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Grape Rootstock Response to Salinity, Water and Combined Salinity and Water Stresses

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Abstract: Diminishing availability of non-saline water in arid and semiarid regions is of concern to all irrigated agricultural producers, including wine and grape producers. Grapes are not a salt tolerant crop and producers often face the choice of either limiting fresh water application, using alternative saline waters or a combination of both. We examined the salt tolerance and effect of restricted water application on three purported salt tolerant rootstocks grafted to Cabernet Sauvignon scion in a 4-year replicated field experiment. ANOVA indicated significant effects of salinity water stress and rootstock on fruit yields. The 140 Ruggeri scion was the top producer across all treatments including control, followed by Salt Creek, with St. George significantly less productive than 140 Ruggeri across all treatments. In terms of salt tolerance, Salt Creek and 140 Ruggeri were not statistically different but St. George was significantly less tolerant than Salt Creek. In terms of drought tolerance (relative yield), there were no statistical differences among rootstocks. Soil salinity profiles and soil moisture sensors indicated reduced water consumption under high salinity, thus no matric stress under 60% of optimal water application when high salt stress was present. The multiplicative stress model where salt and water stress are individually evaluated did not satisfactorily predict yield under combined salinity and reduced water application, likely due to decreased water consumption under saline conditions. Short term (one year) experiments underestimate salt damage to grape vines as salt tolerance decreased over the 4-year experiment.

Keywords: Salinity; grapes; abiotic stress; wine; rootstocks

1. Introduction

Many wine grape production areas throughout the world are facing either increasing salinity in their irrigation water, decreased availability of water, or often a combination of both [1–4]. There is specific information on salt effects on multiple grape rootstock varieties and some information on drought tolerance [2,4], but there is almost no information on the combined effects of water and salt stresses. The effect of salinity on fruit yield has been evaluated in several field studies [5,6]. Grapes are reported as moderately sensitive to salinity with reduction in growth, starting at electrical conductivity of the saturation extract (EC_e) of 1.5 dS m^{-1} and subsequent reduction of 9.6% per each additional dS m^{-1} increase in EC [7]. However, there are significant differences among rootstocks, with yield loss reported to start above 3.3 dS m^{-1} for Ramsey rootstock and above 2.1 dS m^{-1} for Sultana [5]. Most salinity studies on grapes report irrigation water EC rather than EC_e, which is the factor best related to salt response [7]. The EC_e is determined by the irrigation water EC and the leaching fraction. Few studies either measure soil EC_e or provide information on leaching fraction. Some studies report yields under saline conditions without a non-saline control while others evaluate salt tolerance related to relative yield (yield under saline condition/yield under control conditions). Salt tolerant varieties are not necessarily those that produce the highest yield under saline conditions. Thus, for growers,

the critical value will be the absolute yield under specified salinity conditions while plant breeders will be also interested in salt tolerance for development of new varieties. Soultanina vines were reported to have 50% yield loss when irrigated with recycled water (EC 1.9 dS m^{-1}) as compared to fresh water (EC 0.6 dS m^{-1}) [8]. A screening of 25 rootstocks grafted to Colombard scion under saline conditions determined that the top yielding rootstocks were 13-5 EVE Jerez > Ramsey>143-B Mgt>140 Ruggeri, although apparently not statistically significant [9]. Other researchers have evaluated rootstocks with improved salt tolerance [5,6,10]. Most studies on effects of salt or water stress on physiological parameters are based on short term experiments [11–13]. Stevens et al. [14] determined that vines were especially sensitive to salinity during berry development.

There is limited information on potential rootstock differences in tolerance to water stress. The effects of deficit irrigation on fruit yield have been examined [8,15] with the focus of improving water use efficiency as well as yield and pruning mass [16]. Degaris et al. [16] examined deficit irrigation on Shirz under low salinity (EC irrigation of 0.5–1.35 dS m^{-1}). Increased drought survival of Syrah as compared to Grenache grapevines has been attributed to reduced hydraulic conductance in Syrah resulting in reduced shoot cavitation [17].

We conducted a four year experiment under controlled field conditions with the following objectives: (1) The salt tolerance (yield) response of Cabernet Sauvignon grafted on to three rootstocks, (2) Response to irrigation quantities, from meeting crop evapotranspiration (ET$_c$) to application of 80% and 60% of crop evapotranspiration (ET$_c$), and (3) Vine and fruit yield response to combined effects of salt stress and irrigation quantity.

2. Materials and Methods

A field experiment was conducted at the USDA-Agricultural Research Service, U.S. Salinity Laboratory in Riverside, California from June 2013 to Sept 2017 to evaluate the effect of 4 irrigation water salinity levels (EC of 0.7 dS m^{-1}, 1.7 dS m^{-1}, 2.7 dS m^{-1}, and 3.7 dS m^{-1}) designated S0, S1, S2 and S3 respectively and three water treatments 100%, 80%, and 60% of ET$_c$, designated D0, D1 and D2 respectively on three grape rootstocks purported to be salt tolerant. Cabernet Sauvignon scions were grafted onto Salt Creek (Ramsey, *Vitis champini*), 140 Ruggeri (*Vitis berlandieri* x *Vitis rupestris*), and St. George (*Vitis rupestris*) rootstocks and planted in the field in late May 2013. The experiment was a randomized complete block design. The rows were 2.5 m apart and the vine spacing was 2 m within the rows. For each rootstock we had three vines in each treatment with treatments replicated twice. A total of 12 treatments were evaluated for each rootstock.

To measure actual vine evapotranspiration (ET$_a$), we installed an array of 9 weighing lysimeters that were planted with three replicates of each rootstock. A brief description of the lysimeters is given in a previous study [18]. Lysimeters were weighed weekly with a digital scale (accuracy of ± 0.1 kg) and irrigated to replenish consumed water using a graduated cylinder (± 0.01 L). We initially irrigated the treatment vines with volumes above the lysimeter ET$_c$ with non-saline water (0.7 dS m^{-1}) for all treatments in 2013 to ensure good plant establishment.

