Salt Tolerance and Growth of 13 Avocado Rootstocks Related Best to Chloride Uptake

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Abstract. Avocado (Persea americana Mill.) is one of the most salt-sensitive crops and one of the highest value crops per acre. In the United States, avocados are grown primarily in California, in regions experiencing both scarcity of freshwater and salinization of available water supplies. Thus, our objectives were to evaluate avocado rootstocks for salt tolerance and evaluate the relationship between leaf ion concentrations, trunk diameter, leaf burn, and fruit yield. Our field experiment evaluated the salt tolerance of the Hass scion grafted onto 13 different avocado rootstocks using the Brokaw clonal rootstock technique. The experiment consisted of 156 trees arranged in a randomized complete block design with six replications of each saline [electrical conductivity (EC) = 1.5 dS·m⁻¹, Cl⁻ = 4.94 mmol·L⁻¹] and nonsaline (EC = 0.65 dS·m⁻¹, Cl⁻ = 0.73 mmol·L⁻¹) irrigation water treatment. We collected soil samples and leaves, then analyzed them for major ions. The rootstocks R0.06, R0.07, PP14, and R0.17, which had high concentrations of Cl and Na in the leaves, were the least salt tolerant, with 100% mortality in the rows irrigated with saline water for 23 months. The rootstocks R0.05, PP40, R0.18, and Dusa, which had low concentrations of Cl ions in the fully expanded leaves, were least affected by salinity, and these rootstocks exhibited the greatest yields, largest trunk diameters, and greatest survival percentages in the saline treatment. Yield and growth parameters correlated well with leaf Cl concentration, but not Na, indicating that salt damage in avocado is primarily a result of Cl ion toxicity. Under arid inland environments, no variety performed satisfactorily when irrigated with an EC = 1.5 dS·m⁻¹ water (Cl⁻ = 4.94 mmol·L⁻¹). However, the more tolerant varieties survived at soil salinity levels that would apparently be fatal to varieties reported earlier in the literature.

Avocado is considered one of the most salt-sensitive crops (Grieve et al., 2012) and one of the highest value crops per acre. The world avocado production in 2013 was 4.72 million tons—led by Mexico with 1.47 million tons, with the United States as the seventh largest producer at 0.175 million tons (FAO, 2018). Avocado production in California (the major U.S. producer) is increasingly affected by the scarcity of freshwater and relies on more saline waters as well as salinization of existing freshwater supplies. It is thus imperative that we develop not only proper irrigation scheduling and salinity monitoring to maintain productivity, but also to use rootstocks that are more tolerant to salinity.

Salinity has a broad range of effects on plants, and therefore there are also many different mechanisms for plants to tolerate this stress. Roy et al. (2014), classified these mechanisms into three main categories: osmotic tolerance, which is regulated by long-distance signals that reduce shoot growth and is triggered before shoot Na⁺ accumulation; ion exclusion, during which Na⁺ and Cl⁻ in the roots reduce the accumulation of toxic concentrations of Na⁺ and Cl⁻ within leaves; and, last, tissue tolerance, in which high salt concentrations are found in leaves but are compartmentalized at the cellular and intracellular levels (especially in the vacuole). Multiple researchers have found citrus and avocado rootstocks to vary in their capability to absorb and transport Na⁺ and Cl⁻ ions, resulting in differences salt tolerances. Oster and Arpaia (1992) showed that the tolerance level of the avocado scion is dependent on the rootstock used.

Normally, plant injury occurs first at the leaf tips (which is common for Cl toxicity) and progresses from the tip back along the edges as severity increases (Mass, 1984). Excessive necrosis is often accompanied by early leaf drop or defoliation. With sensitive crops, these symptoms occur when leaves accumulate from 0.3% to 1.0% Cl on a dry weight basis, but sensitivity varies among these crops. Many tree crops, for example, begin to show injury at more than 0.3% Cl (dry weight) (Ayers and Westcot, 1985).

Bernstein (1965) pointed out that for many fruit crops, plant damage could be related to the concentration of specific ions, such as Cl⁻ or Na⁺, in the soil solution and/or plant leaves rather than to total soil salinity. Such a tolerance classification is presented in Maas (1984), which shows that, for avocado, the maximum permissible Cl⁻ content in the root zone, expressed as the concentration in the saturation extract (Cl⁻ₑ), is 7.5, 6, and 5 mmol·L⁻¹; and in irrigation water (Cl⁻ₐₑ) is 5.0, 4.0, and 3.3 mmol·L⁻¹, respectively, for West Indian, Guatemalan, and Mexican rootstocks. These correspond to the maximum values to avoid leaf injury and reduction in fruit yield. Other researchers have focused on Na⁺ toxicity of tree crops rather than Cl⁻. For example, Na in the leaf tissue of tree crops in excess of 0.25% to 0.50% (dry weight basis) is often associated with Na⁺ toxicity (Ayers and Westcot, 1985). The ability to maintain low leaf Na⁺ concentration is considered a desirable trait in plants grown under saline conditions.

