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Salinity has a broad range of effects on plants, therefore, there are also many different mechanisms for plants to tolerate this stress. Plants can reduce toxicity by reducing accumulation of toxic ions in the leaf blades (Na^+ and Cl^- exclusion), and/or by increasing their ability to tolerate the salts that they have failed to exclude from the shoot, such as by compartmentation into vacuoles (tissue tolerance; Munns and Tester 2008). The influence of chloride concentrations and other elements in the leaves was studied because California growers are faced with having to use irrigation water high in salts, especially high in sodium and chloride.

Salt tolerance of avocados is complicated because it is a salt sensitive species and ion toxicities cause detrimental effects on growth and yield. Bernstein (1965) pointed out that for many fruit crops, damage to plants can be related to the concentration of specific ions, e.g. chloride or sodium in the soil solution and/or plant leaves rather than to the total soil salinity. A frequent toxicity problem is from chloride in the soil solution. If the chloride concentration in the leaves exceeds the tolerance of the crop, injury symptoms develop such as leaf burn or drying of leaf tissue. Avocados are especially susceptible to leaf injury caused by the toxic accumulation of sodium and chloride in the leaves. Increased varietal or rootstock salt tolerance gives farmers an opportunity to continue growing avocados while using low quality water or planting in salt affected soils. For this reason, evaluation the salt tolerance of avocado rootstocks and varieties is important.

Oster and Arpaia (1992) showed that the tolerance level of the avocado scion is dependent on the rootstock used; in this study, there was large variability among the rootstocks between the sodium and chloride concentrations in the leaves. This variability was imparted by the rootstocks ability to translocate chloride and sodium to the leaves. In the present study, we examined the salinity response and tolerance of 13 clonal rootstocks (Table 1). All trees were grafted with ‘Hass’, a salt sensitive scion.

Based on the work of Oster et al. (2007), they found that there was a yield loss on Mexican seedling rootstocks exposed to saline irrigation water. The threshold for salinity above which yield declined occurred at 0.57 dS m^{-1} and yield declined by 65% per unit of salinity above the threshold. Furthermore, Mickelbart and Arpaia, there were differences in the response of rootstock for Mexican seedling rootstocks. These findings were the basis for our research, pursuing salinity tolerance.

Prior to imposing the salinity treatment, soil samples were collected 30 cm from the tree trunk next to (in wetted area) and in-between trees. The samples were collected using an auger 5 cm in diameter. Samples were taken in 15 cm increments down to 75 cm. The soil samples were collected in prelabeled plastic Ziploc bags and securely sealed to maintain moisture content until analysis and prevent evaporation. The sampling hole was filled back in and the gravimetric water content of the soil samples was measured. The saturation extracts where analyzed for chloride and electrical conductivity (EC). In December of 2013, the two treatment plots were very similar in both EC and chloride concentration as expected because there was no salinity treatment being applied. For the duration of the trial, the trees in both salinity and control rows were irrigated 3 times per week. The amount of water applied was determined using the irrigation calculator on http://avocadosource.com. The avocado crop coefficient 0.86 (Oster et al., 2007) and a leaching fraction of 10% was used from January - August 2014. The leaching fraction was adjusted to 20% in years 2 and 3. The change in leaching fraction was prompted by the overall health of the trees. We found that a 10% leaching fraction was not sufficient in the untreated trees since they showed symptoms of salinity damage due to insufficient leaching.

Table 1. Rootstocks evaluated for tolerance to salinity listed by source of material.

University of California Rootstocks	Westfalia Rootstocks (South Africa)
PP4 (Zentmyer)	Dusa
PP14 (Uzi)	R0.05
PP24 (Steddom)	R0.06
PP40	R0.07
PP45	R0.16
Thomas	R0.17
	R0.18

Field trial design was completely randomized with salinity water applied to entire rows. The recipe for saline water was a blend of salts mirroring Colorado River Water. The recipe is listed in table 2. Salinity treatment was gradually imposed from November 2013 to January 2014 in a step wise manner to enable osmotic adjustment. The salinity treatment was EC 1.5 dS m^{-1} and the control ranged between 0.5 and 0.67 dS m^{-1} (Gage Canal water).

