than of trees on Cleo. Roots of x639 had the highest total Cl content at 30 mM (Fig. 3D). Due to reduced root growth at high salinity, however,

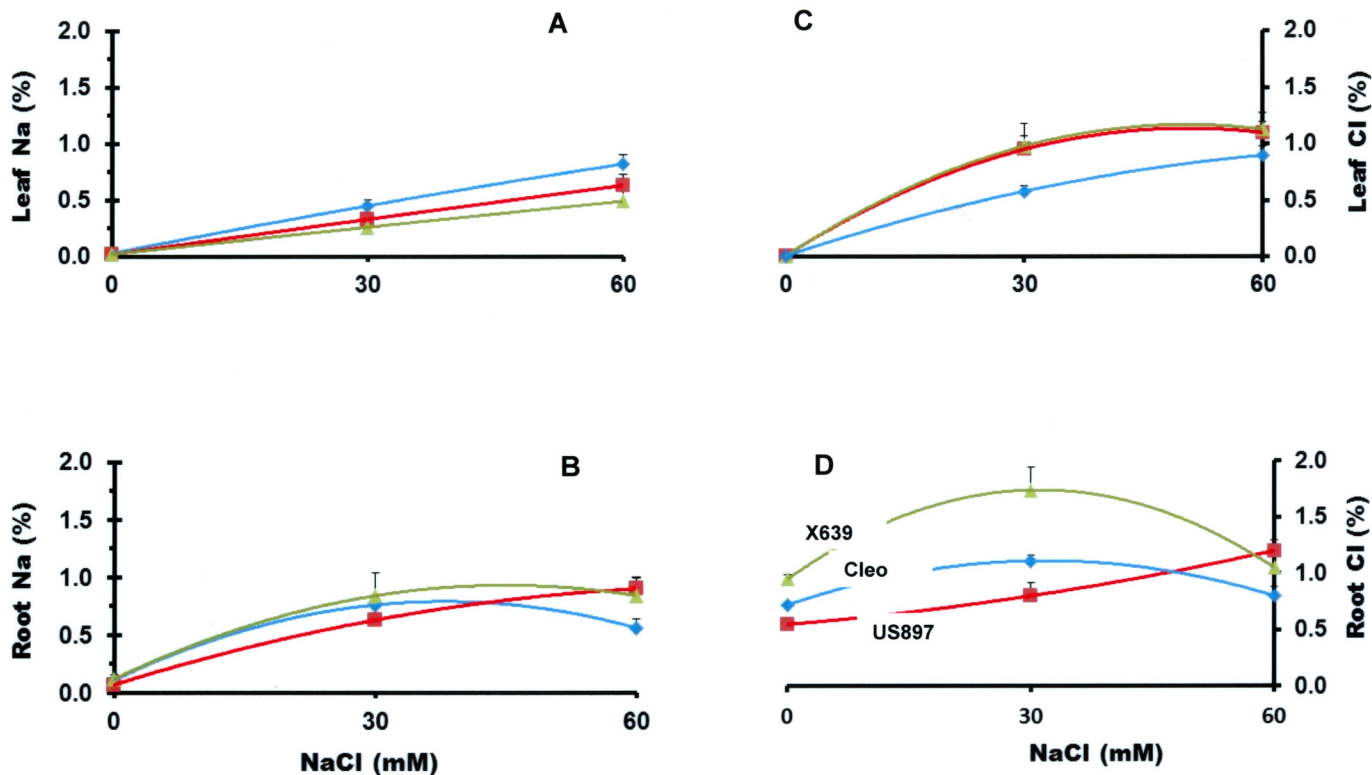


Fig 2. Effects of 0, 30, or 60 mM NaCl added to the irrigation water on (A) leaf Na, (B) root Na, (C) leaf Cl, and (D) root Cl concentrations (percent dry weight) of 2-year-old ‘Hamlin’ orange trees on US-897 (squares), x639 (triangles), or Cleopatra mandarin (diamonds) rootstocks. Each symbol is the mean of n=6 for trees on each rootstock; vertical bars = ±1SE. Best-fit linear or polynomial regression lines were fit to the n=18 data for each rootstock.