Previous studies have not established clearly whether avocado production under elevated salinity is impacted by Cl⁻ toxicity, Na⁺ toxicity, or both. Mickelbart et al. (2002), in an 80-d study with three rootstocks and four salinity levels (EC 1.5–6 dS·m⁻¹), concluded that the “relative tolerance of the various rootstocks appeared due primarily to their ability to exclude Na⁺ and Cl⁻ from the scion” when expected tolerance was evaluated indirectly by net CO₂ assimilation, chlorophyll concentration, and leaf necrosis. In a more detailed analysis from the same experiment, Mickelbart et al. (2007) found a good correlation between leaf necrosis of older leaves and leaf Cl⁻ concentration, as well as leaf necrosis with an “unbalanced” Na:K ratio in older leaves of two of the three rootstocks. Similarly, in a 130-d greenhouse study with Hass grafted to five rootstocks and an EC = 1.6 dS·m⁻¹ saline treatment, Castro et al. (2009) determined that the rootstock with the greatest CO₂ assimilation in the saline treatment was also the one with the lowest Cl⁻ concentration in leaves and the highest Na⁺ and highest Cl⁻ accumulations in the roots. They concluded that restriction of ion transport (Na⁺ and Cl⁻) to the leaves is one of the mechanisms associated with salinity tolerance of avocado. However, they found no interaction between vegetative parameters and salinity treatment, perhaps as a result of the short duration of the experiment. Oster et al. (2007), in a field study, found yield loss above ECₑ = 0.6 dS·m⁻¹, but found no evidence of Cl⁻ or Na⁺ toxicity.
Water savings have been achieved by successful use of more efficient irrigation systems, such as drip and microsprinkler, to irrigate avocado trees. However, there are limitations to reduced water application, as reduced leaching results in increased soil salinity, which impacts yield adversely. With the expectation that freshwater supplies will continue to be scarce in California, it is essential that we also expand the use of more efficient irrigation practices and lower quality waters (such as recycled water) in irrigated agriculture. The recent 3-year drought from 2014 to 2017 in California aggravated the trend of increasing scarcity and cost of freshwater. As a result of these considerations, California growers in the future will likely have to rely on more saline and/or lower quality waters for irrigation to conserve freshwater (Sheikh, 2017). Thus, our objectives were to screen 13 avocado rootstocks for salinity tolerance and study the relationship between leaf Na⁺ and Cl⁻ on growth and yield.

Materials and Methods

Plant material. A field experiment was conducted at the Agricultural Experiment Station at the University of California, Riverside (UCR), from 2013 to Mar. 2016 to evaluate the salt tolerance of the Hass scion grafted onto 13 different avocado rootstocks. The avocado rootstocks that were tested include selections from Westfalia (R0.05, R0.06, R0.07, R0.16, R0.17, and R0.18), UCR experimental selections (PP40 and PP45) and newly released proprietary material from UCR (PP4 ‘Zentmyer’, PP14 ‘Uzi’, and PP24 ‘Steddom’) (Menge et al., 2012), which are available for purchase by any nursery upon request. In addition, Thomas was used as a root rot-resistant standard and Dusa as the current industry standard (root rot resistant as well); Dusa is sold exclusively by Brokaw Nursery (Ventura, CA). All the rootstocks were grafted with scion of the dominant commercial variety, Hass, using the Brokaw clonal rootstock technique (Brokaw, 1987). Hass has the longest harvest season of avocado varieties, is currently the variety preferred by consumers, and therefore is the most used variety for new plantings in coastal zones of southern and central California (Bender, 2012).

Experimental design and treatments. The experiment consisted of 156 trees arranged in a randomized complete block design with a minimum of six replications and up to nine trees per rootstock. Trees were planted in May 2011 with 14 trees per row, 3.35 m between trees, and 6.40 m between rows. The plots were arranged in rows irrigated with non-saline irrigation water (control, EC = 0.65 dS·m⁻¹ and Cl⁻ = 0.73 mmol·L⁻¹) and saline water (EC = 1.5 dS·m⁻¹ and Cl⁻ = 4.94 mmol·L⁻¹). The control water was from Riverside Gauge Canal (groundwater from the Bunker Hill Basin at the base of the San Bernardino Mountains) whereas the saline water was a blend of Gauge Canal water (90%) and a concentrated saltwater solution (10%). The field was irrigated using one 45 L·h⁻¹ microsprinklers located beneath the tree canopy approximately 30 cm from the tree trunks.

Irrigation management. The field plot was irrigated based on the reference evapotranspiration (ET₀) data from the California Irrigation Management Information System (CIMIS). The CIMIS station used in this study is located at the Agricultural Experiment Station at UCR, about 800 m from our plots.

Crop evapotranspiration (ETc) was calculated as the product of the crop coefficient (Kc) and ET₀ from CIMIS:

\[ \text{ETc} = K_c \times \text{ET}_0. \]

A Kc value of 0.55 was used for young avocado trees based on an estimated canopy cover of 50% (Allen et al., 1998). Target irrigation was based on estimated ETc, plus a target of 20% leaching fraction (LF) (Hofshi and Hofshi, 2006), simulating common avocado irrigation practice. The LF was calculated as:

\[ \text{LF} = \frac{\text{Water applied} - \text{ETc}}{\text{Water applied}}. \]

The water applied is equal to the irrigation water plus precipitation. In Sept. 2014, we increased the irrigation water application by 10% to account for increasing tree size and reduced LF.

The field was irrigated two to three times per week depending on the water requirements. From October to April, each tree received between 8.52 and 14 L·d⁻¹ on nonraining days. When a rain event occurred, irrigation was adjusted accordingly. From May to September, each tree received from 24.61 to 46.56 L·d⁻¹. We monitored and recorded water applications to each treatment using water meters. The total precipitation in 2013 was 15.24 cm, 18.03 cm in 2014, and 16.51 cm in 2015.

