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The physiological response of 'Hass' avocado to salinity as influenced by rootstock

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ABSTRACT

With increasing demands on both potable and agricultural water supplies, drought, and extreme temperatures worldwide, agricultural production is challenged with reduced water availability and lower water quality. Salinity, which is associated with low water quality is a critical issue for California avocado growers and, coupled with avocado root rot, threatens the long-term sustainability of the industry since avocados (*Persea americana* Mill.) are known to be extremely salt sensitive. Salt tolerance of the 'Hass' variety, the most commonly grown scion in California, is influenced by rootstock. We investigated 'Hass' scions grafted onto three different avocado rootstocks under control (irrigation using water with EC = 0.65 dS/m) and salinity (irrigation using water with EC = 1.5 dS/m) conditions. Results indicated that, compared to control conditions, the irrigation of avocado trees using water with EC = 1.5 dS/m increased canopy damage by 44%, reduced survival by half of the trees tested, and caused yield losses of more than 63%. Avocado leaves visibly damaged by the salinity treatment (named as partially burned or PB leaves) experienced photoinhibition, and reduction of photosynthetic rate and water-use efficiency, suggesting that the poor performance in carbon assimilation contributed to reductions in yield and increases in mortality. The salinity treatment did not cause water stress and the poor performance of treated trees was attributable to chloride accumulation previously reported. Leaf carbon isotopic composition was affected in trees under salinity treatment by increasing the values of $\delta^{13}\text{C}$ however, this affect was not correlated with water-use efficiency. Overall, 'R0.05', 'PP40' and 'Dusa' performed similarly and, considering the conditions of the experiment and the intrinsic susceptibility of avocado trees to salinity, were superior to all other rootstocks tested. Future screenings for salinity tolerant rootstocks are required to improve yield when poor quality soil or water is used. Overall, our results showed a coordination between the physiological performance, health and productivity of the 'Hass' scion and how these parameters were negatively affected by salinity.

1. Introduction

Water deficit limits plant growth and crop yield more than all other stresses combined (Kramer, 1983). The freshwater resources needed for agricultural irrigation are limited in some areas, and availability is expected to decrease with predicted drying trends associated with

climate change (Field, 2014). Agriculture is a major consumer of water throughout the world (Mendelsohn and Dinar, 2003; Viala, 2008), but during water shortages, supply of high quality water for agriculture cannot always be guaranteed (Gordon et al., 2010; Rosegrant et al., 2009). When water is in short supply, the use of water with increased dissolved solids is often the only option for continued irrigation, but

Abbreviations: dS/m, decisiemens per meter; EC, electroconductivity; DW, dry weight; FG, full green; PB, partially burned (necrotic); A_{area} , photosynthetic CO_2 assimilation per unit leaf area; E , transpiration; g_s , stomatal conductance to water vapor; C_i , internal CO_2 concentration; WUE_i , intrinsic water-use efficiency; SLA , specific leaf area; A_{mass} , maximum rate of CO_2 assimilation per unit mass; LMA , leaf mass per area; F_v/F_m , maximum quantum yield of photosystem II; Leaf, leaf water potential; $\delta^{13}\text{C}$, carbon isotopic composition; VPDB, Vienna Pee Dee Belemnite

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salinity in irrigation water is known to reduce crop growth and yield, or cause outright mortality of crops (Maas and Hoffman, 1977; Munns and Tester, 2008). Thus drought and salinity represent challenges for agriculture that are linked; both are natural phenomena and their intensity are worsened by human activities (McWilliam, 1986). As the human population is expected to surpass 9 billion by 2050, combined with the declining availability of new agricultural land, it is critical to both understand the mechanisms of salinity responses of crops and to evaluate new varieties for increased salinity tolerance.

There is great variation in the types of salts that produce salinity, but the general physiological effects in plants are well documented (Allakhverdiev et al., 2000; Munns and Tester, 2008; Shabala and Munns, 2012). Much of the study of salt stress has been conducted on plants by considering aspects of plant performance such as height or yield as a function of environmental salinity concentration (Maas and Hoffman, 1977). However, it is important to consider that within plants, salinity affects salt metabolism through uptake, transmembrane movement, compartmentalization, and feedbacks to growth and carbon assimilation that result in a problem of mega nutrient availability (Cheeseman, 1988). Among the many responses, osmotic imbalance and ion toxicity due to the accumulation of Na^+ and Cl^- are the first signs of salt stress. However, it is becoming clear that osmolyte biosynthesis and function, water flux control, and membrane transport of ions are critical components of maintenance and re-establishment of ionic balance (Hasegawa et al., 2000). In *Arabidopsis* the isolation and molecular characterization of genes involved in plant salt stress responses have been elucidated, especially in the identification of genes that regulate ion selectivity, transport and accumulation of Na^+ , H^+ , K^+ , Cl^- and Ca^{2+} (+ transporter gene can improve grain yield in wheat (Munns et al., 2012). The germplasm screening for salt tolerance, as well as crop improvement programs using marker assisted selection as a breeding tool are part of the approaches to address the salinity problem in agriculture (Ashraf and Foolad, 2013). In *Arabidopsis* the isolation and molecular characterization of genes involved in plant salt stress responses have been elucidated, especially in the identification of genes that regulate ion selectivity, transport and accumulation of Na^+ , H^+ , K^+ , Cl^- and Ca^{2+} (Golladack et al., 2011). There is also increasing evidence that stress sensing and signaling components play important roles in regulating plant salinity stress responses, as well as novel ion transport, detoxification pathways, and the impact of epigenetic chromatin modifications on salinity tolerance (Deinlein et al., 2014; Golladack et al., 2011; Hanin et al., 2016; Yang et al., 2009). This information is increasingly being used to develop salinity tolerant varieties of key crops. Potassium ion accumulation in roots, for example, has been shown to increase salt tolerance in wheat and barley (Cuin et al., 2012; Wu et al., 2015) and a Na^+ transporter gene can improve grain yield in wheat (Munns et al., 2012). The germplasm screening for salt tolerance, as well as crop improvement programs using marker assisted selection as a breeding tool are part of the approaches to address the salinity problem in agriculture (Ashraf and Foolad, 2013).

