Salinity Tolerance and Leaf Water Use Efficiency in Citrus

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Abstract. In three separate experiments, the growth and water use of salinized citrus rootstock seedlings and grafted trees were modified using different growth substrates, elevated CO\(_2\), or 50\% shade screen under field conditions. By reanalyzing previously published data, we tested the hypothesis that salinity tolerance in citrus can be characterized as the ability to maintain low levels of leaf Cl\(^-\) accumulation through high plant growth and high water use efficiency (WUE) under saline conditions. Well-irrigated salinized seedlings of the relatively salt-sensitive Carrizo citrange [Carr (\textit{Citrus sinensis} \times \textit{Poncirus trifoliata})] were grown in sand, clay, or a peat-based soilless media. Salinity stress reduced plant growth and water use. Leaf Cl\(^-\) concentration was negatively related to plant growth, but leaf Cl\(^-\) increased with transpiration rate in low-saline treatments. In a second experiment using salinized seedlings of the relatively salt-tolerant Cleopatra mandarin [Cleo (\textit{Citrus reticulata})] grown along with Carr seedlings with or without elevated CO\(_2\), leaf Cl\(^-\) was negatively related to growth and to shoot/root dry weight ratio, but was positively related to water use such that leaf Cl\(^-\) was negatively related to leaf WUE. In a third experiment using salinized 2-year-old ‘Valencia’ orange (\textit{C. sinensis}) trees grafted on Cleo or Carr rootstocks and grown with or without shade cloth, leaf Cl\(^-\) was positively related to leaf transpiration as both were higher in the spring than in the fall, regardless of rootstock or shade treatment. Overall, leaf Cl\(^-\) was positively related to water use and was negatively related to leaf WUE. High growth, low water use, and, consequently, high WUE of salinized citrus were related to low leaf Cl\(^-\). Such relationships can be used as indicators of salinity tolerance.

There is a wide range of plant growth and physiological responses to excessive salt ion accumulation and osmotic stress from high salinity (Yeo and Flowers, 1989). Due to the complexity of salt tolerance in crops (Flowers and Flowers, 2005) and trees (Levy and Syvertsen, 2004), attempts to improve the salt tolerance of plants have met with very limited success. Progress in improving salinity tolerance will come through an understanding of processes controlling the uptake and transport of Na\(^+\) and Cl\(^-\) in plants (Munns and James, 2003). In leaves of relatively salt-sensitive citrus (Maas, 1993), the accumulation of Cl\(^-\) is considered to be a more important limitation on growth and physiological responses than Na\(^+\) (Bañuls et al., 1997; Levy and Syvertsen, 2004; Lopez-Climent et al., 2008). Salt-induced defoliation, regulation of Cl\(^-\) transporter genes, and other physiological responses in citrus have been correlated with foliar Cl\(^-\) accumulation rather than with levels of Na\(^+\) or osmotic stress (Brumós et al., 2009). Citrus trees are almost always grafted on rootstocks, which not only affects tree water relations and growth (Castle et al., 1993), but also the amount of Cl\(^-\) and/or Na\(^+\) accumulated in foliage under saline conditions (Maas, 1993). For example, the citrus rootstock Cleopatra mandarin is relatively a Cl\(^-\) excluder, whereas Carrizo citrange is a Cl\(^-\) accumulator but a Na\(^+\) excluder (Storey and Walker, 1999). Although such root characteristics tend to be consistent even when rootstocks are grown as seedlings in a greenhouse, there are often confusing interpretations of the relative rankings of rootstock tolerance to salinity in the literature depending on variations in salinity treatment, duration, scion type, growth substrate, and whether growth or physiological responses were measured (Levy and Syvertsen, 2004). For example, salinity-induced reductions in citrus leaf gas exchange (García-Sánchez and Syvertsen, 2009) or chlorophyll fluorescence parameters (García-Sánchez and Syvertsen, 2006; Lopez-Climent et al., 2008) are not always good indicators of plant growth and leaf Cl\(^-\) accumulation.