The mean seasonal temperatures were as follow: 17 °C in Fall 2013, 15 °C in Winter 2013 and 2014, 19 °C in Spring 2014 and 2015, 24 °C in Summer 2014 and 2015, 19 °C in Fall 2014, 15 °C in Winter 2014, 18 °C in Fall 2015, and 14 °C in Winter 2015. The field was fertilized annually in April with the N fertilizer (UN-32), which was applied at a rate of 0.09 kg·tree⁻¹.

The saline treatment was imposed in three stages: quarter strength in Nov. 2013, half strength in Dec. 2013, and full strength in Jan. 2014. The irrigation water EC (ECᵣ) is just below the level at which yield loss is expected to start for the more sensitive Mexican race; Cl⁻ is slightly above the threshold level presented in Griewe et al. (2012). Ayers and Westcot (1985) indicated that the maximum permissible Cl⁻ in irrigation water without leaf injury was 4 mmol·L⁻¹ for a Guatemalan rootstock. Hass, used as the scion, is a hybrid between the Guatemalan (85%) and Mexican (15%) race (Bergh and Ellstrand, 1986). The rootstocks used in our study are hybrids of varying percentages of these two major races.

Table 1 shows the irrigation water composition for both water treatments. The saline irrigation water had a sodium adsorption ratio (SAR) of 4.7, where SAR is defined as ECNa⁺/ECₙ where concentrations are expressed in millimoles of charge (mmol·L⁻¹) per liter and an SAR of 4.7 is sufficiently low to avoid physical degradation of the soil at an EC = 1.5 dS·m⁻¹.

Soil characteristics. Soil samples were collected prior and during the implementation of the irrigation saline treatment. The gravimetric water content of the soil samples was measured by drying the sample in the oven at 105 °C for 24 h. Saturation paste extracts and 1:1 soil-to-water extracts were prepared and used to analyze for soil pH, EC, moisture content, and Cl⁻. The 1:1 soil extracts were converted to EC of saturation paste extract (ECₛ) using the relationship ECₛ = 3.33EC₁⁻, that we established for this soil. The soil is noncalcareous and does not contain gypsum, so the ECₛ calculation is essentially a correction for the relative water content of the two extracts.

To obtain a representative soil sample that characterizes the soil in which the avocado trees were grown, soil samples were collected 30 cm from the tree trunk in the wetted perimeter as well as in between trees in the row. Soil samples (730 per sampling period) were collected annually using an auger 5 cm in diameter, in 15-cm increments down to 75 cm. The soil samples were placed into plastic Ziploc bags to prevent evaporation and were transported to the laboratory, where moisture content was measured on arrival. Soil texture was determined by the hydrometer method adapted from Bouyoucos (1936) and was classified as an Arlington sandy loam (sand = 50.4%, silt = 41.1%, and clay = 8.5%) (USD, 1987).

Plant measurements. Leaf samples were collected in Oct. 2013, 2014, and 2015. Twenty fully expanded leaves were sampled from each tree from terminals that were not fruiting or flushing. Samples were weighed, washed, oven-dried, and ground by block digester in HNO₃, and subsequently analyzed for Ca, Mg, Na, K, P, S, Fe, Cu, Mn, and Zn using the Perkin Elmer Optima 3300 DV ICP OES (WinLab 32 for ICP Instrument Control Software, 2010; Perkin Elmer, Waltham, MA). Chloride concentrations were determined by wet digestion of the plant material and then analyzed by an amperometric

Table 1. Irrigation water ion composition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC (dS·m⁻¹)</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>HCO₃⁻</th>
<th>SO₂⁻</th>
<th>Cl⁻</th>
<th>NO₃⁻</th>
<th>SAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.65</td>
<td>3.09</td>
<td>0.45</td>
<td>1.65</td>
<td>0.07</td>
<td>1.50</td>
<td>2.68</td>
<td>0.73</td>
<td>0.35</td>
<td>1.24</td>
</tr>
<tr>
<td>Saline</td>
<td>1.50</td>
<td>5.15</td>
<td>1.90</td>
<td>8.89</td>
<td>0.14</td>
<td>1.35</td>
<td>9.41</td>
<td>4.94</td>
<td>0.38</td>
<td>4.74</td>
</tr>
</tbody>
</table>

EC = electrical conductivity; SAR = sodium adsorption ratio.
that of the control at the 0–15 cm depth, with an ECe of 3.20 and 4.10 dS m⁻¹ for the control and saline treatments, respectively (data not shown). There was also a decrease in the mean Cl⁻ concentration in the top 20 cm—from 16.4 mmol L⁻¹ in Aug. 2014 to 10.1 mmol L⁻¹ in 2015 in the saline treatment (data not shown). This decrease in ECe and soil extract Cl⁻ in the saline treatment is explained by the adverse effect of the saline treatment on avocado actual ETc, including extensive tree mortality and, consequently, lower water consumption and greater leaching.

Leaf injury. Based on the ANOVA results, there were significant differences in leaf burn in 2014 and 2015 (P < 0.05), as well as in rootstocks and treatments (saline and control). There were also rootstock × treatment and treatment × year interactions. In 2014, the control treatment had an average leaf burn of 39.6%. Leaf injury in the control is consistent with the Cl⁻ concentrations measured in the soil extracts.

All rootstocks in the salt-treated rows had extensive leaf burn, with Dusa, PP40, R0.18, and R0.05 ranking as the varieties having the least amount of leaf burn (Fig. 2). In 2014, there was a very high correlation (P < 0.004) between leaf burn and survival, with a Pearson correlation coefficient of −0.743, but no significant correlation with other parameters. Also, the leaf burn of the varieties in the saline treatment correlated significantly to the leaf burn in the control treatment. This suggests that soil salinity in the control was sufficiently high to cause varietal differences in leaf burn. It has been indicated that water with an ECw = 0.65 dS m⁻¹ or an ECe above 2 dS m⁻¹ is sufficient to result in yield loss in Hass grafted to Mexican rootstock (Oster et al., 2007).