Table 2. Recipe for saline water based on components of Colorado River Water. Final EC was 1.5 dS m^{-1} and 175 ppm chloride.	
Compound	Concentration g/l
$CaCl_2$	1.738
$MgCl_2$	1.517
NaCl	0.241
KNO_3	0.063
$Na_2(SO_4)$	4.965
KCl	0.008

Once the salinization was imposed, the salts accumulated in the top 20 cm of the soil profile. Based on the calculated leaching fraction in the soil at field moisture content the salts were pushed down and the EC and the chloride remained fairly constant below 40 cm. From August 2014 through September 2014, a total of 730 soil samples were collected. A total of eight holes were sampled from each row and four soil samples were collected 30 cm from the tree and the other four in between the trees. The average EC of these soils in the 7 to 65 cm depth ranged from 3.62 – 2.02 dS m^{-1} and 6.06 – 3.15 dS m^{-1} in the control and salinized rows respectively. The average chloride content of the saturation extracts from the 7 to 65 cm depth ranged from 7.56 to 4.84 $mmol_c$ L^{-1} and 16.41 to 8.78 $mmol_c$ L^{-1} in the control and salinized rows respectively.

On June 15, 2015 an additional 720 soil samples were collected. The soil analyses indicate that soil EC in the salinity treatments had decreased relative to 2014, but it remained above that of the control. The EC in the top 20 cm of soil was at 3.20 dS m^{-1} and 4.10 dS m^{-1} for the control and salinity, respectively. There was also a decrease in the chloride concentration in the top 20 cm from a concentration of 16.41 $mmol_c$ L^{-1} in August 2014 to a 10.07 $mmol_c$ L^{-1} concentration in June 2015 in the salinized row.

Leaf samples were collected in October of 2013 (prior to initiation of treatments), 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, reweighed, digested and subsequently analyzed for calcium, magnesium, sodium, potassium, phosphorus, sulfur, chloride, iron, copper, manganese, and zinc by ICP OES.

The mean chloride content of the leaves varied from 42 to 120 mmol kg^{-1} (dry weight) depending on the rootstock prior to the initiation of treatments. This preliminary analysis showed that the rootstock varieties expected to be more salinity tolerant was either a chloride excluder or did not translocate chloride to the leaves, a trait that is expected for more salinity tolerant plant varieties. From the preliminary leaf analysis, R0.05 and Dusa had the lowest levels of leaf chloride, and thus they were considered to be chloride excluders, they had some of the highest yields and higher survival rates (Figure 1). Slight to severe visual injuries, predominantly leaf tip necrosis, have been reported for mature field grown avocado trees with leaf chloride concentrations from 0.5% to 1.5% dry weight (Bingham et al., 1968). This range translates to 141 mmol kg^{-1}

to 423 mmol kg^{-1} of chloride, thus all of the rootstocks in the control row fall below this range. Salinized rows had significantly greater leaf damage than fresh water treated rows (Fig. 1). We identified leaf injury and necrosis in the trees in the salinized row that had chloride concentration well above the 0.5% to 1.5% dry weight range.

Leaf analysis proved to be a useful method to identify salinity sensitive rootstock such as R0.06, R0.07, PP14, and R0.17. These rootstocks had high chloride and sodium concentrations in the leaves and were the least salinity tolerant with 100% mortality in the salinized rows irrigated with saline water for 23 months. The rootstocks R0.05, Dusa, PP40 and R0.18, accumulated the least amount of chloride in the leaves and were also the rootstocks that accumulated the least chloride in the roots. This indicates that chloride exclusion is occurring at the root interface. This experiment shows, under field conditions, the influence of rootstock on the concentration of chloride and sodium and other elements in the leaves since rootstocks can impart salinity tolerance to the scion of trees, usually by limiting the excessive accumulation of chloride (Cl) and sodium (Na) from the scion (Bañuls et al., 1990). PP14, PP45, R0.06, R0.07, R0.16, and R0.17 all died after about 20 months of being irrigated with 1.5 dS m^{-1} irrigation water. The rootstocks that had the highest survival rate were PP40 and R0.05 with a survival rate of 67%, followed by R0.18 with 63%, and Dusa with 43% (Fig. 2).