The irrigation treatments (salinity and drought) began in July 2014. The S0 (EC 0.7 dS m^{-1}) was the control salinity and was composed of Riverside tap water with 0.5 mmol L^{-1} KNO$_3$. The S1 (EC 1.7 dS m^{-1}) treatment, S2 (EC 2.7 dS m^{-1}) treatment and S3 (EC 3.7 dS m^{-1}) treatment composition of the irrigation waters is given in Table 1. After year two and three in spring 2016 and 2017 we applied 0.20 kg/acre of the systemic imidacloprid to the soil below the vines as treatment against the spread of Pierce's disease. In the late 2016 season several vines were damaged due to Pierce's disease, hence they were subsequently removed from the subsequent statistical analysis.

Table 1. Chemical composition of irrigation waters.

Treatment	EC dS m^{-1}	Irrigation Water Composition (mmol$_c$ L^{-1})							SAR
		Ca^{2+}	Mg^{2+}	Na$^+$	K$^+$	SO$_4$$^{2-}$	Cl$^-$	NO$_3$$^-$	
S0 (Control)	0.65	3.09	0.45	1.65	0.07	2.68	0.73	0.35	1.24
S1	1.7	3.65	12.74	7.30	1.00	4.73	9.46	0.50	4.08
S2	2.7	6.50	4.90	13.00	1.00	8.30	16.60	0.50	5.45
S3	3.7	9.30	7.00	18.60	1.00	11.80	23.60	0.50	6.52

The vines that were irrigated at 100% of ET$_c$ (based on the lysimeter data) had two 1.0 L h^{-1} and one 0.5 L drippers, the 80% ET$_c$ treatment had two 1.0 L h^{-1} drippers and the 60% ET$_c$ treatments had one 1.0 L h^{-1} and one 0.5 L h^{-1} drippers, with all treatments receiving equal irrigation times. All vines were irrigated with Netafim pressure compensated drippers that were tested for accuracy before the start of the experiment and yearly thereafter; all drippers were within 5% of stated rates throughout the experiment.

ET$_c$ was also calculated from combining local reference ET (ET$_0$) [18] with crop coefficients (K$_c$) calculated based on shaded fraction/leaf area index (LAI) weekly data using the relation,

$$ET_c = K_c \times ET_0 = (F_{SA} (100 \times 0.017) - 0.008) \times ET_0 \tag{1}$$

where F$_{SA}$ is the fraction of the area shaded [19].

Soils were initially evaluated to ensure that salinity was below 1.5 dS m^{-1}. Soil samples were collected from the plots after harvest in 2016 and 2017. At each hole, samples were collected at 15 cm intervals down to a depth of 105 cm. Samples were air dried, saturation pastes prepared, and subsequently, extracts analyzed. The electrical conductivity values of the extracts, EC$_e$ were reported. Soil water content was measured using Decagon 5TE sensors installed at a depth of 15 cm.

Fruit yield was collected yearly and measured individually from each vine within 24 h of collection using an analytical balance (±0.1 g). Trunk diameter measurements were taken at a height of 75 cm in June of each year using calipers (±0.05 mm). Pruning was performed in the winter during dormancy. Material was collected, oven dried at 105 °C for 72 h and weighed (±0.1 g). Yield, trunk diameter and pruning weight data were analyzed using SAS statistical package (SAS Institute. 2011. The SAS system for Windows. Release 9.4. SAS Inst., Cary, NC, USA) We utilized ANOVA as well as Duncan's test for pairwise comparisons.

3. Results
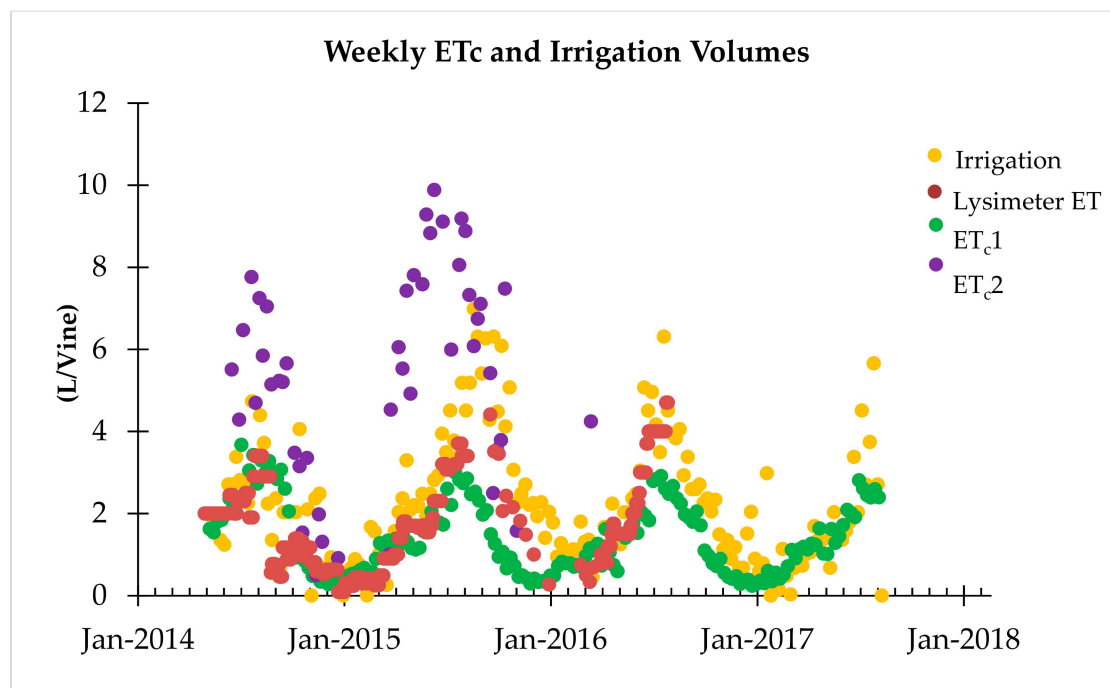
3.1. Water Application and Evapotranspiration

The applied irrigation volumes over time are shown in Figure 1 for the three application rates. Applied volumes were consistent with the desired ratios of the three application rates throughout the experiment.