Our control irrigation water was just below this ECw threshold, but the ECe was above the ECe threshold at 3.11 dS m⁻¹ in...
died after about 20 months of application of PP45, R0.06, R0.07, R0.16, and R0.17 all the saline treatment. The varieties PP14, but a very large number of trees died under control survived to the end of the experiment, –0.388. The weak correlation in 2015 is with a Pearson correlation coefficient of \( P(2015) \), there was only a weak correlation between leaf burn and survival (\( P = 0.20 \)) with a Pearson correlation coefficient of –0.388. The weak correlation in 2015 is explained by the observation that the salt-treated trees with greater leaf burn in 2014 were dead in 2015, thus no leaves could be sampled. Nonetheless, in 2015, leaf burn in the saline treatment also correlated weakly with relative fruit number (\( P = 0.10 \)), with a Pearson correlation coefficient of –0.476.

**Yield.** Avocado fruit was harvested in Feb. 2014; yield was low, as expected for young trees. Differences in yield were related primarily to fruit weight rather than fruit number. There were no significant varietal differences in the control, but Dusa (3.47 kg/tree) and PP40 (3.34 kg) were the highest yielding varieties in terms of fruit yield (measured in kilograms per tree) and number of fruit (Fig. 5). In the saline treatment only, Dusa yield was significantly greater than Thomas, PP14, PP5, PP14, and PP4. As shown in Fig. 5, the greatest fruit yield in the saline treatment was produced by Dusa (5.53 kg/tree), R0.18, R0.06, PP40, R0.05, and R0.18—mostly varieties that had the lowest leaf burn and highest survival.

In 2015, there were no significant yield differences among varieties in the control, but Dusa, R0.05, PP24, and PP40 were the highest yielding (Fig. 6A). Dusa, PP40, and R0.05 were also among the highest producing in the control in 2014. In 2015, there was further mortality of trees under the saline treatment. All yields in the saline treatment were low, with the top producers being R0.05, Dusa, PP40, and R0.18 (Fig. 6A).

Based on survival in 2015 (Fig. 4) and total yield (Fig. 6A), it is evident that no variety examined (with an initial soil ECe of 6–7 dS·m⁻¹) can be irrigated sustainably with an ECw = 1.5 dS·m⁻¹ with currently recommended water management under the climatic regime of inland southern California. These results are consistent with results of others (Oster et al., 2007; Shalhevet, 1994).

In 2016, the yields of the control increased significantly and dramatically relative to 2015, consistent with alternate bearing cycles of avocado trees and growth of young trees. The top five rootstocks with the highest number of fruit and yield (respectively) in the control treatment at the end of the experiment followed the order of Dusa (161 and 27.9 kg), PP40 (159 and 26 kg), R0.18 (146 and 24.0 kg), and R0.05 (137 fruit and 25.2 kg total yield). Dusa was significantly greater than PP14, R0.16, Thomas, and R0.17. In 2016 yields declined in the surviving trees in the saline treatment (Fig. 6B) and there were no significant rootstock differences. At that time, only five varieties (more salt tolerant) had surviving trees in the saline treatment.

The rootstocks with the highest yield per surviving tree were PP40 followed by R0.18, R0.05, and Dusa. Four of five of the surviving trees in the saline treatment also were in the groupings of the highest yields in the 2014 harvest in the saline treatment. Also, the fact that the surviving varieties were also the highest yielding in the controls suggests that salinity also impacted yield in the control treatment.

Relative yield (yield of saline treatment/yield control) is the commonly used criteria for evaluation of salt tolerance (Grieve et al., 2012). In 2014, the yield was very low for both the control and saline treatments; thus, salt tolerance could not be evaluated based on relative yield. We also consider that because relative yield depended on fruit number and not fruit size, it would be best reflected after at least 1 year of salinity treatment. Using the relative yield criterion, the salt tolerance ranking in 2015 was R0.18 > R0.05 > Dusa > PP40,
with all others having low or zero salt tolerance (Fig. 7A). In 2016, the salt tolerance ranking was (Fig. 7B) R0.18 > PP40 > R0.05 > Dusa > PP4, with four of these five also being the top varieties in 2015, but the relative yields were all below 0.15.

In terms of salt tolerance and high absolute yield under saline conditions, the preferred varieties were R0.05 and Dusa. These data are in contrast to those of Shalhevet (1994) and Oster et al. (2007), who calculated a threshold loss above EC_e = 0.6 dS m⁻¹ and zero yield when EC_e = 1.85 and 2.2 dS m⁻¹, respectively, based on the Maas Hoffman (Maas 1984) salt tolerance model using Mexican rootstock. Nevertheless, our data showed that good yields could be achieved for young trees on some newer, apparently salt-tolerant varieties at EC_e = 3.0 dS m⁻¹ for young trees on some newer, apparently salt-tolerant varieties at EC_e = 3.0 dS m⁻¹, harvested Feb. 2014. Uppercase letters designate differences among varieties in the saline treatment. There were no significant differences among varieties in the control. Rootstocks with different letters signify significant differences according to the Tukey-Kramer test at α = 0.05. As a result of differences in survival, the number of samples varied from five to nine in 2014.