Fruit were harvested from all trees in April 2014 after 3 months of treatment. The number of fruit per tree ranged from 0-77, and the average was 7.1 fruit (std dev: 12.5). There were no significant differences in the number of fruit between salinity treated and control trees ($p>0.05$), however, there were significant differences between the rootstock varieties ($p=0.0006$). The total weight of fruit per tree ranged from 0-15.28 kg, and the average was 1.6 kg (std dev: 2.6). There were no significant differences in the weight of fruit between treated and non-treated trees ($p>0.05$), however, there were significant differences between the rootstock varieties ($p=0.0004$). This may be due to the fact that the fruit set prior to any salinity treatments and were nearing maturity when the full salinity treatment was started in January 2014. Fruit were harvested in April 2014 after only 3 months of exposure to salts.

Fruit harvested in 2015 showed that there were significant differences in the number of fruit, and weight of the fruit between treated and non-treated trees after 1 year of salinity treatment ($p< 0.0001$). There were significant differences in the number of fruit, and weight of the fruit among the rootstock varieties ($p= 0.0081$, $P= 0.0074$) in control, and salinity treated group ($p= 0.0032$, $P= 0.0033$), with R0.05 and Dusa had the highest number of fruit and fruit weight in the control group, and R0.05 and PP40 had the highest number of fruit and fruit weight in salinity treated group. This may be due to the long period of the salinity treatment. The number of fruit per tree ranged from 0-149, and the average was 18.76 fruit (std dev: 2.0). The total weight of fruit per tree ranged from 0-20.55 kg, and the average was 2.59 kg (std dev: 0.28).

Figure 1. Percent survival of rootstocks after 1 year of exposure to salinity treatment.