Traditionally, the sensitivity of crop plants to salinity has been measured as the relative yield as a function of the electrical conductivity (EC) measured in deciSiemens per meter (dS/m). In agriculture, the highest quality water has EC values lower than 0.5 dS/m. However, growers commonly use water with EC values within a range of 0–3 dS/m (Ayers and Westcot, 1985). Avocado is known to be extremely sensitive to salinity; yield begins to decline at irrigation water EC above 0.75 dS/m with chloride concentrations > 100 ppm and the general recommendation is to maintain a 10%–20% leaching fraction to keep EC_{sw} lower than 2.0 dS/m (Crowley, 2008; Maas and Hoffman, 1977; Mickelbart et al., 2007). With increasing water demands and droughts, avocado growers are faced with both reduced water availability and lower water quality. Salinity is a critical challenge for avocado growers and, coupled with avocado root rot, threatens the long-term sustainability of the industry. For example, since the 2009 California water management regulations were established (California

Department of Water Resources, 2009; State of California, 2009), water allocated to agriculture has been reduced or become more expensive, affecting water supply to avocado orchards in Orange, Riverside, Santa Barbara, San Bernardino, San Diego and Ventura Counties. Since avocado is not adapted to very hot and dry climates, avocado growers have sometimes “stumped” or completely removed groves (Medellín-azuara et al., 2012; Spann, 2014). In other cases, growers were supplied with low quality or reclaimed water with EC ranging 1–2 dS/m, associated with increased concentrations of dissolved solids, which can cause accumulation of toxic elements in soil, leading to accumulation in leaves, stomatal closure, reduced productivity and soil salinization (Branson and Gustafson, 1971; Grattan et al., 1997).

Specific studies on salinity responses of avocado have shown that salt sensitivity of avocado is influenced by rootstock selection. Oster and Arpaia (1992) found that rootstock affects fruit weight and health of ‘Hass’ avocado trees exposed to saline water. Chloride toxicity is correlated with reduction in yield and survival rates and the ability of the rootstock to exclude Cl^- and/or Na^+ from stems and leaves in avocado, such that new growth becomes the primary mechanism of salinity tolerance (Celis et al., 2018; Mickelbart and Arpaia, 2002; Oster et al., 2007). Celis et al. (2018) determined that the adverse effect on avocado yield and trunk diameter growth for 13 rootstocks was primarily related to leaf Cl^- toxicity. Rootstock also affects leaf area and biomass accumulation in leaves and stems of avocado trees exposed to a salinity treatment, which suggests that leaf biomass production per branch could be a good predictor of salinity tolerance in avocado (Bernstein et al., 2001). Mickelbart et al. (2007) analyzed the effect of salinity in the tissue-ion concentration of ‘Hass’ avocado trees grafted onto different rootstocks. Low Cl^- concentration and reduced $\text{Na}^+:\text{K}^+$ ratios in old leaves represent good markers to identify the ion exclusion ability of avocado rootstocks as well as salinity tolerance (Mickelbart et al., 2007). Little is known regarding the relationship between physiological performance, survival and yield in avocado trees under salinity conditions and how rootstock influences these factors. We investigated the physiological performance of ‘Hass’ avocado scions grafted onto a select group of rootstocks. Our main objectives were to: 1) determine effects of salinity on canopy damage, photosynthesis and plant water relations of selected rootstocks grafted with ‘Hass’; and 2) identify the effect of salinity on leaf-scale metrics of water-use efficiency.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted at the University of California Agricultural Experiment Station-Citrus Research Center, Riverside, California, USA (Parcel 13-C; 33.9737°N, 117.3281°W), administered by the Department of Agricultural Operations. The mean annual temperature is 18.6°C and ranges from a monthly mean of 11.7°C in January to 24.9°C in August. Mean annual precipitation is 280 mm and the soil is classified as an Arlington fine sandy loam, Haplic Durixeralf (Saito et al., 2006).