Trunk diameter. There were significant differences between the trunk diameter in the saline and control rows. The average rate of increase in trunk diameter from 2014 to 2015 was 21% in the control and 0.7% in the saline treatment. These results are consistent with the findings of Oster and Arpaia (1992) that tree height, trunk diameter, and fresh weight decrease with increasing salinity. However, we did not find significant differences using Tukey’s test (α = 0.05) between the interaction of rootstock and treatment. Under the control condition, only PP40 and R0.18 had significantly greater trunk diameters than R0.06 and Thomas in 2015. We also found significant differences in trunk diameter in the saline treatment in 2015, with rootstocks PP40, R0.18, and R0.05 having significantly larger trunk diameter than those of PP4, PP14, PP24, PP45, R0.06, R0.07, R0.16, R0.17, and Thomas (Fig. 8). We also evaluated relative trunk diameter (ratio of salt/control). The trees with the greatest relative diameter (salt/control) were PP40, R0.05, and R0.18 (data not shown).

Leaf ion analysis. As expected, there were no significant differences for Na⁺ as well as Cl⁻ concentrations in the rows designated as future control and saline treatment before application of saline water (data not shown). Leaf Na⁺ concentration from the Oct. 2013 sampling before imposition of the treatments from the individual rootstocks is presented in Fig. 9A. At that time, EC_w was 0.65 dS m⁻¹, but the average soil EC_e was 6.83 dS m⁻¹. The Na⁺ concentration in the leaves ranged between 51.7 mmol kg⁻¹ for R0.05 and 68.7 mmol kg⁻¹ in Thomas; however, there were no significant differences (P > 0.05) in leaf Na⁺ concentration among varieties (Fig. 9A). These leaf Na⁺ concentrations are all below the 109–218 mmol kg⁻¹ concentrations at which Na⁺ toxicity generally occurs in trees (Ayers and Westcot, 1985).

In contrast to Na⁺, there were significant differences in leaf Cl⁻ concentrations among the varieties, as shown in Fig. 9B. In Oct. 2013, before imposition of saline treatment, leaf Cl⁻ concentrations ranged between 76.6 mmol kg⁻¹ for R0.05 and 226.6 mmol kg⁻¹ in PP14. At this time, the average root zone Cl⁻ concentration was 8.3 mmol·L⁻¹, above the soil extract Cl⁻ values cited by Maas (1984) for the three rootstock races, at which leaf damage occurs.

The role of Na⁺ and Cl⁻ ions in avocado salt tolerance is not clear. Bernstein et al. (2001) stated that under saline conditions, selection of rootstocks based on minimized leaf burn and fruit yield have resulted in rootstocks that “include reduced transport of Cl and exclusion of Na.” Cooper (1951) found that the salt tolerance of avocado, oranges, and grapefruit was closely related to the Cl⁻ accumulation properties of the rootstock. Prior investigators have reported that the mechanism of salinity tolerance in avocado appears to include reduced transport of Cl⁻ and exclusion of Na⁺ by the rootstock (Ben-Ya'acov, 1970; Downton, 1978; Gustafson et al., 1970; Kadman, 1963, 1964; Oster et al., 1985). Maas (1990) stated that rootstocks influence the salt tolerance of fruit trees and vine crops because of differences in absorption and transport of Na⁺ and Cl⁻; however, there is no detailed information to determine which ion is most responsible for reduced yields and mortality.

Based on our analyses from pre-experiment conditions (Oct. 2013), there were varietal differences in leaf Cl⁻ but not in leaf Na⁺ concentration. The R0.05 rootstock was significantly lower in accumulation of Cl⁻ in the leaves compared with the other 12 rootstocks (Fig. 9B). Dusa was also low in Cl⁻ accumulation, because it was not statistically different from R0.05. In contrast, PP14 and Thomas were in a grouping with the greatest leaf Cl⁻ accumulation.

Comparison of the Cl⁻ accumulation in the control in 2013 (Fig. 9B) with survival in the saline treatment (Fig. 4) shows that the low Cl⁻ accumulators—Dusa and R0.05—also had high survival rates and high yields under saline conditions in 2015 and 2016 (Fig. 6A and B, respectively). The Cl⁻ accumulators in the control—PP14, PP45, R0.17, and Thomas—had either zero or very low survival in the saline treatment by 2015.
In 2015, the top-yielding varieties in the control were Dusa, R0.05, PP24, PP40, and R0.06. Before the imposition of the saline treatment, R0.05, Dusa, PP40, and R0.06 had the lowest leaf Cl– concentrations (Fig. 9B). Leaf ion composition for the other elements from the sampling in Oct. 2013 indicated that all the varieties had elemental concentrations within adequate nutrient ranges for avocado (data not shown).

The mean varietal elemental analysis of leaf samples from all surviving trees (10–15 leaves/tree) collected in Oct. 2014 from the control (data not shown) and saline treatments is presented in Supplemental Table 1 (except for Na and Cl presented in Fig. 9). In the control, the Na+ concentrations varied considerably, but the only significant differences in leaf Na+ were seen in R0.06, R0.07, and Thomas. They had significantly greater Na+ concentrations than that of PP40 (Fig. 9A). All Na+ concentrations were below the level of 100–200 mmol·kg–1, at which Na+ toxicity is expected.