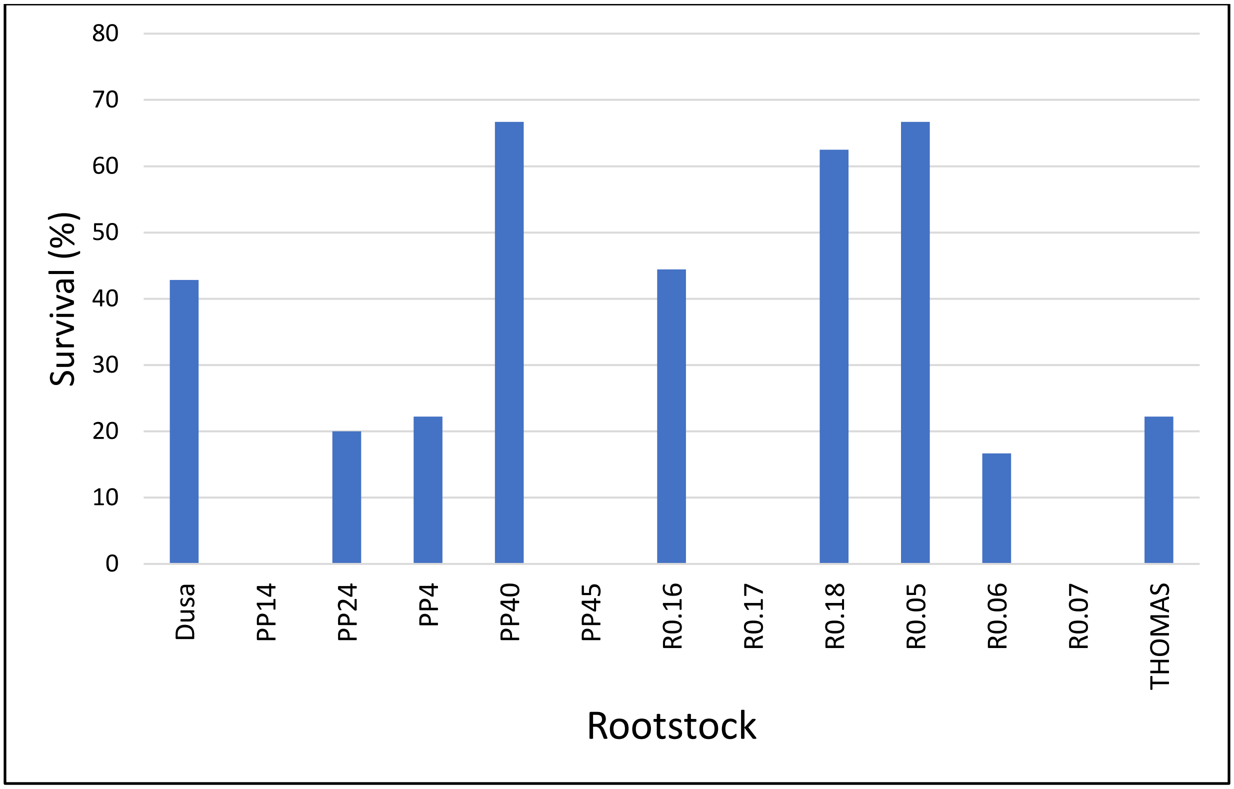
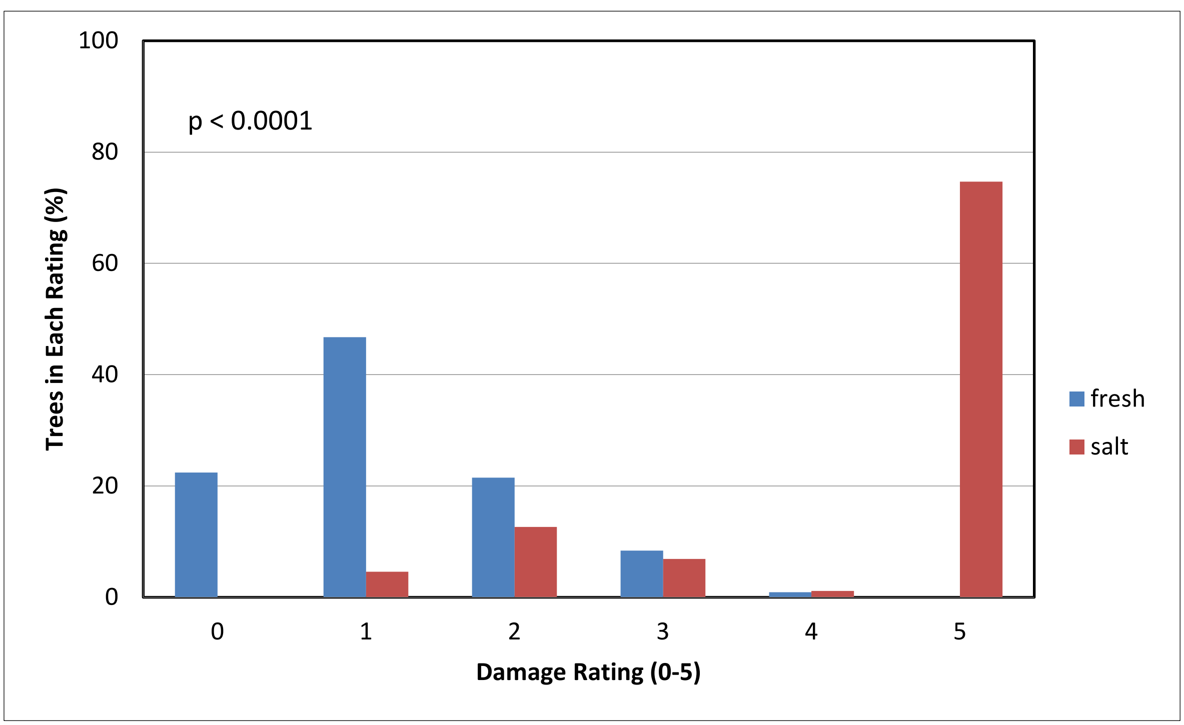


Figure 2. Average leaf burn by irrigation treatment one year after full salinization. Severity rating, 0=healthy leaf without necrotic margins to 5=severe leaf burn and defoliation.



In the final harvest in 2016, there were significant differences in the number of fruit (Fig. 3), and weight of the fruit (Fig. 4) between treated and non-treated trees after 2 years of salinity treatment ($p < 0.0001$). There were significant differences in the number of fruit, and weight of the fruit among the rootstock varieties ($P = 0.0002$, and $P < 0.0001$) in control group, with Dusa and PP40 had the highest number of fruit and fruit weight in the control group. However, after 2 years of treatment, there were no significant differences in the number of fruit, and weight of the fruit among the rootstock varieties in salinity treated group ($P = 0.2143$ and $P = 0.5471$), and PP40 and R0.05 had the highest number of fruit and fruit weight in salinity treated group. With the increasing length of the salinity treatment, the fruiting capacity in the tolerant rootstock significantly decreased. This may due to the decrease of the photosynthesis rate, the growth of the tree, or the ability to keep the fruit under the long-term salinity stress.

Figure 3. Number of fruit harvested by rootstocks under fresh water and salinity treatments harvested in March 2016.