In April 2011, rootstocks from California, USA and South Africa with purported resistance to *Phytophthora* (*Phytophthora cinnamomi* Rands) grafted with ‘Hass’ scions were planted at a spacing of 3.4 × 6.4 m (11 × 21 ft.) and allowed to grow for 2 years and 8 months. The trees were randomly assigned a position and planted in 18 rows. Beginning in November 2013, selected rows were transitioned to salinity incrementally until the full salinity treatment (irrigation water with EC = 1.5 dS/m, $\text{Cl}^- = 4.94 \text{ mmol L}^{-1}$) was implemented in January 2014. The rows were randomly selected with salinity treatments originating from a 10X tank of saline water that was diluted using a Mazzei injector (Mazzei, Bakersfield, California). The remaining rows were irrigated with standard well water from the Gage Canal, used as control treatment (irrigation water with EC = 0.65 dS/m, Cl^-

= 0.73 mmol L⁻¹). Details of the experimental design are presented in an earlier publication (Celis et al., 2018).

This study was focused on three rootstocks: two avocado rootstocks from South Africa, 'R0.05' (experimental from Westfalia Technological Services) and 'Dusa' (commercially available), and one UC Riverside experimental selection 'PP40' rootstock breeding program. The rootstocks were chosen based on their ability for chlorine ion exclusion (Celis et al., 2018). Sample sizes varied between 6–9 individual trees per rootstock in the salinity treatment and between 5–8 individual trees per rootstock in the control treatment, depending on variety.

2.2. Plant survival and fruit production

Survival of experimental trees was calculated as the number of surviving individuals divided by the number planted × 100% for each rootstock variety 18 months after the start of the treatment. At the same time, canopy damage was measured as percent of canopy with necrotic leaves. In February 2015, fruit was harvested and total number of fruit and yield (kg fresh weight of all fruit) per tree were recorded (Celis et al., 2018). Using control trees as a reference, percent reduction in number of fruit and yield, and percent of canopy damaged by salinity was calculated for trees irrigated with saline water. Effects of salinity on fruit maturity were tested by measuring fruit dry weight (Fruit-DW) according to Arpaia et al. (2001). Fruit-DW was determined post-harvest by coring the flesh from the fruit, recording fresh mass and then evaporating water from the cut pieces in a microwave oven until constant mass.

2.3. Physiological measurements

All physiological measurements were performed in summer 2015. Photosynthetic gas exchange was measured using a portable photosynthesis system (LI-6400, Li-Cor Biosciences, Lincoln, Nebraska, USA), on mature, fully expanded leaves of the most recent flush for each experimental tree. In the control treatment, we measured two leaves per tree. In the salinity treatment, we separated leaves into classes based on leaf burn, a brown region of dry necrotic leaf tissue emanating from the leaf tip, and measured two leaves per tree that were fully green (FG) and two leaves per tree that were partially burned (PB). We measured and compared physiological traits in both types of leaves to identify differences between them and compared them with the leaves from the control trees. Photosynthetic measurements were taken at 25 °C controlled by integrated Peltier plates, 1500 μmol m⁻² s⁻¹ of photosynthetically active radiation supplied by a red/blue light source (Li-Cor 6400-02B, Li-Cor Biosciences), with CO₂ concentration maintained at 400 μmol mol⁻¹. Measurements were conducted from shortly after sunrise until 10:00 h, before stomatal closure, so that maximum rates of photosynthetic CO₂ assimilation per unit leaf area (A_{area}), transpiration (E), stomatal conductance to water vapor (g_s) and internal CO₂ concentration (C_i) could be determined. Intrinsic water-use efficiency (WUE_i) was calculated as A_{area}/E . Leaves measured with the portable photosynthesis system were harvested to determine specific leaf area (SLA). SLA was calculated as leaf area (cm²) measured with a leaf area meter (LI-3100; Li-Cor Biosciences) divided by dry mass (g), after drying leaves at 65 °C for 48 h. SLA was used to calculate maximum rate of CO₂ assimilation per unit mass (A_{mass}). Leaf mass per area (LMA) was calculated as $1/SLA$. The dark-adapted maximum quantum yield of photosystem II (F_v/F_m) was measured to monitor photosynthetic energy conversion, using a portable pulse amplitude modulated fluorometer (*Mini-PAM*, Heinz Walz GmbH, Effeltrich, Germany) at predawn on three leaves on each experimental tree. Leaves were exposed to modulated weak far-red irradiance, followed by exposure to a 0.8-s saturating flash (2,000–3,000 μmol m⁻² s⁻¹) of actinic white light. To maintain a constant distance and angle (60°) relative to the leaf plane, the fiber-optic probe that delivered the measuring beam and saturating pulse was mounted above the leaf with a leaf clip holder (2030-B, Heinz

Walz GmbH). Leaf water potential at predawn (Leaf Ψ_{predawn}) and midday (Leaf Ψ_{midday}) was measured with a pressure chamber (1001, PMS Instruments, Albany, Oregon, USA). Leaves were cut and immediately placed inside the chamber with the cut end exposed. Pressurized N₂ gas was gradually added to the chamber until sap exited the cut end as viewed with a lighted magnifying glass. This balancing pressure was taken as equal to bulk leaf water potential.