The data for lysimeter ET, calculated ET$_c$ from leaf area index (LAI) and weather station data, ET$_c$, calculated from the Food and Agriculture Organization (FAO 56) with local windspeeds [18,20], crop coefficients and weather station data, and the D0 actual water application (100%) are shown in Figure 2. The measured lysimeter water use was initially less than the FAO based ET$_c$ calculations (ET$_c$1) until mid-summer 2015, when the lysimeter values became greater than the calculated FAO ET$_c$ as shown in Figure 2. The ET$_c$ values based on LAI were much greater than the lysimeter measurements and FAO ET values. Part of the discrepancy between lysimeter values and LAI based ET was attributed to a larger LAI in the experimental field vines as compared to LAI from the lysimeter vines. As shown in Figure 2, in response to these differences we irrigated above lysimeter values after June 2015. For the full water application treatments, actual water applications were intermediate between these lysimeter and LAI based estimates of water requirements. These values assumed a slight excess above ET$_a$ for leaching.
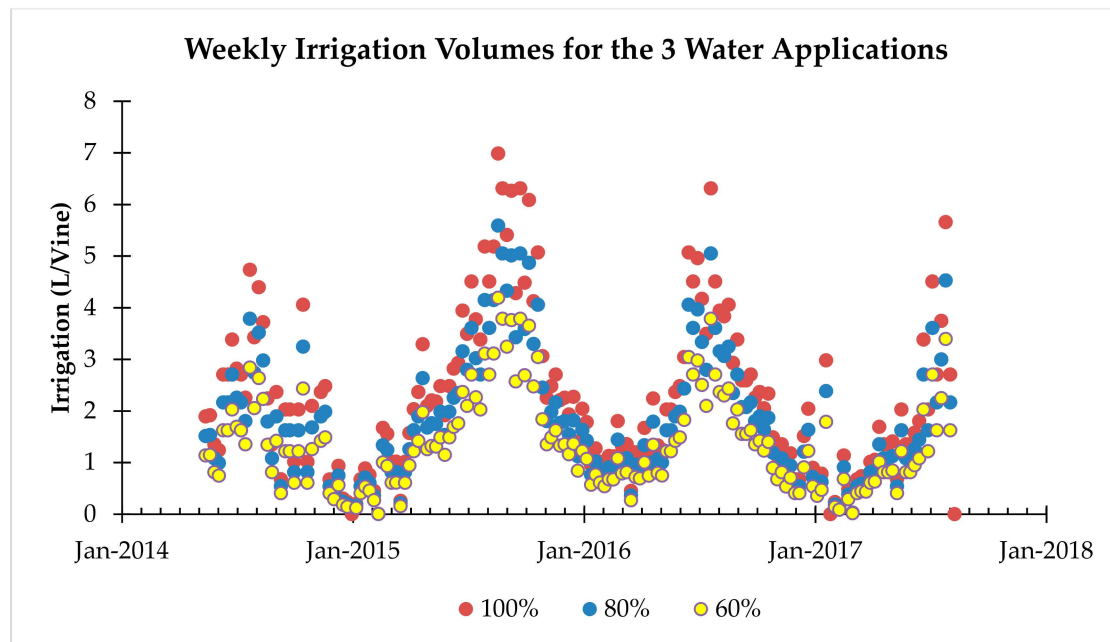
**Figure 1.** Weekly irrigation applications calculated from irrigation rates and times.

Figure 2. Water consumption per vine, from lysimeter measurements, water application for D0 treatments (100%), ET_c1 crop evapotranspiration (ET) based on k_c from Allen et al. [14] and weather station ET_0, and ET_c2, crop ET based on leaf area index (LAI) and weather station ET_0.

Based on water applications and calculations of water consumption, the full ET water applications (D0) had no water stress while 80% (D1) and 60% (D2) treatments were irrigated below water requirements, (Supplementary Materials Figure S1). Additional estimates of water application in comparison to water use were obtained based on salinity profiles and estimates of leaching as described below.

3.2. Soil Salinity

Soil extract data from the 2016 and 2017 is shown in Figure 3 for selected treatments. In 2016 the control salinity levels (S0), as expected, had the lowest soil EC values with a peak value at 45 cm, as shown in Figure 3a. The peak salinity value for the S0 treatment is 2.2 dS m^{-1}, corresponding to a soil water EC (EC$_{sw}$) at field capacity of 4.55 dS m^{-1}, using the conversion EC$_{sw}$ = 2.07 EC$_e$ determined for this soil earlier [21].
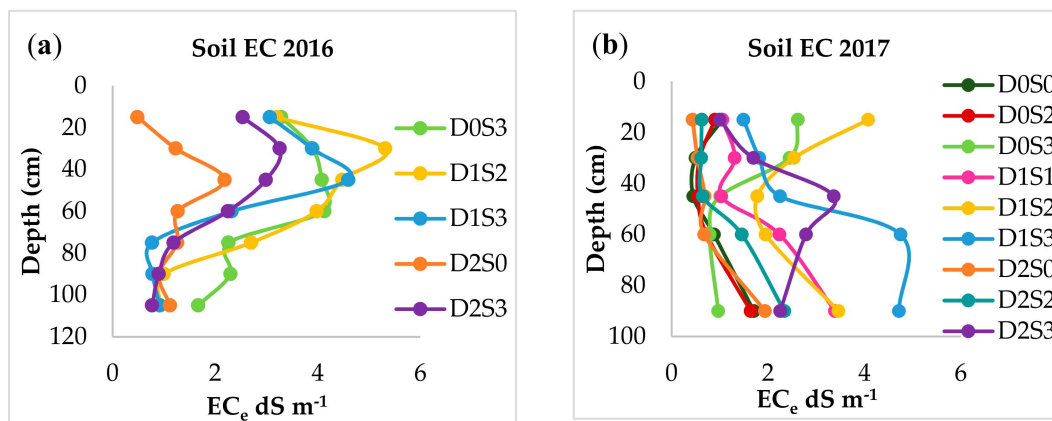
Figure 3. Saturation extract electrical conductivity of select treatments with depth for (**a**) 2016 and (**b**) 2017. The designated symbols represent the treatment water applications (D0 = ET$_c$, D1= 80% ET$_c$, D2 = 60% ET$_c$), S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC = 1.7 dS m^{-1} water, S2 = irrigation with EC = 2.7 dS m^{-1} water and S3 = irrigation with EC = 3.7 dS m^{-1} water.