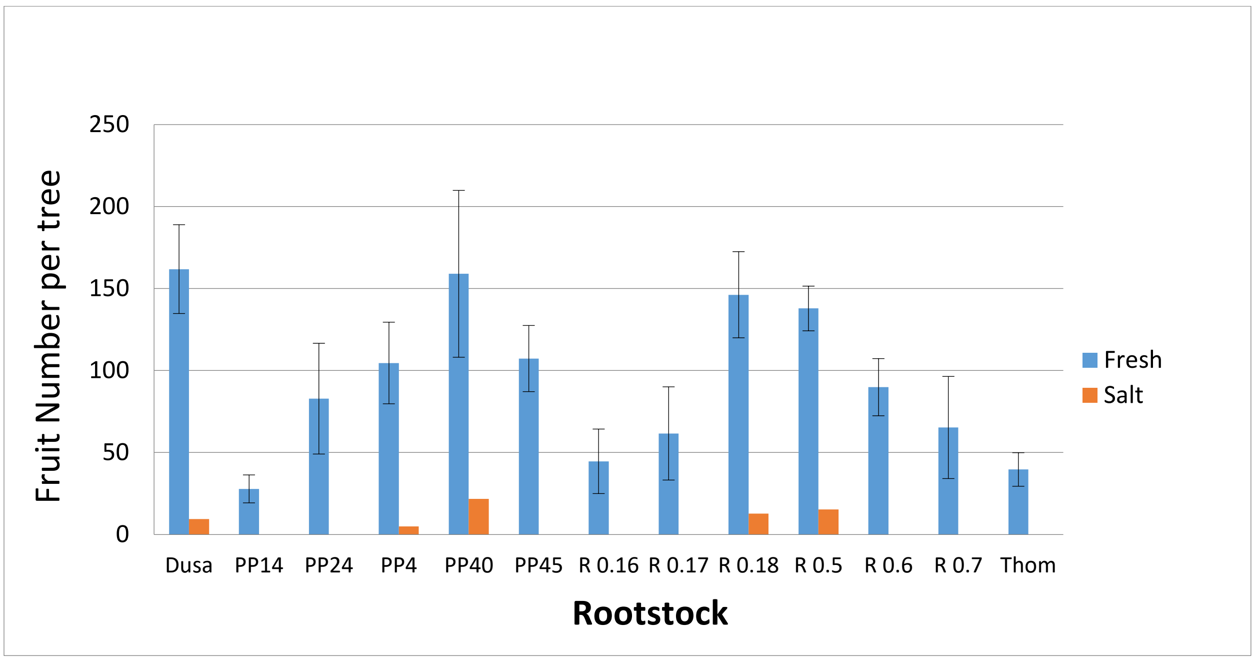
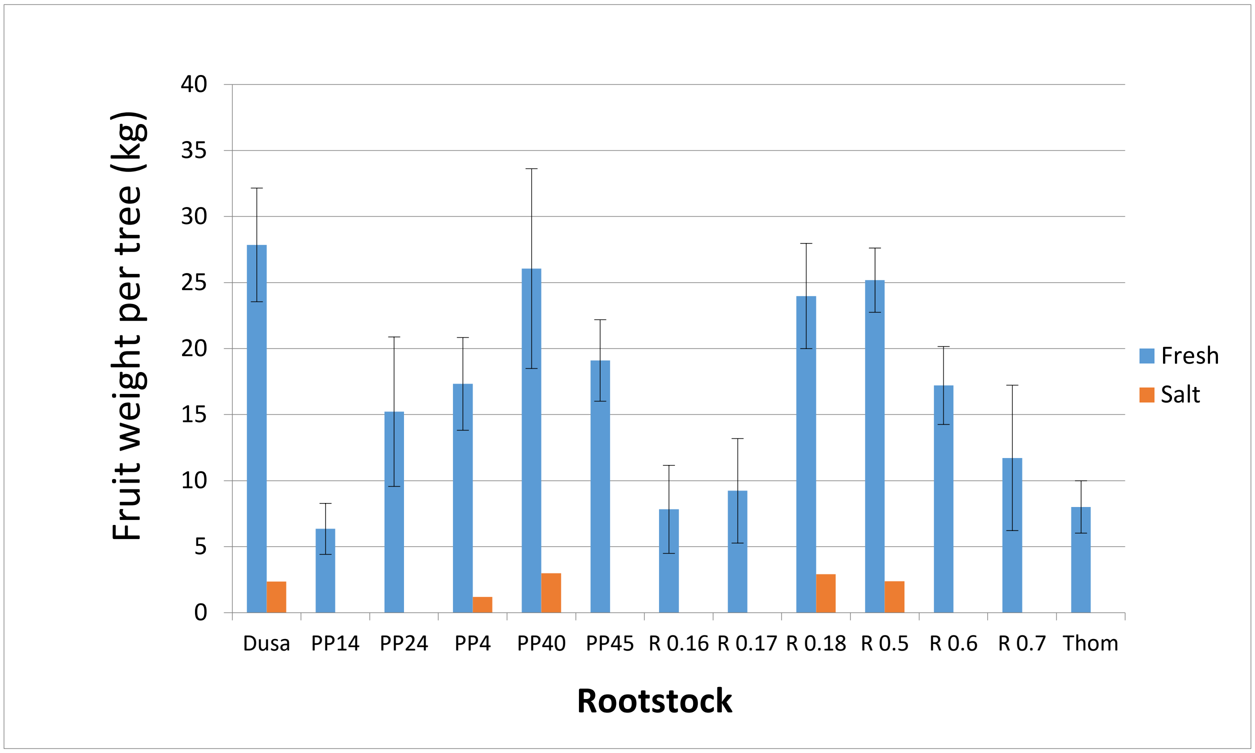