2.4. Leaf carbon isotopic composition

We used bulk leaf carbon isotopic composition ($\delta^{13}\text{C}$) as a measure of long-term integrated water-use efficiency. This technique is based on the observation that conditions causing plants to reduce stomatal aperture cause an increase in water-use efficiency and also a reduction of CO₂ concentration at the site of carboxylation, forcing Rubisco to assimilate more ¹³CO₂ (Farquhar and Richards, 1984). Thus, larger $\delta^{13}\text{C}$ values are interpreted as greater water-use efficiency (Cernusak et al., 2013). $\delta^{13}\text{C}$ has become an important tool for comparing water-use efficiency among agricultural varieties, including wheat, barley and cowpea (Farquhar and Richards, 1984; Hall et al., 1990; Hubick and Farquhar, 1989), and more recently avocado (Acosta-Rangel et al., 2018). Twenty sun-exposed and fully expanded leaves, from control trees and FG leaves from treated trees, but not PB leaves, were sampled from terminal branches that were not fruiting or flushing in October 2015. Samples were weighed, oven dried at 105 °C for 24 h until completely dry and then ground using a mortar and a pestle. Sub-samples of 0.5 mg ± 0.05 (dry wt) leaf tissue were loaded into tin capsules. Values of $\delta^{13}\text{C}$ were determined with a stable isotope ratio mass spectrometer (Isoprime Ltd., Cheadle, United Kingdom). Isotopes are reported in per mil (‰) relative to the standard VPDB (Vienna Pee Dee Belemnite), and verified with EDTA and USGS40 as working standards, which have $\delta^{13}\text{C}$ values of -32.24 and -26.39‰, respectively.

2.5. Data analysis

Statistical analysis was performed using R software. The data were tested for normality using a Shapiro-Wilk test and homocedasticity using Levene's test. Averages of each variable were calculated to compare the effect of rootstock and salinity using one-way nested ANOVA, with Tukey post-hoc tests for parametric variables and Kruskal-wallis post-hoc tests for non-parametric variables with significance of $p < 0.05$. Pearson product-moment analysis was performed to identify correlations among all variables.

3. Results

3.1. Effect of salinity on the canopy damage, survival rate and yield of 'Hass' avocado grafted to different rootstocks

The salinity treatment produced a progressive health decline to avocado trees over time that resulted in canopy damage ($p < 0.001$) and reductions in survival rate ($p = 0.0153$) and production ($p < 0.05$) (Table 1). In contrast, the three rootstock varieties had no effect on survival nor health over the 'Hass' scion ($p > 0.05$). The canopy damage induced by the saline treatment increased by 42%, 48% and 43% for 'R0.05', 'PP40' and 'Dusa', respectively, compared to control trees, which also had a percent of damage inflicted by heat (Table 1). In terms of tree survival, 100% of the trees from the control treatment survived regardless of rootstock, whereas, 33% of trees grafted in 'R0.05' or 'PP40' and 57% of trees grafted in 'Dusa' died under the salinity treatment. Salinity also reduced the productivity of all avocado trees independently of rootstock. The number of fruit per tree ranged from 43 to 53 in control trees and decreased in all trees exposed to salinity (11–29 fruit/tree), representing an average reduction of 63% compared to the control (Celis et al., 2018). Similar results were found

Table 1

Canopy damage, survival rate, reduction in number of fruits/tree and yield reduction/tree of 'Hass' avocado scions grafted onto different rootstocks after 13 months of salinity treatment. All trees in control treatment survived.

Rootstock varieties	Canopy damage (%)			Survival (%) ^a	Reduction in number of fruit/tree (%)	Reduction in kg fruit/tree (%)
	Control	Saline treatment	Difference			
'R 0.05'	15	57	42	67	45	60
'PP 40'	12	69	48	67	74	75
'Dusa'	31	74	43	43	69	68
Average	20	64	44	54	63	68

^a Survival and yield data from Celis et al. (2018).

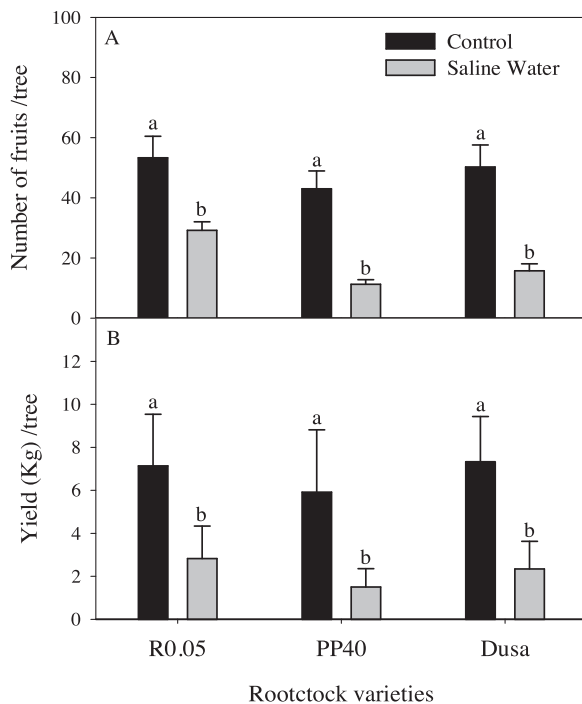


Fig. 1. Number of fruit (A) and yield (kg) (B) produced by trees grafted onto different avocado rootstocks (Celis et al., 2018). Bars represent mean \pm SE of control (black) treatment (EC = 0.65 dS/m) and salinity (light gray) treatment (EC = 1.5 dS/m). Different letters shared by the bars indicate significant differences within rootstocks at $p < 0.05$.