Potential leaching or water stress can be calculated from the following equation:

$$LF = (V_a - ET_a)/V_a \qquad (2)$$

where LF is the leaching fraction, V_a is volume of water applied and ET_a is actual crop water consumption. Using salinity data, we can also calculate LF from irrigation water salinity and soil salinity at the bottom of the rootzone as follows

$$LF = EC_i/EC_d \qquad (3)$$

where EC_i is the irrigation water salinity and EC_d is the drainage water salinity, or EC at the bottom of the rootzone, assuming no soil mineral dissolution/ precipitation. This assumption is reasonable for our non-calcareous and non-gypsiferous soil and our low bicarbonate, acid-treated irrigation water.

Considering that the irrigation water of the S0D0 treatment is 0.65 dS m^{-1}, the soil peak EC soil water value of 4.55 dS m^{-1} corresponds to a calculated leaching fraction of 0.14. This value is greater than lysimeter estimates but much lower than ET$_c$ based on LAI measurements, which indicated all vines were underirrigated. Based on leaching calculations (0.14 for the D0 treatment), the D1 and D2 treatments with corresponding reductions of 20% and 40% of the water applied, would be expected to experience water stress. However, under saline conditions the salinity stress would be expected to reduce ET, which we observed for our more saline treatments. For the S3D0 treatments, the peak mean soil EC$_e$ is 4.13 dS m^{-1}, corresponding to an EC$_{sw}$ of 8.55 dS m^{-1} and a leaching fraction of 0.48, based on the irrigation water salinity of 3.7 dS m^{-1}. Thus, based on the soil EC data of the S3D0 treatment, irrigation water salinity of 3.7 dS m^{-1} reduced crop water consumption by approximately 45% relative to the non-saline control (S0D0). Thus, under the maximum salt stress the D1 and likely D2 treatments would have no water stress.

The S3 treatments (D0, D1, D2) show the maximum peak value occurring at increasing soil depth with increasing water application, as expected. The peak soil salinity value did not increase with decreasing water application, as would be expected. These data suggest that at the highest salinity and reduced water application the ET_a was reduced below that of the saline no water stress treatment (S0D0). The S2D1 soil salinity value was greater than expected but the peak was at 30 cm rather than 45 cm (D0 treatments) consistent with reduced water application (Figure 3a).

The soil salinity data for samples collected in 2017 is shown in Figure 3b. The salinity distribution for the S0D0 treatment is consistent with both leaching and a deeper root zone with vine age. The maximum EC occurred at the deepest depth at 90 cm, thus the rootzone peak ECe is 1.7 dS m^{-1}. This EC value corresponds to a calculated EC_{sw} of 3.52 dS m^{-1} and a leaching fraction of 0.18 for the full water application, meaning that the applied water volume was 22% above the estimated ET_a. These data suggest that in 2017 the S0D1 treatment with 20% less water might still have had sufficient water for optimal needs, while D2 treatments would clearly have deficit irrigation and water stress (under non-saline conditions).

At the highest irrigation water salinity and full water application (S3D0) the peak EC_e was 2.62 dS m^{-1}, corresponding to a leaching fraction of 0.68. When compared to the LF of the S0D0 treatment (0.18), this value indicates that high salinity in the absence of drought stress (S3D0) reduced water consumption to 39% of that for the non-saline treatment (S0D0). In this instance at EC = 3.7 dS m^{-1} irrigation water salinity, the reduced water applications (80% and 60%) of the D1 and D2 treatments would not be expected to result in drought stress, because of the reduced water uptake associated with the saline water.

The peak salinity value of EC 4.75 dS m^{-1} for the S3D1 treatment corresponds to a calculated 0.31 leaching fraction. The value for this treatment is less than expected (LF = 0.63) if water consumption for S3D1 were equal to water consumption for S3D0, but in any instance it indicates that sufficient water was available for all the S3D1 treatments. The S3D2 treatments had a maximum soil EC of 3.37 dS m^{-1} corresponding to a leaching fraction of 0.53 based on soil salinity. If water consumption were the same as in S3D0, the expected LF for this treatment would be 0.46 indicating that the S3D2 treatment consumed approximately the same amount of water as the S3D0, non-water stress treatment. Thus in 2017, there was no evidence of reduced ET as a result of water stress at the high salinity treatments with reduced water application.

3.3. Soil Water Content

The average soil water content in 2016 is shown in Figure 4. At full water application (D0) treatments the higher salinity treatments (S2 and S3) had greater soil water content than did the non-saline (S0) and first salinity level (S1). As all these treatments had equal water application this suggests decreased water uptake at the two highest salinity levels and no reduction in water uptake for the first salinity treatment (EC_{iw} = 1.7 dS m^{-1}). This pattern was repeated for the 80% and 60% water treatments—the water content was greater at higher salinity. Interestingly, the highest water content was at 60% water application and EC 2.7 dS m^{-1} (D2S2), suggesting that earlier salt stress greatly reduced vine water uptake. These data as a whole indicate that under salt stress water uptake was reduced below water application, even at 60% of the maximum water application. These data are also consistent with estimates of relative water consumption based on soil salinity, as discussed above.
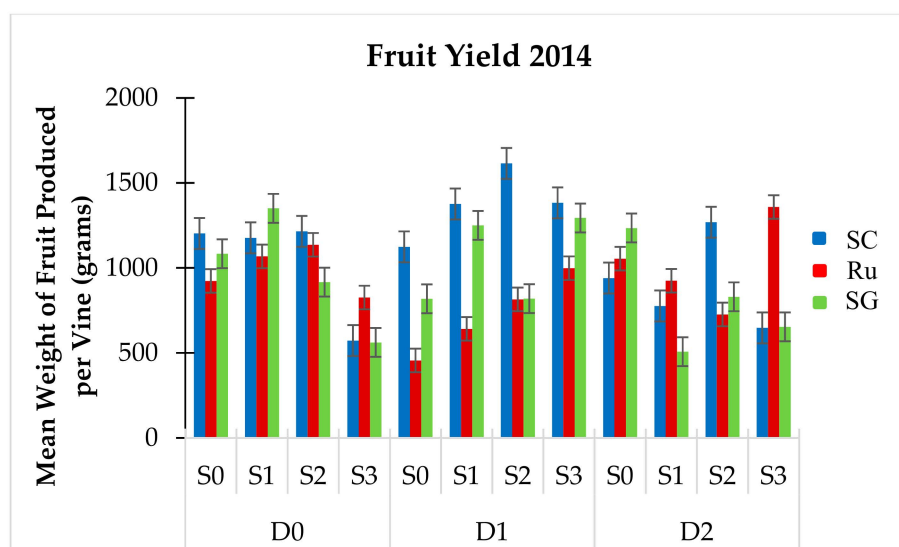
3.4. Fruit Yield