Figure 4. Fruit Weight of Different Rootstocks under Different Treatments harvested in March 2016.



Physiological parameters were measured in avocado trees with Dusa, PP4 (Zentmyer), PP40 and R0.05 rootstocks in 2014 and 2015. Consistent with the finding of Mickelbart and Arpaia (2002), leaf necrosis was evident in trees exposed to salinity, especially in the sensitive rootstocks. Salinity treatment reduced photosynthesis (Fig. 6) and significantly reduced carbon assimilation rate in damaged leaves compared with leaves from control trees and healthy leaves from trees. In this trial, the damage was more severe in PP40 and least severe in R0.05. Salinity treatment, when compared with the untreated controls, also affected Water Use Efficiency (WUE) in avocado, by reducing its performance in Dusa and R0.05 trees. PP40 had the highest transpiration and the lowest WUE comparing to Dusa and R0.05. Water potential was measured at predawn and midday for trees with Dusa, R0.05, PP4 (Zentmyer) and PP24 rootstocks. No significant differences were found in varieties or treatments. Chlorophyll fluorescence was also measured in these trees. Only damaged leaves from treated trees in all rootstocks showed photoinhibition (Fig. 6), with F_v/F_m values significantly reduced compared with control and healthy leaves from treated trees. Our findings showed that rootstocks affected the physiological performance in avocados with Dusa and R0.05 the most tolerant rootstocks under stress conditions.

Figure 5. Photosynthesis of Hass grafted to selected rootstocks. Leaves were selected by leaf health (visibly necrotic area areas=damaged leaves) and irrigation treatment.