for yield, in which control trees produced 5.9–7.3 kg of fruit/tree and trees under the salinity treatment produced 1.5–2.8 kg of fruit/tree with a mean reduction of 68% compared to the control (Table 1, Fig. 1, (Celis et al., 2018)). In contrast, no effect of rootstock on fruit maturity was detected ($p > 0.05$).

3.2. Effect of salinity on the physiological performance of 'Hass' avocado scions grafted on different rootstocks

Salinity negatively affected 'Hass' avocado scion physiological performance (Table 2). In most cases, the fully green (FG) leaves of trees in the salinity treatment showed similar physiological values compared to leaves of control trees, but the partially burned (PB) leaves in the salinity treatment showed significantly reduced physiological values. Values for A_{area} , A_{mass} and F_v/F_m , were statistically similar for control and FG leaves, whereas PB leaves had significantly lower values ($p < 0.001$, Fig. 2). Leaf A_{area} for the control, FG and PB leaves averaged 15.28 ± 0.92 , 12.35 ± 0.74 and $5.95 \pm 1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. PB leaves had a 50% reduction of carbon uptake compared to FG leaves, where both were subjected to the same salinity treatment. Leaf A_{mass} in control, FG and PB leaves averaged 142.87 ± 17.28 , 128.15 ± 8.71 and $59.11 \text{ nmol g}^{-1} \text{s}^{-1}$, respectively, and similar to

A_{area} , PB leaves had more than a 50% reduction in A_{mass} compared to FG leaves. F_v/F_m in control, FG and PB leaves averaged 0.80 ± 0.02 , 0.78 ± 0.04 and 0.53 ± 0.07 , respectively. The F_v/F_m values of PB leaves were far below 0.75, considered the minimum value for healthy leaves (Fig. 2). Avocado rootstock varieties had no significant effect on these physiological traits ($p > 0.05$, Table 2).

Water relations in avocado leaves were also affected by the salinity treatment, however, the responses were modulated by rootstock variety (Table 2). For each rootstock, control and FG leaves had similar values for g_s , E , WUE_i and C_i ($p < 0.05$), whereas PB leaves had variable responses. In 'PP40', for example, PB leaves had a reduction in g_s and E greater than 50% relative to the control and FG leaves. 'R0.05' and 'Dusa' also had a significant reduction of ~50% in WUE_i for PB leaves compared to control leaves, but contrastingly ~2-fold greater values in C_i . Under the control conditions, a natural variation was found among the rootstock varieties regarding water relations. 'PP40' had ~2-fold greater rates of g_s compared to 'R0.05' and 'Dusa' ($g_s = 0.29 \pm 0.04$, 0.147 ± 0.06 and $0.128 \pm 0.06 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$, respectively). C_i was also ~2-fold greater in 'PP40' compared to 'R0.05' and 'Dusa' ($C_i = 284 \pm 2$, 154 ± 24 and $140 \pm 20 \text{ mm CO}_2 \text{ m}^{-2} \text{s}^{-1}$, respectively) (Fig. 3). Under salinity treatment, FG leaves from the 'R0.05' rootstock had significantly greater water-use efficiency compared to 'PP40', likely due to high rates of stomatal conductance in control and FG leaves of 'PP40'. The general low performance of PB leaves remained similar across the rootstocks.

Analysis of the effects of salinity and rootstock on other physiological traits, like LMA and leaf water potential, did not show significant differences among treatment or rootstocks varieties ($p > 0.05$) (Table 2). Leaf Ψ_{predawn} ranged from -0.11 to -0.21 MPa and leaf Ψ_{midday} ranged from -0.92 to -1.38 MPa (Fig. 4), showing that water was available and being used by treated trees.

3.3. Effect of salinity on leaf carbon isotopic composition

The analysis of carbon isotopic composition showed significant differences among treatments and rootstock varieties in the control treatment ($p < 0.001$, Table 2, Fig. 5). The salinity treatment increased $\delta^{13}\text{C}$ in FG leaves compared to leaves from control trees in 'Dusa' and 'PP40', but not in 'R0.05' (Fig. 5).