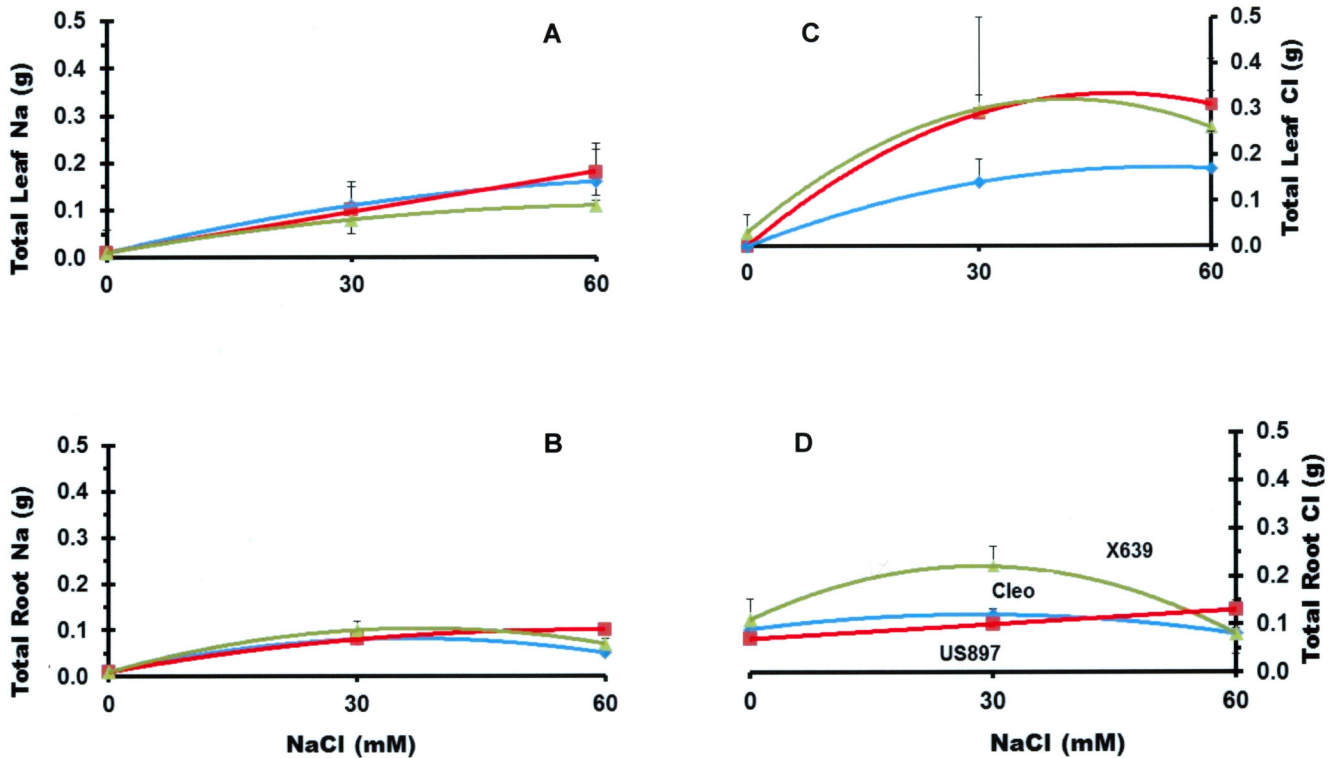


Fig 3. Effects of 0, 30, or 60 mM NaCl added to the irrigation water on total (A) leaf Na, (B) root Na, (C) leaf Cl, and (D) root Cl contents (g) of 2-year-old 'Hamlin' orange trees on US-897 (squares), x639 (triangles), or Cleopatra mandarin (diamonds) rootstocks. Each symbol is the mean of n=6 for trees on each rootstock; vertical bars = ±1SE. Best-fit linear or polynomial regression lines were fit to the n=18 data for each rootstock.

total root Cl contents at 60 mM NaCl differed little from root Cl contents at 0 mM and were not affected by rootstock.

Net assimilation of CO₂ by leaves was reduced similarly by salinity in trees on all three rootstocks but overall, leaf ACO₂ was more strongly related to leaf Cl concentration ($r = -0.47$, Fig. 4A) than to leaf Na concentration ($r = -0.38$, $P < 0.05$). Average leaf ACO₂ was highest in leaves on US-897 at high salinity (data not shown). Leaf transpiration (E_{tr}) was also negatively related to leaf Cl concentration (Fig. 4B). Overall, the measured EWP from weight loss measurements were correlated E_{tr} measurements from leaf gas exchange ($r = 0.27$ at $P < 0.05$). Leaf WUE also decreased with increased leaf Cl concentration (Fig. 4C). Although high leaf WUE can be related to high salt tolerance (Syvertsen et al., 2010), there was no significant effect of rootstock on leaf WUE. Calculated C_i increased from an average of 282 $\mu\text{mol}\cdot\text{mol}^{-1}$ of CO₂ in 0 mM NaCl leaves to 298 $\mu\text{mol}\cdot\text{mol}^{-1}$ of CO₂ in 60 mM leaves regardless of rootstock (data not shown). The increased C_i with salinity supports the idea that the decreases in net gas exchange were not due to decreases in stomatal conductance but rather to direct effects of salinity on ACO₂. In previous studies, salinity stress in well watered citrus seedlings reduced both leaf water potential and osmotic potential such that leaf turgor was increased above that on non-salinized seedlings (García-Sánchez and Syvertsen, 2009). Thus, the salinity-induced reductions in growth and net gas exchange in this and in previous studies were probably not the result of loss of turgor but more likely the result of toxic Cl ion accumulation in leaves.