Citrus leaves can osmotically adjust to saline conditions such that the relative water content of leaves is maintained even under concomitant drought stress (Pérez-Pérez et al., 2007), and under well-watered saline conditions, osmotic adjustment often increases leaf turgor above that of non-salinized leaves (García-Sánchez and Syvertsen, 2006). Leaf Cl\(^-\) concentration and, thus, relative salt tolerance has been linked to plant growth, water use (Castle and Krezdorn, 1975; Syvertsen et al., 1989), and transpiration (Moya et al., 1999, 2003). Because rapidly growing trees always use more water than slower-growing trees, leaves on rapidly growing trees would be exposed to relatively more Cl\(^-\) in the transpiration stream than leaves on slower-growing trees (Syvertsen et al., 1989). Thus, at least part of the mechanism underlying high salt tolerance or the accumulation of relatively low leaf Cl\(^-\) in some citrus rootstocks may be related to their low growth vigor, low water use, and/or a high shoot/root ratio (Moya et al., 2003).

Plant growth, net CO\(_2\) assimilation (\(A_c\)), and water use efficiency (WUE) of most crop plants can be increased when plants are grown in elevated CO\(_2\) (eCO\(_2\)), but at the same time,
leaf transpiration ($E_{\text{lit}}$) and plant water use usually are decreased (Bowes, 1991; Chen and Lenz, 1997). Therefore, growing plants at eCO2 offers a mechanism to separate plant growth from water use, two processes that are normally tightly coupled. Even salt-stressed plants generally grow more at eCO2 than at ambient levels of CO2 (Ball and Munns, 1992). If water use and salt uptake are indeed linked (Moya et al., 2003), then increasing WUE in eCO2 should result in reduced rates of salt accumulation in the leaves. Elevated CO2 increased growth and decreased leaf Cl$^-$ and Na$^+$ in the salt-sensitive Carr seedlings, but tended to increase both ions in leaves of the more salt-tolerant Cleo (García-Sánchez and Syvertsen, 2009).

The effects of salinity stress can be worse in full sun than in shade in evergreen species (Tattini et al., 2006). In citrus, 50% shade screens reduced excessively high leaf temperatures and leaf-to-air vapor pressure differences (VPD) at midday such that leaf $A_C$ and WUE were increased above that of unshaded leaves (Syvertsen et al., 2003). Although $E_{\text{lit}}$ and whole plant water use are changed little by shade (Jifon and Syvertsen, 2003a, 2003b), shade-induced increases in WUE (García-Sánchez et al., 2006) may be related to differences in leaf Cl$^-$ of salinized ‘Valencia’ orange trees as determined by rootstocks with different abilities to exclude Cl$^-$. We used the modified growth and water use of salinized citrus rootstock seedlings (García-Sánchez and Syvertsen, 2006, 2009) and grafted trees (García-Sánchez et al., 2006) to test the hypothesis that modified growth, water use, and WUE of salinized citrus reflects salinity tolerance as determined by leaf Cl$^-$ concentration. Such responses may yield insights into mechanisms of salinity tolerance, including processes controlling the uptake and transport of Cl$^-$ in citrus, and may provide clear indices of salinity tolerance.

**Materials and Methods**

All experiments were carried out at the University of Florida/Institute of Food and Agricultural Sciences Citrus Research and Education Center, Lake Alfred (lat. 28°9’ N, long. 81°73’ W; elevation 51 m). Salinity stress was imposed using 50 mM Cl$^-$, a level known to induce physiological damage (Candler and Syvertsen, 2009). Candler soil is a well-drained typic quartzipsamment that is about 97% sand with 1% organic matter, whereas Floridana clay soil is a poorly drained loamy Argiaquoll that is 30% clay, 54% sand, and 1% organic matter (Table 1).

Seedlings were grown in a greenhouse under natural photoperiods during the late summer when maximum photosynthetically active radiation (PAR) at plant level was about 1200 mmol·m$^{-2}$·s$^{-1}$ (LI-170; LICOR, Lincoln, NE). The average daytime temperature was 36/21 °C and relative humidity varied diurnally from 40% to 100%.

Plants were irrigated every other day with a dilute solution of a complete fertilizer (8N–0.7P–6.6K) plus 6% iron chelate in a sufficient volume to leach from the bottom of all pots. Plants received about 21 mg of N per week. Two months after transplanting, 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. There were six replicate plants in each substrate and salinity treatment. After 9 weeks of treatment, salinized clay soil had a higher total electrical conductivity (EC) and Cl$^-$ concentration in the leaching fraction than the other substrates (Table 1). Leachates from the non-saline treatments had low EC and Cl$^-$ concentrations that were unaffected by substrate.