The ANOVA data indicated that for 2014 there were no significant differences in fruit yield among rootstocks, water treatments or salinity, although high salinity yield was significantly lower than the control when all rootstocks were averaged. Across treatments the order of yield was Salt Creek (SC) > St. George (SG) > *140* Ruggeri (Ru). Lack of statistical significance of treatment effects ($p > 0.05$) in 2014 is not surprising considering that the applications were initiated shortly before veraison and

that vines may experience a delayed response to abiotic stress. The data for individual treatments and rootstocks are shown in Figure 5.

Average Soil Moisture
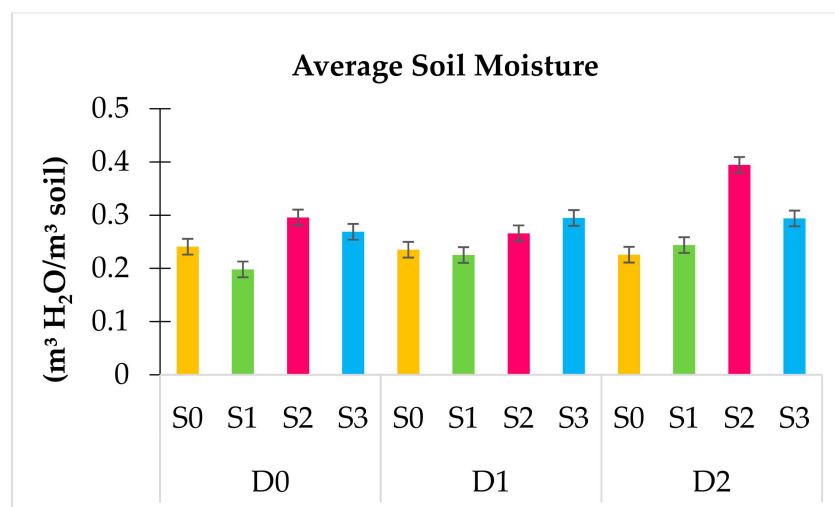
Figure 4. Available soil water measured by soil moisture sensors (Decagon 5TE Soil Moisture probes) installed at 15 cm depth during September 2016. The designated symbols represent the treatment water applications (D0 = ET_c, D1 = 80% ET_c, D2 = 60% ET_c), S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC = 1.7 dS m^{-1} water, S2 = irrigation with EC = 2.7 dS m^{-1} water and S3 = irrigation with EC = 3.7 dS m^{-1} water.

Fruit Yield 2014

Figure 5. Mean grape fruit yield as related to salinity, water application and rootstock, 2014 harvest. The designated symbols represent the treatment water applications (D0 = ET_c, D1 = 80% ET_c, D2 = 60% ET_c.), S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC = 1.7 dS m^{-1} water, S2 = irrigation with EC = 2.7 dS m^{-1} water and S3 = irrigation with EC = 3.7 dS m^{-1} water. SC represents Salt Creek rootstock, Ru represents 140 Ruggeri rootstock, SG represents St. George rootstock.

In contrast to 2014, the ANOVA for fruit yield in 2015 indicated highly significantly differences among rootstocks, water application, and salinity ($p < 0.01$). Rootstock-water and rootstock-salinity interactions were not significant, indicating no significant differences in salt or water stress tolerance among rootstocks. This is not unexpected in the early phases of the experiment. Salinity-water interactions were significant ($p < 0.05$), indicating that the water and salt stress were not additive but rather interactive. Across all salinity and water applications, Ru yield was significantly greater than SC, and SC was significantly greater than SG. Mean fruit yield of Ru across all treatments was almost twice