3.4. Integration of traits that promote scion productivity

Physiological traits in avocado trees were correlated with survival rates and production. A_{mass} was positively correlated with survival ($r = 0.84$, $p < 0.038$). High A_{mass} values belonged to leaves from the control treatment, in which all trees survived, and low values of A_{mass} belonged to leaves from trees under salinity treatment, in which the survival rate was reduced to nearly 60% on average. A_{area} values were positively correlated with greater production in terms of number of fruit ($r = 0.95$, $p < 0.004$) and yield ($r = 0.94$, $p < 0.005$). The correlation between physiological traits showed a coordination between carbon assimilation and water movement where high photosynthetic rates and photosynthetic yield were associated with higher transpiration

Table 2

Effect of salinity treatment and avocado rootstocks on 'Hass' avocado leaf physiological traits. Numbers in bold type represent *p*-values with significance at an alpha of 0.05.

Variable	Test	Normality	Homoscedasticity	Treatment	Rootstock	Interaction (Treatment x Rootstock)
A_{area}	Anova	0.676	0.860	< 0.001	0.088	0.917
A_{mass}	Anova	0.159	0.312	< 0.001	0.099	0.777
F_v/F_m	Kruskal-Wallis	0.6243	0.018	< 0.001	0.946	–
g_s	Anova	0.080	0.776	0.028	< 0.001	0.015
E	Anova	0.568	0.093	< 0.001	0.052	0.115
C_i	Kruskal-Wallis	0.015	0.426	0.010	< 0.001	–
WUE_i	Anova	0.983	0.328	< 0.001	< 0.001	0.279
$\delta^{13}\text{C}$	Anova	0.069	0.050	< 0.001	< 0.001	0.055
LMA	Anova	0.299	0.439	0.093	0.281	0.332
Leaf Ψ_{predawn}	Anova	0.514	0.492	0.212	0.417	0.652
Leaf Ψ_{midday}	Anova	0.214	0.243	0.096	0.657	0.519

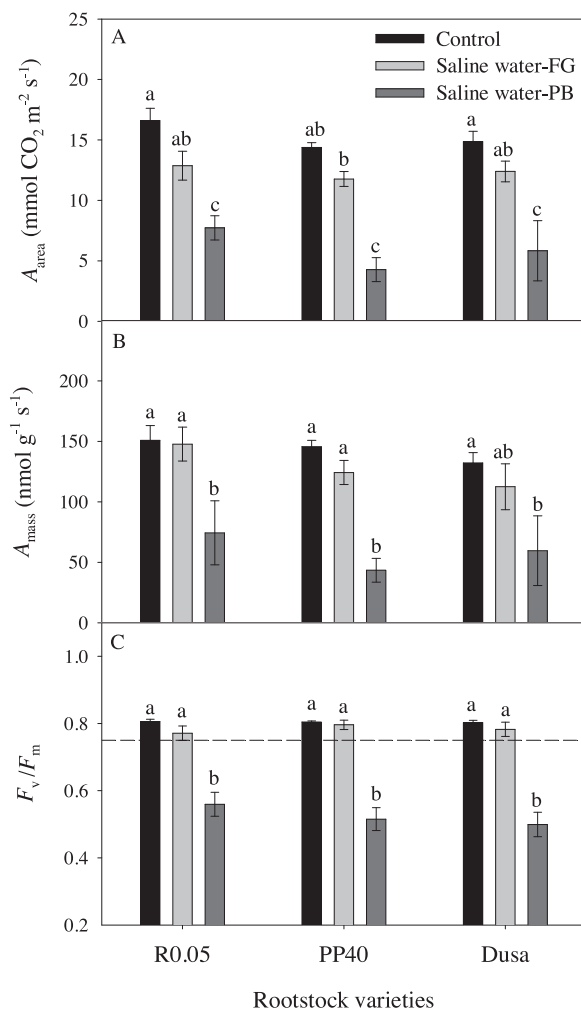


Fig. 2. Net photosynthetic rate per unit leaf area (A), net photosynthesis per unit mass (B) and maximum quantum yield of PSII (C) in leaves of 'Hass' scion grafted onto different avocado rootstock varieties. Different colored bars represent mean \pm SE of control (black) treatment (EC = 0.65 dS/m) and salinity treatment (EC = 1.5 dS/m) of both fully green (FG) (light gray) and partially burned (PB) (dark gray) leaves. Different letters shared by the bars indicate significant differences at $p < 0.05$.

($r = 0.87$, $p < 0.002$) and water-use efficiency ($r = 0.82$, $p < 0.015$), and low internal CO_2 concentration ($r = -0.73$, $p < 0.026$). Water potential was not correlated with any other traits and Pearson correlation values ranged from -0.64 to 0.44 ($p > 0.05$). Surprisingly, $\delta^{13}\text{C}$ was not correlated with WUE_i or any other physiological trait.

4. Discussion

This field-based salinity trial on established avocado plants identified varying levels of salinity tolerance in rootstocks when plants were irrigated with 1.5 EC water as compared to the control (0.65 EC). Survival and yield were related to photosynthetic rate, suggesting that the reduction in carbon assimilation from leaves in salinity treatments contributed to reductions in yield and increases in mortality. In general, rootstock varieties showed similar behavior with regards to carbon uptake but 'PP40' had poor stomatal control that reduced its water-use efficiency. Physiological results indicated that damaged partially burned (PB) leaves present in the canopies of 'Hass' scions on all rootstocks had a reduction in carbon assimilation and a loss of stomatal control under salinity treatment. Because all three rootstock varieties investigated herein have been previously verified as root rot tolerant the survival and yield of 'Hass' scions in this study should make these three rootstocks key candidates for further trials and show potential for incorporation into commercial growing operations. Overall, the results are promising for identifying potential germplasm material for future breeding projects and further investigations of the underlying mechanisms and points of control for genetic improvement of avocados in California.