All leaf measurements used fully expanded mature leaves from the mid-stem area on each 5-month-old seedling. Net gas exchange (including $A_C$, $E_{\text{lit}}$, and WUE) of mature leaves was determined with a LI-COR portable photosynthesis system (LI-6200) using a well-stirred 0.25-L cuvette. All measurements were made in the morning from about 0800 to 1100 hr to avoid high afternoon temperatures and low humidity, which can cause midday depression of net gas exchange (Hu et al., 2009; Jifon and Syvertsen, 2003a). All measurements were made under saturating PAR ($\geq$800 µmol·m$^{-2}$·s$^{-1}$), leaf temperatures of 32 ± 2 °C, and VPD of 2.4 ± 0.4 kPa within the cuvette. Measurements were made on a single mature leaf on each of the six replicate plants in each substrate and salt treatment.

At the end of the experiment, whole plant transpiration ($E_{\text{wp}}$) was also measured gravimetrically by daily weight loss from each pot sealed in a plastic bag at the base of the stem, divided by the total leaf area at harvest, expressed in units of grams per square meter per hour. At harvest, plants were separated into leaves, stems, and roots, briefly rinsed in deionized water, and oven dried at 60 °C for at least 48 h.

**EXPT. 2: SALINITY AND ELEVATED CO2.** Uniform 2-month-old citrus rootstock seedlings of Cleo and Carr were transplanted into Candler sand and fertilized with a complete nutrient solution (8N–0.9P–6.6K + Fe-chelate), with or without additional

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**Table 1.** (Expt. 1) Percentages of sand, silt, clay, and organic matter [OM (from García-Sánchez and Syvertsen, 2009)] along with mean (n = 6), electrical conductivity (EC), and Cl$^-$ concentration in the leaching fraction collected from salinized (+S) and non-salinized (–S) Candler sand, Floridana clay soil, and a soilless peat mix.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>OM (%)</th>
<th>EC –S (dS·m$^{-1}$)</th>
<th>EC +S (dS·m$^{-1}$)</th>
<th>Cl$^-$ –S (ms)</th>
<th>Cl$^-$ +S (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candler sand</td>
<td>97</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2.4</td>
<td>10.0 b</td>
<td>2.3</td>
<td>82.2 b</td>
</tr>
<tr>
<td>Floridana clay</td>
<td>54</td>
<td>15</td>
<td>30</td>
<td>1</td>
<td>2.3</td>
<td>12.0 a</td>
<td>2.5</td>
<td>102.7 a</td>
</tr>
<tr>
<td>Soiless peat mix*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>81</td>
<td>2.0</td>
<td>9.8 b</td>
<td>2.0</td>
<td>82.6 b</td>
</tr>
</tbody>
</table>

*Pro-Mix (Premier Horticulture, Red Hill, PA).

*Means within a column followed by different lower case letter are significantly different via Duncan’s multiple range test at $P < 0.05$. 

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Table 2. (Expt. 3) The effect of the shade treatment above ‘Valencia’ orange trees on midday PAR, leaf temperature ($T_l$), and leaf-to-air vapor pressure difference (VPD) along with daylength (lat. 28°9’N) on the measurement dates at the end of the spring and fall salinization period.

<table>
<thead>
<tr>
<th>Date</th>
<th>Period</th>
<th>Treatment</th>
<th>PAR ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$T_l$ (°C)</th>
<th>VPD (kPa)</th>
<th>Day length (h:min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 June 2003</td>
<td>Spring</td>
<td>Sun</td>
<td>1884</td>
<td>37.5</td>
<td>3.53</td>
<td>13:56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shade</td>
<td>773</td>
<td>34.6</td>
<td>2.65</td>
<td></td>
</tr>
<tr>
<td>7 Nov. 2003</td>
<td>Fall</td>
<td>Sun</td>
<td>1302</td>
<td>34.7</td>
<td>3.03</td>
<td>10:57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shade</td>
<td>614</td>
<td>32.3</td>
<td>2.38</td>
<td></td>
</tr>
</tbody>
</table>

50 mM NaCl, and were grown at ambient CO$_2$ (360 μL·L$^{-1}$) or eCO$_2$ (700 μL·L$^{-1}$) in controlled environment greenhouses for 8 weeks (García-Sánchez and Syvertsen, 2006). Net gas exchange was evaluated as above on a single mature leaf on each of the eight replicate plants in each rootstock, salt, and CO$_2$ treatment. At the end of the experiment, $E_{wp}$ was measured gravimetrically and plants were harvested as above.