that of SG (1.10 kg/vine vs. 0. 562 kg/vine). Across all salinity and rootstocks, yield of D0 treatments were significantly greater than those in D2 treatments and the yields were in the order, D0 > D1 > D2, indicating that there was water stress and it had a negative impact on yield. Based on this response, Ru rootstock provided higher fruit yield across salinity and water applications (Figure 6). Across all treatments, yield decreased with increasing salinity S0 > S1 > S2 > S3 (950, 868, 783 and 491 g/vine, respectively), but only S0 and S3 were significantly different.
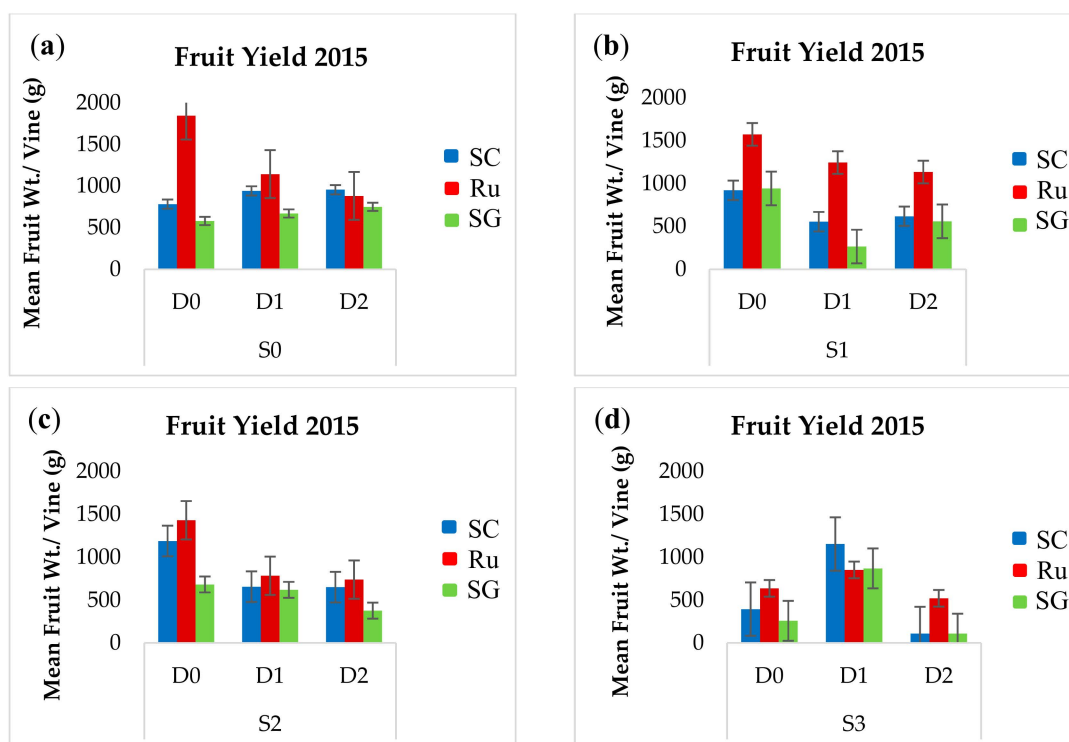
Figure 6. Mean grape fruit yield as related to salinity, water application and rootstock, 2015 harvest. The designated symbols represent the treatment water applications (D0 = ET_c, D1 = 80% ET_c, D2 = 60% ET_c). (**a**) S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, (**b**) S1 = irrigation with EC = 1.7 dS m^{-1} water, (**c**) S2 = irrigation with EC = 2.7 dS m^{-1} water and (**d**) S3 = irrigation with EC = 3.7 dS m^{-1} water. SC represents Salt Creek rootstock, Ru represents 140 Ruggeri rootstock, SG represents St. George rootstock.

The data for the individual treatments, shown in Figure 6, show the decrease in yield with reduced water application for all rootstocks, except at the highest salinity level. At the highest salinity level the yields are greatest at D1 water treatment, suggesting again that at high salinity, water demand was reduced. These data are consistent with the soil salinity and leaching data discussed above. Under non-saline conditions across all water treatments, yield of Ru was significantly greater than yield for SC and SG (1845, 783, and 580 g/vine), respectively. For the S2 treatments (EC_w = 2.7 dS m^{-1}) yield for Ru was significantly greater than for SG. SC with intermediate yield, was not significantly different from Ru or SG.

In 2016 the ANOVA results indicated that fruit yield was significantly different among rootstocks ($p < 0.02$) across all treatments and highly significant for water application and salinity ($p = 0.006$). These results are similar to those obtained in 2015. As in 2015, rootstock-water and rootstock-salinity interactions were not significant, but in contrast to 2015, salinity water interactions were not significant.

Across all salinity and water applications Ru fruit yield (1014 g/vine) was greater than SC (941 g/vine) and significantly greater than SG yield (683 g/vine); this was similar to results from 2015. Across all rootstocks and salinity levels fruit yields of D0 treatments were significantly greater than D2 treatments and the order was D0 > D1 > D2 (1034 g/plant, 837 g/plant and 729 g/plant, respectively).

Fruit yield across water and rootstocks decreased in the order S0 (1036 g/plant) > S1 (923 g/plant) > S2 (841 g/plant) > S3 (574 g/plant), with S0 being significantly greater than S3.

The yield data for individual treatments and rootstocks in 2016 is shown in Figure 7. Effects of water stress are evident in the low salinity treatments but not in the higher salinity treatments. This is consistent with the observation of reduced yield at the two highest salinity levels. Reduced yield is related to reduced water uptake and thus at high salinity with these observed reductions in yield we would not expect water stress associated with the reduced water application. These data are also consistent with the soil salinity and leaching data in 2016, that indicated expected water stress under non-saline conditions and very reduced water uptake under highly saline conditions, with no water stress under saline conditions.
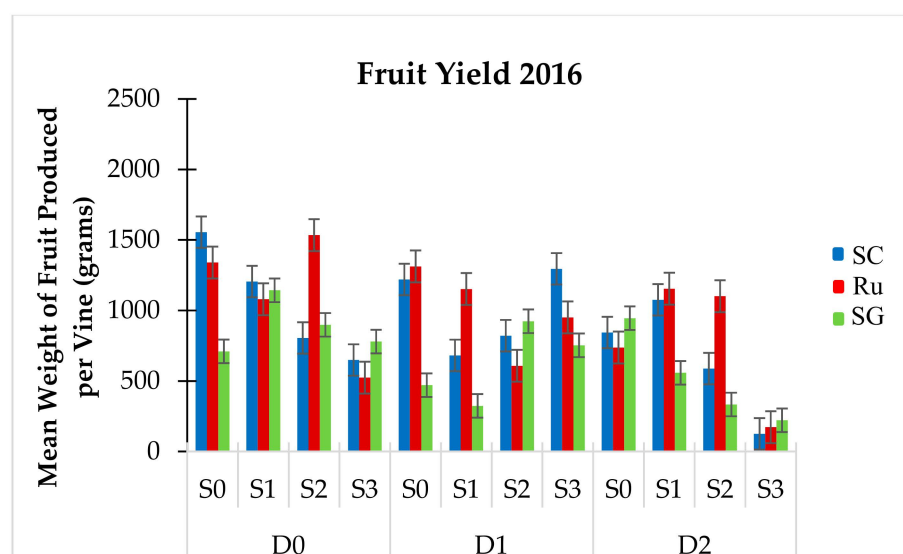
Figure 7. Mean grape fruit yield as related to salinity, water application and rootstock, 2016 harvest. The designated symbols represent the treatment water applications (D0 = ET_c, D1 = 80% ET_c, D2 = 60% ET_c). S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC = 1.7 dS m^{-1} water, S2 = irrigation with EC = 2.7 dS m^{-1} water and S3 = irrigation with EC = 3.7 dS m^{-1} water. SC represents Salt Creek rootstock, Ru represents 140 Ruggeri rootstock, SG represents St. George rootstock.