Previous studies on avocado and citrus traits have shown variation in the performance of scions grafted with different rootstock varieties (Bañuls et al., 1990; Mickelbart and Arpaia, 2002). Celis et al. (2018) measured leaf ion concentrations in the same trees as in the present study, finding that avocado trees with the levels of survival rate and yield correlated well with lower leaf chloride concentrations. The most vigorous rootstock varieties were chosen for testing salinity tolerance in this study to understand what characteristics potentially contribute to maintaining output in salinity conditions. The results of this study indicate that reduced canopy damage and good stomatal control mitigate the effect of salinity on avocado production.

One of the most striking results of our study is the consistent statistical relationship that A_{area} showed with survival and yield. These data suggest that the carbon income from photosynthesis promotes survival and yield and that the reduction in photosynthetic carbon income, due to canopy damage in the salinity treatment, contributes to poor performance. The reduction in photosynthetic rate in the scions of rootstock varieties that performed relatively well in salinity treatments is consistent with a number of studies on reduced gas exchange under salinity conditions (Ball and Farquhar, 1984; Ishikawa et al., 1991). In PB leaves, there was a strong reduction in F_v/F_m , below the 0.75 value that is considered healthy for leaves (Bolhar-Nordenkamp et al., 1989). Therefore, damage to photosystems II is a component of the reduction in carbon gain of PB leaves. However, for FG leaves, F_v/F_m values were similar to that of control leaves, indicating that any reduced photosynthetic rates resulted from tighter stomatal control of gas exchange rather than damage to the photosystem or a change in carboxylation efficiency (Santiago et al., 2000). Therefore, if it is a consistent pattern

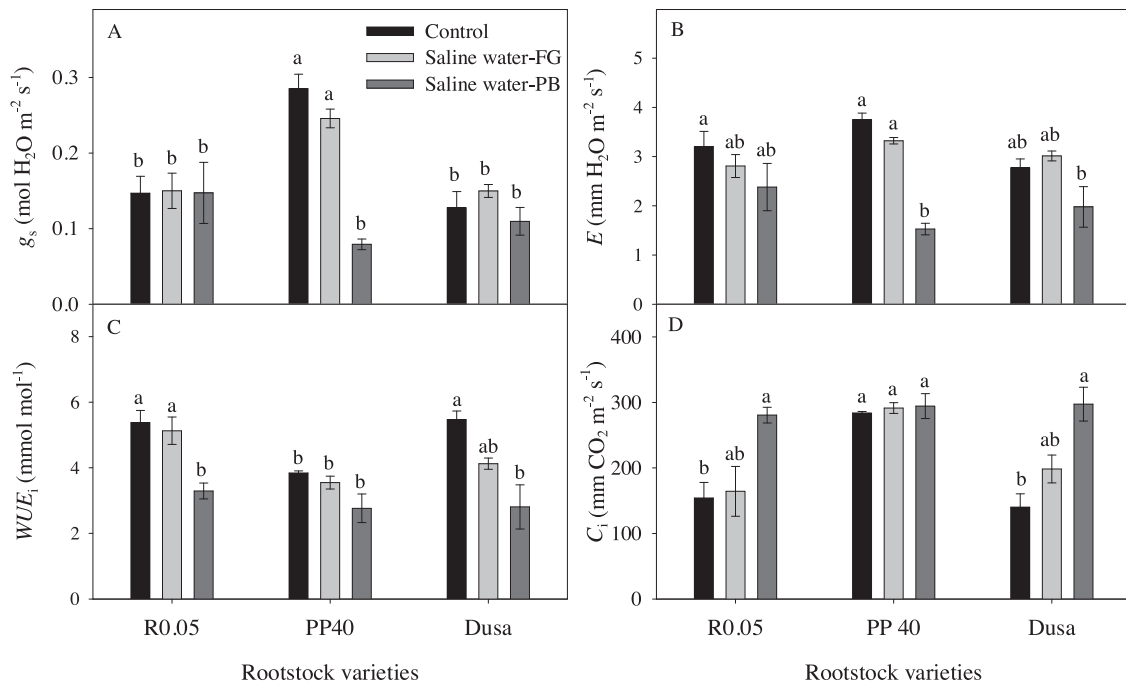


Fig. 3. Stomatal conductance (A), transpiration (B), water-use efficiency (C) and internal CO₂ concentration (D) in leaves of ‘Hass’ scions grafted on different avocado rootstock varieties. Different colored bars represent mean ± SE of control (black) treatment (EC = 0.65 dS/m) and salinity treatment (EC = 1.5 dS/m) of both fully green (FG) (light gray) and partially burned (PB) (dark gray) leaves. Bars with different letters are significantly different across rootstocks at *p* < 0.05.