**Expt. 3: Salinity and shade.** We determined physiological responses to shade and salinity of well-watered and fertilized 2-year-old ‘Valencia’ orange trees grafted on Cleo or Carr rootstocks throughout one growing season (García-Sánchez et al., 2006). Twenty uniform trees on each rootstock were grown outdoors in 5-L plastic containers filled with native Candler sand. Trees were watered three times per week with 1 L of water with soluble fertilizer (9N–0.9P–7.5K), Ca(NO$_3$)$_2$, and iron-chelate (6%) with an N concentration of 66 mg L$^{-1}$. The 1-L volume of nutrient solution was enough to achieve leaching from the bottom of all containers.

The shade treatment was conducted from April to Nov. 2003 by placing 50% shade screens (Aluminet-50; Polysack Plastic Industries, Nir Yitzhak, Israel) on top of 2.2-m-tall PVC frames constructed over the trees (García-Sánchez et al., 2006). Two salinity treatments, 0 and 50 mM Cl$^-$ [NaCl and CaCl$_2$ (3:1)] added to the nutrient solutions, were evaluated on five trees on each rootstock in full sun or under the shade. The salinity treatment was begun at the same time as the shade treatment, but the salinity was applied during the 2-week dry period, 23 Apr. to 24 June and 18 Sept. to 21 Nov. At the beginning of each period, the salinity treatment was added in increasing increments of 15 mM Cl$^-$ per day during 2 d (consecutive) to avoid osmotic shock. On the third day, salinity was increased by 20 mM Cl$^-$ to reach the final concentration of 50 mM Cl$^-$. Although the shade treatment was maintained during the intervening typical summer rainy period (25 June–17 Sept.), the previously salinized trees were irrigated only as necessary with the standard nutrient solution without salt.

Leaf gas exchange was measured as above on selected clear days near the end of each salinity period under ambient full sun or shade. Measurements were made on two mature leaves chosen from the mid-shoot area of each tree, giving 10 replicate leaves per treatment. Leaves measured at the end of the dry salinity period (22 June) were spring flush leaves about 3 months old and leaves at the end of the fall salinity period (7 Nov.) were summer flush leaves about 4 months old. Average measurement conditions are summarized in Table 2. At the end of the experiment, trees were harvested and dried as above.

**Tissue Cl$^-$.** In all three experiments, near the end of each salinity period, dried mature leaves and dried fibrous roots were ground to a powder. Tissue Cl$^-$ concentration, expressed in percentage dry weight (DW), was measured using a silver ion titration chloridometer (Haake Buchler, Sandle Brook, NJ) after the tissue had been extracted with 0.1 N solution of nitric acid and 10% acetic acid. Titrations were calibrated against known chloride standards bracketing the range of Cl$^-$ in tissues. As an estimate of total Cl$^-$ accumulation, and leaf and root Cl$^-$ contents (in milligrams) were calculated by multiplying tissue DW by their Cl$^-$ concentration.

**Data analysis.** Data were analyzed using factorial analysis of variance (ANOVA; SAS version 9.1; SAS Institute, Cary, NC) and means were separated using Duncan’s multiple range test at $P < 0.05$. Within each of the three experiments, data were combined across treatments, and linear regression and Pearson’s correlation coefficients were used to investigate relationships between selected variables.

**Results**

**Expt. 1: Salinity and substrate type.** Salinized Carr seedlings growing in Floridana clay soil had higher leaf Cl$^-$ concentrations and grew less than seedlings in the other two substrates (García-Sánchez and Syvertsen, 2009; Fig. 1A). Across all substrates and salinity treatments, there was a negative relationship between leaf Cl$^-$ and total plant DW ($r = -0.85, P < 0.001$). In addition, there was a significant increase in leaf Cl$^-$ with increased $E_{wp}$ rates across the low salinity

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**Fig. 1.** (Expt. 1) Effects of growing Carrizo citrange seedlings in salinized [+S (solid symbols)] or non-salinized [-S (open symbols)] Candler sand, Floridana clay soil, or a soilless peat mix on relationships between leaf Cl$^-$ concentration and (A) total plant DW and (B) whole plant leaf transpiration ($E_{wp}$); +S = dashed line, all data and –S = solid line; ***, **, or * = correlations coefficients that are significant at $P < 0.001, 0.01,$ or 0.05, respectively.
treatments, but there was no relationship between leaf Cl− and 
E_{wp} in high salinity seedlings (Fig. 1B). Overall, larger plants, 
of course, used more water, as E_{wp} was correlated (r = 0.44, P < 0.01) to total plant DW. Leaf Cl− concentrations were correlated 
with leaf Cl− contents within low salinity treatments and overall salinity and substrate treatments (Fig. 2A). Root Cl− concentrations 
were similarly correlated with root Cl− contents regardless of substrate or salinity treatments (Fig. 2B).