There was also variability in the leaf Na+ concentrations among the different rootstocks in the saline treatment in 2014 (Fig. 10B). R0.06 accumulated significantly higher Na+ than the group that accumulated the least Na+: PP40, R0.05, R0.18, and PP24. The R0.07 variety was also very high in leaf Na+ in the one surviving tree. After 8 months of irrigating with saline water, rootstocks PP45 and R0.16 died, and consequently these samples could not be collected. The rootstocks that accumulated the least amount of Cl– and Na+ in the leaves in Oct. 2014 were also the ones that had the highest survival percentage in 2015, as discussed earlier.

Oster and Arpaia (1992) found differences among rootstocks for plant appearance based on Cl– and Na+ contents of the plant tissues. Cooper and Gorton (1950) found that the visual symptoms of leaf burn of avocados in the Rio Grande Valley in Texas was associated with a large Cl– accumulation in leaves. Goodall et al. (1981) reported that leaf Cl– values above 140 mmol·kg–1 caused leaf injury. Slight to severe visual injuries, predominantly leaf tip necrosis, have also been reported for field-grown avocado trees, with leaf Cl– concentrations in the range from 0.5% to 1.5% dry weight (Bingham et al., 1968), or 140 to 420 mmol·kg–1. Thus, all the rootstocks in the control (Fig. 10C) and saline treatment (Fig. 10D) were above the 140 mmol·kg–1 level, and all the saline treatments except R0.17 were above 400 mmol·kg–1, indicating that even Cl– in the controls may be sufficiently high to impact physiological parameters adversely, which is confirmed by the leaf burn data discussed earlier. The Cl– concentrations in the control for R0.06, Dusa, PP4, PP24, PP40, R0.18, R0.05, Thomas, and PP40 were significantly lower than the Cl– in the leaves of PP14. There were no significant varietal differences in the leaf Cl– concentrations in the saline treatment in Oct. 2014. Nonetheless, the rootstocks that accumulated the least amount of Cl– after 1 year of irrigation with saline water were PP40 (445 mmol·kg–1), PP24 (490 mmol·kg–1), Dusa (506 mmol·kg–1), R0.18 (529 mmol·kg–1), and R0.05 (574 mmol·kg–1) (Fig. 10D). In this group, PP40, PP24, and R0.05 were also the varieties that accumulated significantly less Cl– in the control in 2014. Similarly, R0.05 and Dusa were the varieties that accumulated the least amount of Cl– in 2013 before the start of the experiment. Thus, Cl– accumulation under our control conditions was an indicator of Cl– under more saline conditions. This suggests...
that screening for varietal differences among rootstocks for Cl– exclusion might be done even when irrigating with an ECw of 0.65 dS·m–1, as long as soil Cl– concentration is relatively uniform across the field.

The elemental concentrations of Ca, Mg, K, P, S, Fe, Cu, Mn, and Zn in the control treatment in 2014 (data not shown) fall within the adequate nutrient ranges for avocado (Lee, 1980). The leaves collected from the saline treatment in Oct. 2014 (after 9 months of saline water irrigation) showed leaf Ca concentration deficiency for R0.06, PP4, and R0.07 (<247 mmol·kg–1). Leaf Mg concentrations were deficient for R0.06, R0.07, and Thomas (<102 mmol·kg–1); K was deficient in Dusa, PP24, and Thomas (<520 mmol·kg–1); Fe was deficient for R0.06, Dusa, PP24, Thomas, and PP40 (<49 mmol·kg–1); Cu was deficient in PP24 (<5 mg·kg–1); and Mn was deficient in R0.07 (<30 mg·kg–1). Sulfur and Zn were within adequate ranges for all the rootstocks (Lee, 1980). After 8 months of irrigating with saline water, rootstocks PP45 and R0.16 died, and consequently there were no leaves to collect from the saline treatment of these varieties in Oct. 2014.

Leaf ion analysis was performed on samples collected in Oct. 2015. There were no significant varietal differences in the Na+ concentration in the control for the Oct. 2015 leaf sampling because all the varieties were similar in concentration (Fig. 10A). Analysis of the data in the saline treatment in terms of rootstock variations is difficult because, by that time, six of the original rootstocks died as a result of salt sensitivity. Of the seven surviving rootstocks in Oct. 2015, Thomas and PP4 accumulated significantly more Na+ in the leaves compared with the other surviving varieties (Fig. 11A); however, all were below levels associated with Na ion toxicity.

The very low Na concentrations for all leaves in Oct. 2015 are likely a result of the rain event that occurred in July 2015. The Cl– concentration in the leaves sampled in Oct. 2015 from the control treatment were also lower than in 2014 and ranged between 142 mmol·kg–1 for R0.05 and 306 mmol·kg–1 for R0.18 (Fig. 11B). The R0.05 variety had a significantly lower Cl– concentration than the PP4 variety. In general, varieties with Cl– leaf concentrations greater than 550 mmol·kg–1 in 2014 did not survive by 2015. This is also seen in the data in Fig. 11B, where no varieties have leaf Cl– concentrations above 500 mmol·kg–1.

Our results show that, under field conditions, rootstocks limit the uptake or Fig. 10. Average ion concentration in leaves of individual rootstocks collected in Oct. 2014 for (A) Na in the control, (B) Na in the saline treatment, (C) Cl in the control, and (D) Cl in the saline treatment. *Only one tree remained and was not used in the statistical analysis. The two lines indicate leaf Cl concentration above which there is expected to be visual leaf injury ranging from slight to severe. Uppercase letters designate differences among varieties in the saline treatment; lowercase letters indicate differences among varieties in the control. Rootstocks with different letters signify significant differences according to the Tukey-Kramer test at \( \alpha = 0.05 \).