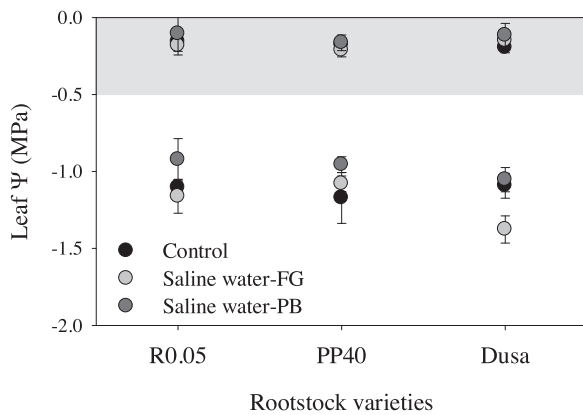


Fig. 4. Leaf Ψ_{predawn} (shaded area) and Leaf Ψ_{midday} (clear area) of ‘Hass’ scions grafted onto different avocado rootstock varieties. Different colored symbols represent mean ± SE of control (black) treatment (EC = 0.65 dS/m) and salinity treatment (EC = 1.5 dS/m) of both fully green (FG) (light gray) and partially burned (PB) (dark gray) leaves. No differences between treatments or among rootstocks were found (*p* > 0.05).

that FG leaves in salinity treatment are as healthy as they look, it may be possible to visually assess the degree of salinity stress and potential effects on survival and yield by measuring the percent of damage in the canopy. Furthermore, leaf water potential was not affected by treatment and stomatal closure in the salinity treatment probably helped to maintain plant water status and reduce ion accumulation. The role of changing water status in salinity-induced mortality could have had a greater role in varieties that suffered complete mortality that were outside of the vigorous varieties chosen in this study.

Because nearly all commercial production of avocados in California uses ‘Hass’ scions, there have been relatively few recent studies on variation in scion physiological performance. However, one recent study showed that among 24 avocado scions, there was 2-fold variation in WUE_i (Acosta-Rangel et al., 2018), and that much of this variation was related to differences in leaf sapwood area ratio (LA:SA), g_s and C_i .

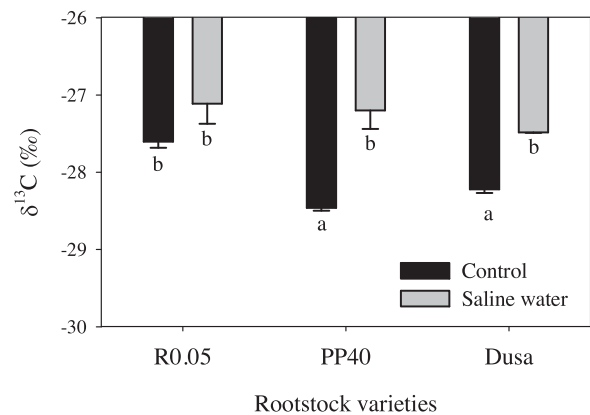


Fig. 5. $\delta^{13}\text{C}$ in leaves of ‘Hass’ scion grafted on different avocado rootstock varieties. Different colored bars represent mean ± SE of control (black) treatment (EC = 0.65 dS/m) and salinity treatment (EC = 1.5 dS/m) of fully green (FG) leaves (light gray). Bars with different letters are significantly different across rootstocks at *p* < 0.05.

LA:SA ratio varied up to 2.5-fold in the amount of leaf area supported by a given cross-sectional area of sapwood. Plant species or varieties that tend to maintain lower LA:SA ratio, g_s and C_i tend to be more conservative and show higher WUE_i , indicating that there may be further ways to overcome salinity by combining successful rootstocks with the right scion.

Overall, ‘R0.05’, ‘PP40’ and ‘Dusa’ performed well considering the conditions of the experiment. These three rootstocks in the present study had the highest survival rate, yield and toxic ion exclusion among 13 avocado rootstocks reported by Celis et al. (2018). During the first year of the saline treatment, there was a significant salinity stress due to accumulation of salts. During the time of these measurements, leaching fraction had been adjusted. However, the trees had not fully recovered, which could explain low yield in salinity treated rows despite canopy recovery. Taken together, the results suggest that ‘Hass’ scions on ‘R0.05’, ‘PP40’ or ‘Dusa’ would perform uniformly under the levels of

salinity currently encountered in avocado-growing areas in California and globally and may be able to tolerate anticipated near-term increases in salinity in irrigation and reclaimed water available to growers. With the appropriate leaching fraction, these rootstocks could outperform other rootstocks grown under saline conditions.

To conclude, the physiological responses of the trees under salt stress provide an indication of how well the trees might do as the quality of water for agriculture worsens. Currently, California growers use water with EC > 0.75 dS/m, but the threshold of water quality to prevent yield reduction in avocado is considered to be EC = 0.75 dS/m (Oster et al., 2007). Future screenings for salinity tolerant rootstocks are required to improve yield when poor quality soil or water is used.

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