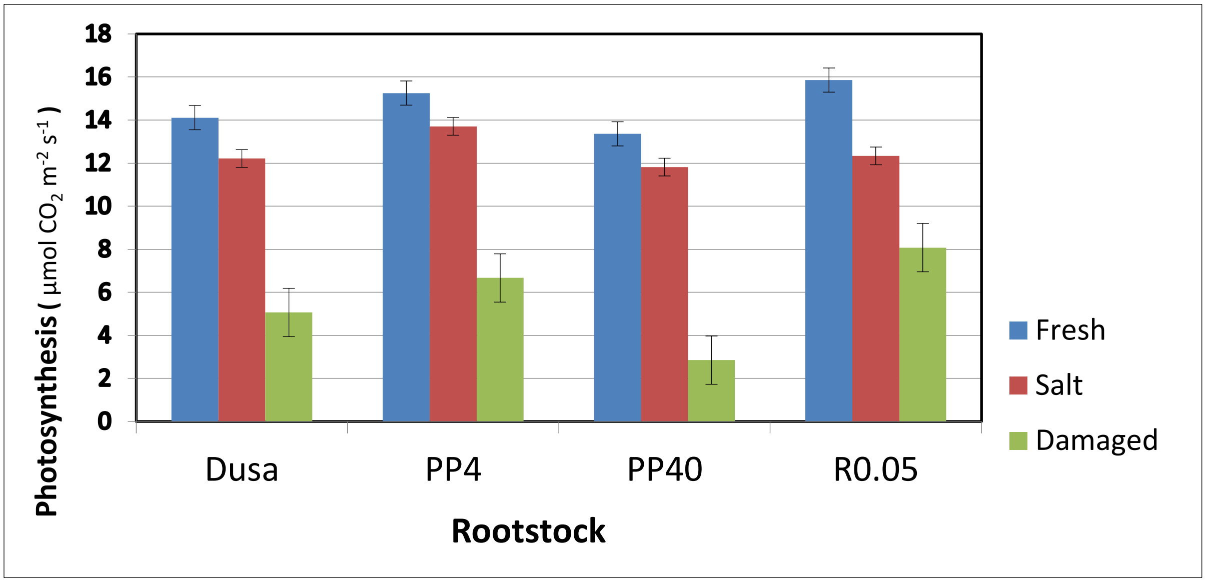
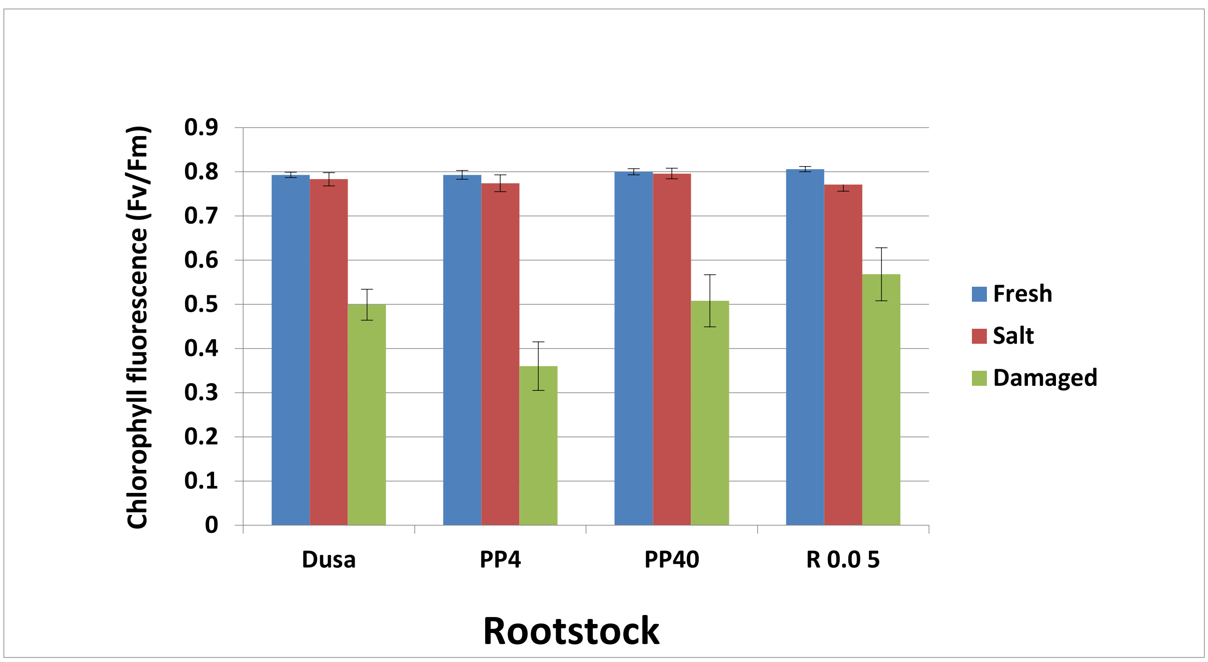


Figure 6. Chlorophyll fluorescence by rootstock and irrigation treatment (2015).



According to Mickelbart and Arpaia (2002) the differences in responses to salinity among rootstocks were found primarily in morphological traits such as growth and leaf necrosis, rather than physiological traits such as gas-exchange and water relations. Leaf burn is due to salt injury which is evident in older leaves and is the result of vacuoles dying and no longer being able to sequester salts (Kozlowski, 1997). In this trial, when leaf burn was first evaluated in February 2014, there were no significant differences in damage rating between salinity treated and non-treated trees ($p>0.05$), but there were significant differences in leaf burn between the rootstocks ($p=0.0001$). The damage in the non-treated trees is because they have different tolerance to extreme heat, lack of mulch, and possibly insufficient irrigation duration/frequency prior to initiating the experiment. Although the salinity treatment had been imposed for one month, it is likely that the differences in rootstock response to salinity was caused by extreme heat, lack of mulch, and possibly insufficient irrigation duration/frequency prior to initiating the experiment. The rating scale ranged from 0 (no leaf burn) to 5 (tree defoliated), but field data ranged from 0-3. However, when reevaluated after 8 months, and 1 year of salinity treatment, there were significant differences in leaf damage between varieties within salinity treatment but no differences between varieties in the fresh treatment.