For the 2017 harvest the ANOVA results for fruit yield showed significant differences among rootstocks with yield in the order SC = Ru > SG and significant differences for water treatments with yield in the order D0 > D1 > D2. This was similar to other years with the exception that SC and Ru were not significantly different in 2017. In contrast to earlier years, yield was not significantly different with salinity at the ($P < 0.05$) level. This is mostly attributed to the total mortality of the S3 salinity level. The non-saline treatments had the highest yield and the order of yield was S0 > S1 > S2 as in earlier years.

The results from the individual treatments are shown in Figure 8. SC had significantly greater yield under non-saline conditions as compared to Ru and SG that had only 35% of the yield of SC. Drought stress was still evident under high low salinity, but not under high salinity, again consistent with the estimates of water consumption based on soil salinity.

3.5. Trunk Diameter

Trunk diameter provides an indicator of vine vigor and is an overall indicator of vine growth. Based on the ANOVA results across all years, the rootstock, water application and salinity all had a significant effect on trunk diameter. There were no significant interactions among these variables. The trunk diameters were in the order Ru > SC > SG with Ru being significantly greater than SC and SG. Similarly, for trunk diameters related to water treatment, D0 > D1 > D2 with D0 being significantly

greater than D1 and D2. Salinity had an adverse effect on trunk diameter; the order was S1 > S2 > S0 > S3 with S1 and S2 being significantly greater than S3. The trunk diameter data for 2015 is shown in Supplementary Materials Figure S2 and the 2016 data in Figure S3.

Based on the ANOVA results in 2016 water application and salinity had a significant effect on trunk diameter but rootstock did not. The interactions were all non-significant. The trunk diameters as related to salinity were S0, S1, and S2 not significantly different from each other but all significantly different (greater) than S3. The individual data for all treatments and rootstocks is shown in Figure S3. The trunk diameter data is consistent with the fruit yield response but trunk growth was not as adversely impacted by salinity and water stress as compared to fruit yield.
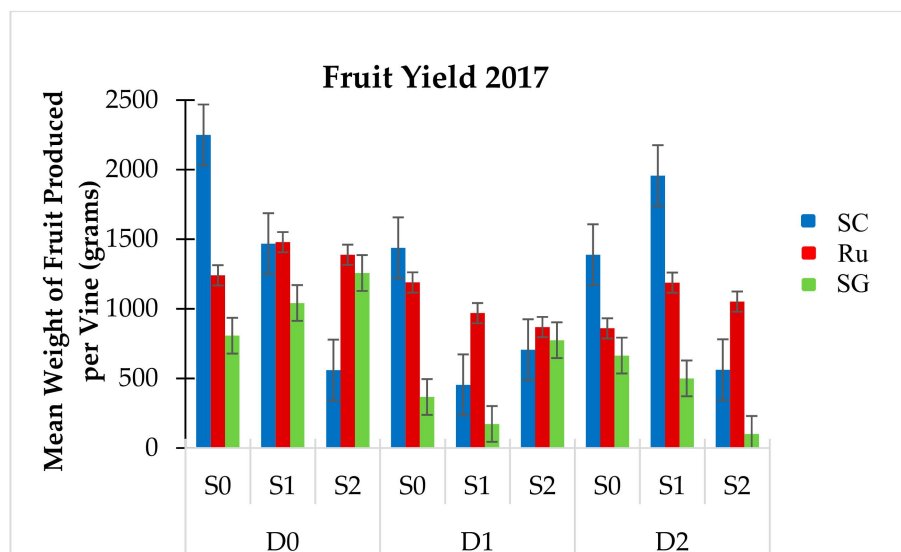
Figure 8. Mean grape fruit yield as related to salinity, water application and rootstock, 2017 harvest. Data are not available for vines irrigated with 3.7 dS m^{-1} water due to vine mortality. The designated symbols represent the treatment water applications (D0 = ET$_c$, D1= 80% ET$_c$, D2 = 60% ET$_c$), S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC = 1.7 dS m^{-1} water and S2 = irrigation with EC = 2.7 dS m^{-1} water. SC represents Salt Creek rootstock, Ru represents 140 Ruggeri rootstock, SG represents St. George rootstock.
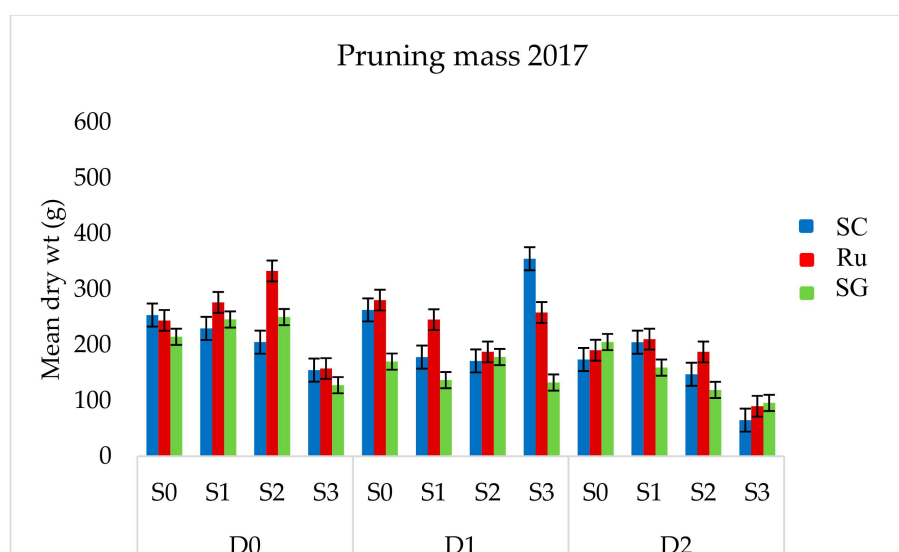
3.6. Pruning Weight

Pruning weight is a direct indicator of vegetative response to stress as well as an evaluation of the relative vigor of the various rootstocks. Across all salinity levels, water and rootstocks, pruning weights in 2014 showed no significant differences (data not shown).