Fig. 11. Average concentration in leaves per rootstock collected in Oct. 2015 for (A) Na and (B) Cl. Average root concentrations for rootstocks collected in Mar. 2016 for (C) Na and (D) Cl. *Only one tree remained and was not used in the statistical analysis. There were no significant differences in Na concentration among varieties in the saline treatment of the avocado roots in 2016. Rootstocks with different letters signify significant differences according to the Tukey-Kramer test at \( \alpha = 0.05 \). Uppercase letters designate differences among varieties in the saline treatment; lowercase letters indicate differences among varieties in the control. The missing samples (saline treatment) are rootstocks with trees that died before we sampled in 2015.
translocation of Cl– and Na+ to a varying extent, which consequently increases salt tolerance in the scion, as noted by Castro et al. (2009). In their experiment, rootstock ‘Duke 7’ and ‘Nebal’ accumulated the highest quantity of Cl– in the roots. ‘Duke 7’ had a significantly high concentration of Cl– in the leaves whereas ‘Nebal’ showed low concentrations at the foliar level under the same treatment, suggesting that restriction of Cl– translocation was important for ‘Nebal’, at least in the short term. Both root exclusion as well as restriction of ion translocation can serve to increase salt tolerance. However, in the long-term life cycle of the tree, it is likely that only varieties that are able to restrict Na+ uptake (exclusion) will remain salt tolerant. The results of the root ion accumulation at the end of the experiment are presented in Fig. 11. Of the surviving varieties, the rootstocks in the saline treatment Dusa, Thomas, and R0.18 accumulated the most Na+ in the roots. Compared to the leaf ion analyses in 2015, two of the three varieties (excluding Thomas) were in the lower statistical grouping in terms of leaf ion Na+’, suggesting these varieties may restrict Na+ translocation primarily within the tree, rather than restrict root Na+ uptake. The rootstocks R0.05, Dusa, and R0.18 in the saline treatment accumulated the least amount of Cl– in the roots (Fig. 11D) and were statistically significantly different from PP4 (only surviving rootstocks could be analyzed). The rootstocks with low Cl– in the roots were also the rootstocks with high survival (Fig. 4). The tolerance mechanism thus appears to be Cl– exclusion rather than reduced translocation of Cl– to the leaves, because if reduced translocation were the important process, then the tolerant rootstocks would have higher Cl– in the roots and lower Cl– in the leaves, but this was not the case. **Trunk diameter and yield related to leaf ion composition.** Differences in ion accumulation were next compared with differences in yield and growth parameters. High soil salinity and root Na+ levels have been reported to cause reductions in fruit yield and tree size, lowered leaf chlorophyll content, decreased photosynthesis, poor root growth, and leaf scorching (Mickelbart and Arpaia, 2002). There was only a very weak relationship between trunk diameter and Na+ leaf ion composition (data not shown). In contrast, Fig. 12 shows a good correlation between trunk diameter and leaf Cl– concentration ($R^2 = 0.49$), which again supports the concept that Cl– is the toxic ion for salt damage in avocado. There was also a correlation between trunk diameter and Cl– when the control and saline treatments were considered separately. As shown in Fig. 13A, the number of fruit correlated poorly with leaf Na+ concentration ($R^2 = 0.17$). In our experiment, fruit number rather than mean fruit weight was the primary factor reducing yield. Thus, varietal differences in leaf Na+ do not explain yield differences among the treatments. Therefore, Na+ is not likely the most important factor in avocado salt damage, which is consistent with the trunk diameter data discussed earlier. In contrast, leaf Cl– correlated well with fruit number ($R^2 = 0.58$) (Fig. 13B). These data support the idea that Cl– is a specific toxic ion for avocado. It also appears (Fig. 13B) that 250 mmol·kg$^{-1}$ is the critical Cl– concentration at which avocado fruit number and thus yield declines. This leaf Cl– concentration is much more than the reported level (around 140 mmol·kg$^{-1}$) for initial leaf burn. Thus, initial leaf burn is a good diagnostic criterion for avoiding avocado yield loss, but it was not indicative of actual loss, as shown by our data. **Summary and Conclusions** All rootstocks grafted on Hass showed a high sensitivity to salinity, with very low survival and very low fruit yield for surviving trees under irrigation with saline water (EC = 1.5 dS·m$^{-1}$). Salt damage and mortality increased with time. Nonetheless, there was considerable variability in tolerance as some varieties such as R0.06, R0.07, R0.16, R0.17, PP4, and PP45 had 0% survival after 2 years under the saline treatment, whereas PP40 and R0.05 had up to 67% survival. After year 3, only a few trees survived in the saline treatment. The commercially available rootstock variety Dusa was among the top producers under irrigation with an EC$_w$ = 1.5 dS·m$^{-1}$, followed by PP40, R0.05, and R0.18. These four varieties performed at EC$_w$ values that other varieties did not survive. Although we evaluated only one salt level above the control, we used the variability among varieties in response to salinity and examined the relationships between ion uptake and growth and yield parameters. The more tolerant rootstocks (evaluated by trunk diameter growth, fruit yield, and survival rate) were characterized as Cl– ion excluders, based on leaf and root ion accumulation. In contrast, there was no correlation between Na+ accumulation and yield or trunk growth. Leaf Cl– analysis thus proved to be a useful method to identify salt-sensitive rootstocks rapidly and serves as a diagnostic tool for salt damage. Further development of salt-tolerant rootstock is merited because no variety did well under salinity levels common to recycled water (EC = 1.5 dS·m$^{-1}$) and with a soil EC$_w$ range of 4.1 to 6.7 dS·m$^{-1}$. **Literature Cited** Allen, R.G., L.A. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper no. 56. FAO, Rome, Italy. Ayers, R.S. and D.W. Westcott. 1985. Water quality for agriculture. FAO Irrigation and Drainage Paper no. 29. FAO, Rome, Italy. Bender, G.S. 2012. Avocado botany and commercial cultivars grown in California, p. 23–37. In: Book 1: Avocado production in California: A cultural handbook for growers. 2nd ed. Ben-Ya’acov, A. 1970. Characteristics associated with salt tolerance in avocados grafted on Mexican and West-Indian rootstocks. Proc. 18th Inter. Hort. Cong. Vol. 1. p. 135. Bergh, B. and N. Ellstrand. 1986. Taxonomy of the avocado. Calif. Avocado Soc. Yrbk. 70:135–145. Bernstein, L. 1965. Salt tolerance of fruit crops. USDA-ARS, Agr. Inform. Bul. Bernstein, N., M. Ioffe, and M. Zilberstaine. 2001. Salt-stress effects on avocado rootstock growth: 1. Establishing criteria for determination of shoot PPFD growth sensitivity to the stress. Plant Soil 233:1–11. Bingham, F.T., L.B. Fenn, and J.J. Oertli. 1968. A sandculture study of chloride toxicity to mature avocado trees. Soil Sci. Soc. Amer. Proc. 32:249–252. Bouyoucos, G.J. 1936. Directions for making mechanical analysis of soils by the hydrometer method. Soil Sci. 4:225–228.
Supplemental Table 1. Average leaf ion composition in saline treatment for samples collected in Oct. 2014.