**EXPT. 2: SALINITY AND ECO2.** Cleo seedlings were larger and 
had higher shoot/root DW ratio (Sh/Rt) than the same aged Carr seedlings regardless of CO2 level, and salinized Cleo seedlings 
had lower leaf Cl− concentrations than Carr seedlings (García-Sánchez and Syvertsen, 2006). Combining salinized data from 
both seedling types and both CO2 levels, leaf Cl− was negatively 
related to total plant DW (r = −0.56, P < 0.01) as larger plants 
had lower leaf Cl− than smaller plants (Fig. 3A). Leaf Cl− also 
was negatively related to Sh/Rt (r = −0.71, P < 0.001; Fig. 3B).

Across all CO2 and salinity treatments, leaf Cl− concentrations 
were again strongly correlated with leaf Cl− contents (r = 0.65, P < 0.001) and root Cl− concentrations were correlated with root Cl− contents (r = 0.75, P < 0.001; data not shown).

Elevated CO2 increased A_{C} and decreased E_{lf} in both species 
(García-Sánchez and Syvertsen, 2006). Overall, leaf Cl− increased with E_{wp} in salinized seedlings (r = 0.79, P < 0.001; Fig. 4A). In addition, because gravimetric E_{wp} was correlated to instantaneous E_{lf} (r = 0.53, P < 0.001), relationships between leaf Cl− and E_{wp} (data not shown) were very similar to those of leaf Cl− versus E_{lf}. Leaf Cl− decreased with increasing leaf WUE in salinized Carr (r = −0.71, P < 0.01), but the decrease in leaf Cl− with increasing WUE was not significant in salinized Cleo seedlings (Fig. 4B).

**EXPT. 3: SALINITY AND SHADE.** The shade treatment lowered midday PAR by 59% during the spring period and by 53% in the fall relative to the unshaded (sun) treatment (Table 2). Shade also decreased average leaf temperatures more than 2 °C and leaf-to-air VPD in the spring and fall. The daylength at this location (lat. 28°9'N) was almost 14 h at the end of the spring salinity period, but only about 11 h at the end of the fall period. The shade-induced decreases in midday leaf temperature and VPD did not affect ‘Valencia’ orange tree growth, Sh/Rt, or E_{lf}, but salinity stress did reduce leaf growth and E_{lf} (García-Sánchez et al., 2006). At the end of the spring period, ‘Valencia’ orange E_{lf} was almost three-fold higher than E_{lf} in the fall, regardless of rootstock or salinity treatment (Fig. 5A). In salinized trees, average leaf Cl− was about two-fold higher in the spring than in the fall. Combining all salinized data from the two seasons, leaf Cl− increased with E_{lf} (r = 0.78, P < 0.001) regardless of rootstock or shade treatment. In addition, leaf Cl− was also correlated with E_{lf} (r = 0.45, P < 0.01) in salinized trees in the fall, but not within the higher data from the spring. In salinized trees, leaf Cl− decreased with increasing leaf WUE regardless of rootstock or shade condition during growth (dashed line, r = −0.61, P < 0.01; Fig. 5B). In addition, when all data were combined regardless of treatment, the relationship between leaf Cl− and WUE was similar (solid line, r = −0.66, P < 0.01). Across all shade and salinity treatments, leaf Cl− concentrations again were strongly correlated with total leaf Cl− contents.
Discussed

Across all treatments within all three experiments, concentrations of Cl– in salinized leaves and roots were strongly correlated with total leaf and root Cl– contents, respectively (see Fig. 2). Thus, the overall negative relationships between tissue Cl– concentrations and growth characteristics in Expts. 1 and 2 appeared similar to relationships with total Cl– accumulation as estimated from tissue Cl– contents (data not shown).