In analyzing the interaction of rootstock and treatment in respect to trunk diameter, there were no significant differences using Tukey's test alpha = 0.05. However, when analyzing the differences between salinity and control trees, there were significant differences in trunk diameter with the salinity treatment having smaller diameters. There were no significant differences in rootstock diameters for the different rootstocks in the control. In contrast to this, there were differences in rootstock trunk diameters in the salinity treatments. With PP40, R0.18, and R0.05 having the largest trunk diameter.

Based on leaf analyses and the correlation of chloride in leaf tissue with tree survival data, we conclude that chloride accumulation in the leaves from both the control and salinity treatments provided a good indicator of survival under the salinity treatment or in turn salinity tolerance. We also determined that sodium content in leaves was not a good marker for salinity tolerance of avocado rootstock. There is a reduction in avocado yield at about a chloride concentration 280 mmol kg^{-1} dry weight in the leaf tissue. In this experiment, the rootstocks that restricted chloride ion uptake and translocation to the mature fully expanded leaves were R0.05, PP40, R0.18 and Dusa which were also the rootstocks that had minimal effect on growth and yield exhibiting the highest yield, highest trunk diameter and highest survival percentage.

Crowley and Arpaia (2011) showed that the avocado yields decrease in linear proportion to the levels of chloride that are contained in the leaf tissues. Salinity and chloride strongly interact with more than additive effects in decreasing avocado yields. Although our trial did not include all the rootstocks they investigated, our results also indicated that fruiting capacity significantly decreased with the salinity treatment with high chloride concentrations (175 ppm). This is consistent with their findings. Of commercially available rootstocks tested, Dusa is the preferred rootstock for high salinity.

Based on the currently available rootstocks that were evaluated in this study, Dusa had the highest salinity tolerance. Of rootstocks tested that are currently experimental, R0.05 and PP40 showed the most promise under these conditions. Further testing is needed to determine suitability in the various avocado production areas in California.

References

Bernstein, L. 1965. Salt tolerance of fruit crops. Washington, D.C.: Agriculture Research Service, U.S. Dept. of Agriculture.

Bingham, F.T., Fenn, L.B. and Oertli, J.J. 1968. A sandculture study of chloride toxicity to mature avocado trees. Soil Sci. Soc. Amer. Proc. 32:249-252.

Bañuls, J., F. Legaz, and Primo-Millo, E. 1990. Effect of salinity on uptake and distribution of chloride and sodium in some citrus scion--rootstock combinations. J. Hortic. Sci. 65:715--724.

Crowley, D. and Arpaia M.L. 2011. Salinity-Chloride Interactions Effects on Yield. Production Research Report California Avocado Commission.

Kozlowski T. T. 1997. Responses of woody plants to flooding and salinity. Tree Physiology, 17(7):490, doi:10.1093/treephys/17.7.490

Mickelbart, M. and Arpaia, M.L. 2002. Rootstocks influence changes in ion concentrations, growth, and photosynthesis of Hass avocado trees in response to salinity. J. Amer. Soc. Hort. Sci. 127: 649-655.

Mickelbart, M.V., Melser, S., and Arpaia, M.L. 2007. Salinity-Induced Changes in Ion Concentrations of ‘Hass’ Avocado Trees on Three Rootstocks, Journal of Plant Nutrition, 30:1, 105-122, DOI: 10.1080/01904160601055137

Munns, R., and Tester, M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol, 59, (2008), pp. 651–681.

Oster, J.D. Stottlmyer, D.E., Arpaia, M.L. 2007. Salinity and Water Effects on ‘Hass’ Avocado Yields. J. Amer. Soc. Hort. Sci. 132(2):253–261.

Oster, J.D. and Arpaia, M.L. 1992. ‘Hass’ avocado response to salinity as influenced by clonal rootstocks. Proc. 2nd World Avocado Congr. 1:209-214.