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Ca (mmol·kg⁻¹)</th>
<th>Mg (mmol·kg⁻¹)</th>
<th>K (mmol·kg⁻¹)</th>
<th>P (mmol·kg⁻¹)</th>
<th>S (mmol·kg⁻¹)</th>
<th>Fe (mmol·kg⁻¹)</th>
<th>Cu (mg·kg⁻¹)</th>
<th>Mn (mg·kg⁻¹)</th>
<th>Zn (mg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0.06</td>
<td>154 b</td>
<td>89.8 b</td>
<td>902 a</td>
<td>143 a</td>
<td>163 a</td>
<td>25.8 a</td>
<td>20.6 a</td>
<td>81.6 a</td>
<td>64.5 a</td>
</tr>
<tr>
<td>Dusa</td>
<td>380 ab</td>
<td>151 ab</td>
<td>435 a</td>
<td>56.6 a</td>
<td>88.8 a</td>
<td>41.7 a</td>
<td>9.78 a</td>
<td>65.6 a</td>
<td>35.3 a</td>
</tr>
<tr>
<td>PP4</td>
<td>217 ab</td>
<td>121 ab</td>
<td>782 a</td>
<td>124 a</td>
<td>114 a</td>
<td>74.6 a</td>
<td>19.8 a</td>
<td>79.6 a</td>
<td>58.4 a</td>
</tr>
<tr>
<td>PP14 y</td>
<td>415</td>
<td>120</td>
<td>894</td>
<td>111</td>
<td>126</td>
<td>153</td>
<td>13.9</td>
<td>62.9</td>
<td>67.7</td>
</tr>
<tr>
<td>PP24</td>
<td>355 ab</td>
<td>160 ab</td>
<td>516 a</td>
<td>32.7 a</td>
<td>80.7 a</td>
<td>21.0 a</td>
<td>4.88 a</td>
<td>60.6 a</td>
<td>39.3 a</td>
</tr>
<tr>
<td>PP45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>R0.07 y</td>
<td>139</td>
<td>78.7</td>
<td>1,108</td>
<td>122</td>
<td>124</td>
<td>105</td>
<td>16.7</td>
<td>26.9</td>
<td>56.6</td>
</tr>
<tr>
<td>R0.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0.18</td>
<td>400 ab</td>
<td>152 ab</td>
<td>564 a</td>
<td>65.8 a</td>
<td>81.5 a</td>
<td>98.7 a</td>
<td>10.8 a</td>
<td>52.7 a</td>
<td>42.3 a</td>
</tr>
<tr>
<td>R0.05</td>
<td>445 a</td>
<td>164 ab</td>
<td>599 a</td>
<td>73.6 a</td>
<td>100 a</td>
<td>136 a</td>
<td>11.0 a</td>
<td>72.4 a</td>
<td>57.6 a</td>
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<tr>
<td>Thomas</td>
<td>266 ab</td>
<td>91.6 b</td>
<td>356 a</td>
<td>44.2 a</td>
<td>76.9 a</td>
<td>38.9 a</td>
<td>7.46 a</td>
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<tr>
<td>R0.17 y</td>
<td>289</td>
<td>103</td>
<td>881</td>
<td>171</td>
<td>117</td>
<td>105</td>
<td>20.5</td>
<td>110</td>
<td>88.1</td>
</tr>
<tr>
<td>PP 40</td>
<td>352 ab</td>
<td>197 a</td>
<td>571 a</td>
<td>39.4 a</td>
<td>85.1 a</td>
<td>45.5 a</td>
<td>6.41 a</td>
<td>67.3 a</td>
<td>37.7 a</td>
</tr>
</tbody>
</table>

Rootstocks with different letters signify significant differences according to Tukey-Kramer test at alpha = 0.05.

Rootstocks that only had one tree alive and therefore were not included in the statistical analysis.

No leaf samples were collected for these rootstocks because of tree mortality.