All plants were well-irrigated. Leaf Cl– accumulated with water use because there were positive relationships between leaf Cl– and water use ($E_{lf}$ or $E_{wp}$) in all three experiments. However, the higher EC and Cl– concentration in the clay soil grown as seedlings (Levy and Syvertsen, 2004). Nonetheless, the growth of rootstock seedlings had higher leaf area growth of rootstock seedlings and lower water use at eCO2 were not only species dependent, but also involved whole plant growth and allocations of Na+ and Cl– (García-Sánchez and Syvertsen, 2006). Elevated CO2 also reduced leaf Cl– accumulation by the increased growth in the eCO2. In addition, patterns of Cl– and Na+ responses in leaves were opposite in direction to their respective responses in leaves. The modifications of citrus seedling responses to salinity by the higher growth and lower water use at eCO2 were not only species dependent, but also involved whole plant growth and allocations of Na+ and Cl– (García-Sánchez and Syvertsen, 2006). Altho...
WUE in salinized seedlings (Fig. 4). To the extent that leaf WUE can be used as a surrogate for whole plant growth or yield per water use, increased leaf WUE under salinity stress can be an indicator of tolerance to salinity. This is an important result as it shows that variations in leaf WUE, a potential integrator of growth and water use, can be used as an index of salt tolerance based on Cl– accumulation in citrus.

The regulation of leaf Cl– concentration in citrus leaves has been associated with $E_{L}$, total water absorbed per plant (Moya et al., 1999, 2003), Sh/Rt, and the efficiency of the root system for limiting the Cl– uptake (Storey and Walker, 1999). The higher exclusion of Cl– from shoots of trees on Cleo than on Carr was also related to the ability of roots to restrict the movement of Cl– because their Sh/Rt, leaf DW, and $E_{L}$ were similar (García-Sánchez et al., 2006). Citrus leaves growing in full sun experience high temperatures that decreased midday $A_{c}$, stomatal conductance ($g_{s}$), and WUE. Lowered leaf temperature from shading increased midday $A_{c}$, $g_{s}$, and leaf WUE but did not affect $E_{L}$. Nonetheless, seasonal variations in $E_{L}$ were related to variations in leaf Cl– concentrations in salinized ‘Valencia’ orange trees regardless of rootstock or shade treatment (Fig. 5). This is remarkable because such seasonal differences in instantaneously measured $E_{L}$ by gas exchange would be exaggerated if integrated over the longer days in the spring than in the fall. In addition, at the end of the fall salinization period, there were variations in $E_{L}$ that were attributable to differences in rootstock, but independent of shade. Leaves on trees with higher transpiration rates over the longer daylengths in the spring also had higher concentrations of leaf Cl–. There was a negative relationship between leaf Cl– and leaf WUE, again supporting the idea that increased leaf WUE under salinity stress can be an indicator of salt tolerance. These data, therefore, support our original hypothesis that high growth, low water use, and, consequently, high WUE of salinized citrus can result in low leaf Cl– and high salinity tolerance.

There have been many similar reports of physiological responses to salinity in trees and crop plants (Yeo and Flowers, 1989) and even reports underscoring the importance of WUE contributing to salt tolerance (Ball and Munns, 1992). However, this is the first study that summarizes the use of soil type, elevated CO2, and shade to modify growth and water use of salinized citrus and to link high WUE with low Cl– accumulation and salinity tolerance.

Although higher salinities over longer durations may have elicited different responses, it is clear that salinity stress from 50 mM NaCl for 8 to 9 weeks reduced growth and water use in well-irrigated citrus seedlings and grafted trees. Growth reductions by salinity were greater in the more salt-sensitive Carr seedlings and in ‘Valencia’ orange trees grafted on Carr rootstock than for Cleo seedlings and trees on Cleo, which was related to leaf Cl– concentrations (García-Sánchez et al., 2006). Such different effects of salinity on growth and leaf Cl– between Cleo and Carr were consistent with earlier findings (García-Sánchez et al., 2002) and underscore the importance of whole plant growth and salt ion allocation between root and shoots. Regardless of whether substrate type, eCO2, or shade modified growth and water use of salinized Carr or Cleo seedlings or of grafted ‘Valencia’ orange trees on these contrasting rootstocks, leaf Cl– accumulation was positively related to water use and negatively related to growth, Sh/Rt, and WUE. Thus, based on Cl– accumulation, the ability to maintain high WUE under salinity stress can be an important indicator of salt tolerance.

**Literature Cited**