Conclusions

'Hamlin' trees on Cleo accumulated the lowest concentrations of Cl in leaves, supporting the previously known relatively high salt tolerance of trees on Cleo (Castle et al., 2006). However, the low accumulation of Cl in leaves on Cleo and also in Cleo roots was a function of the lower growth, lower water use and higher Rt/Sh ratio of trees on Cleo than of trees on the two rootstock hybrids. Trees on both x639 and US-897 allocated relatively more growth to shoots than to roots than trees on Cleo regardless of salinity. Although salinized trees on both x639 and US-897 accumulated higher levels of Cl than trees on Cleo, they continued to grow better than trees on Cleo and produced more total leaf area especially trees on US-897. Leaf ACO₂ and WUE were reduced similarly by salinity across the three rootstocks and leaf Cl was more important than leaf Na in reducing leaf function.

Relative growth and physiological responses to salinity by trees on these rootstocks was similar to responses of these rootstocks as seedlings (Syvertsen et al., 2010). Based on total plant growth and tolerance to high leaf Cl, 'Hamlin' trees on x639 and US-897 had higher salt tolerance than trees on Cleo.

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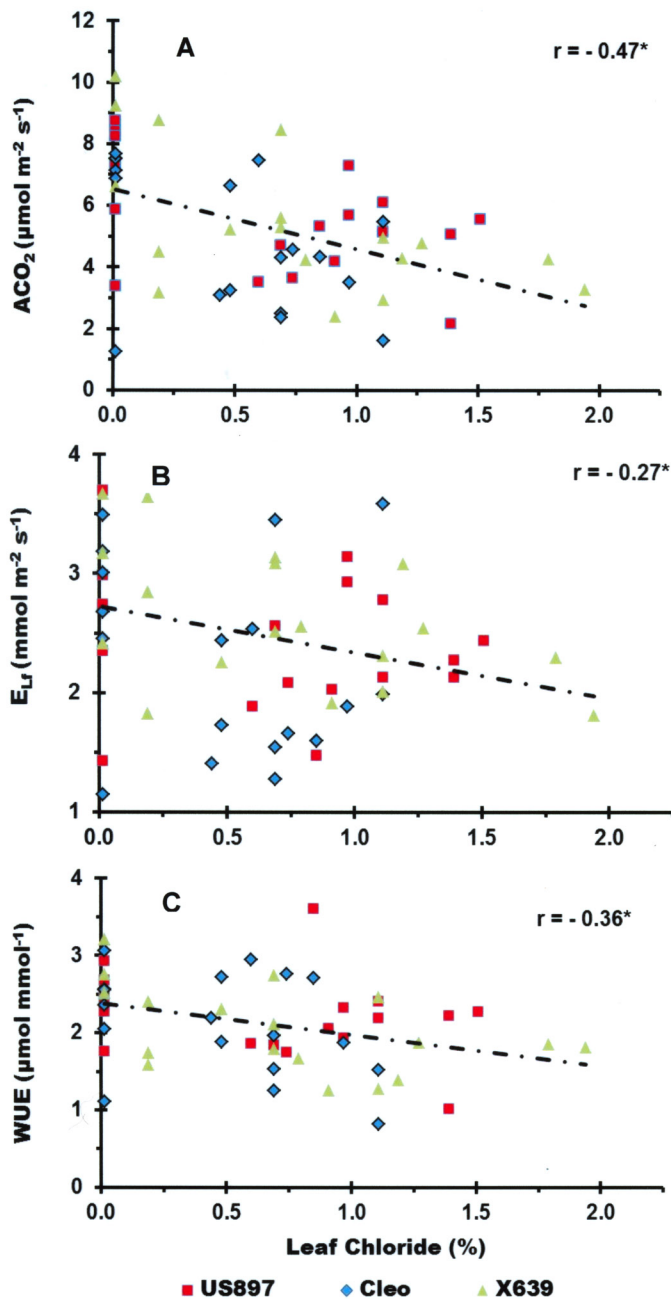


Fig 4. Effects of leaf Cl concentrations (percent dry weight) on (A) net assimilation of CO₂ (ACO₂), (B) leaf transpiration (E_{tr}), and (C) water use efficiency (WUE) of leaves on 2-year-old 'Hamlin' orange trees on US-897 (squares), x639 (triangles), or Cleopatra mandarin (diamonds) rootstocks. Each symbol represents a single tree; best-fit linear regression lines were fit to the n=54 combined data for trees on all three rootstocks. Critical r (n - 2 = 52) at P < 0.05 = ±0.27.

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