The ANOVA analysis for 2016 data across all rootstocks, salinity and water treatments indicated no rootstock differences but significant adverse differences related to salinity and water application. The data for individual treatments show the overall adverse effect of salinity and reduced water on pruning weights, with S1 > S0 > S2 > S3 and D0 > D1 > D2 (Figure 9). Treatments at 60% of water requirements and EC 3.7 dS m^{-1} (S3D2) irrigation water had an approximately 50% reduction in pruning weights relative to non-stress conditions (S0D0).

The ANOVA data for 2017 across all treatments indicated significant differences for rootstock, salinity and water application. The pruning weights were in the order Ru > SC > SG. Both SG and SC had decreased pruning weights with salinity above the control level, while Ru maintained pruning weight up to EC 2.7 dS m^{-1} (Figure 10), consistent with the yield data. The pruning weights were in the order S0 > S1 > S2 > S3, consistent with salt and the water treatments in the order D0 > D1 > D2.
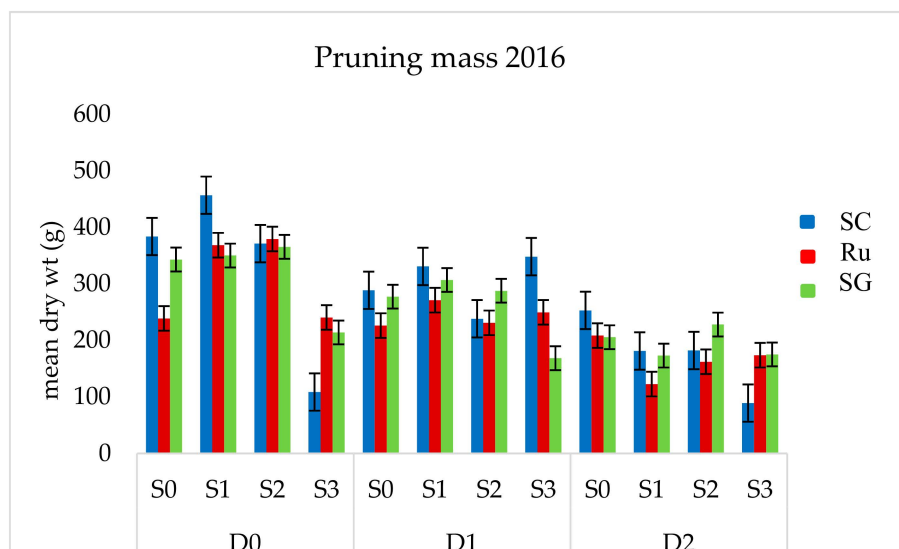
Figure 9. Mean pruning weight (dry) as related to salinity, water application and rootstock, 2016. The designated symbols represent the treatment water applications (D0 = ET_c, D1 = 80% ET_c, D2 = 60% ET_c, S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC= 1.7 dS m^{-1} water, S2 = irrigation with EC = 2.7 dS m^{-1} water and S3 = irrigation with EC = 3.7 dS m^{-1} water. SC represents Salt Creek rootstock, Ru represents 140 Ruggeri rootstock, SG represents St. George rootstock.

Figure 10. Mean pruning weight (dry) as related to salinity, water application, and rootstock, 2017. The designated symbols represent the treatment water applications (D0 = ET_c, D1= 80% ET_c, D2 = 60% ET_c. S0 represents irrigation treatments with EC = 0.7 dS m^{-1} water, S1 = irrigation with EC= 1.7 dS m^{-1} water, S2 = irrigation with EC = 2.7 dS m^{-1} water and S3 = irrigation with EC = 3.7 dS m^{-1} water. SC represents Salt Creek rootstock, Ru represents 140 Ruggeri rootstock, SG represents St. George rootstock.

4. Discussion

4.1. Relative Fruit Yield

The results indicated that the Ru rootstock produced the highest yield when evaluated across all treatments and years, and Ru was significantly greater than SG. In general, Ru rootstock produced the highest yield under control, drought and saline conditions, significantly greater than SG but not significantly greater than SC rootstock. The observed mortality especially at the highest salinity level

after four years is consistent with the results of a previous study where mortality was delayed relative to yield loss, with mortality still increasing in year four [22].

Our results indicate much greater sensitivity to salinity than indicated previously [6]. They examined 8 rootstocks with a control and two salinity levels (EC 1.75 and 3.5 dS m^{-1}). They did not report individual yearly yields, but cv Saltana, grafted on Ramsey (Salt Creek) rootstock, had no significant reduction in 5 year average yield even at EC irrigation water of 3.5 dS m^{-1} despite 48% reduction in pruning weight [6]. In contrast, we had complete mortality after year four when cv Cabernet Sauvignon, grafted on Ramsey was irrigated with 3.5 dS m^{-1} irrigation water. In our salinity treatments, in the absence of water stress we had small yield losses until EC 3.5 dS m^{-1} then a very rapid decline in yield. Under combined stress we had yield losses at the first salinity level (EC 1.7 dS m^{-1}). Among the differences between our study and theirs, their region had substantial rainfall, and they examined table grapes, where yield is optimized, in contrast to wine grapes where pruning is more extensive. However, salt tolerance is generally related to soil salinity and their average rootzone EC$_e$ values were somewhat greater than ours. We had no yield loss on Ramsey rootstock, in year one. We had significant yield loss in year two and three only at EC 3.7 dS m^{-1}, in the treatments without water stress, with subsequent mortality in year four. Based on the data from [22] (cv Sugarone grafted on Salt Creek rootstock) we would have expected about 30% yield loss with irrigation water of EC 3.7 dS m^{-1} after four years; however they also observed increasing mortality in year four as compared to year three.

After 25 days of exposure to water stress (50% of control), Jogaiah et al. [11] reported that young rootstocks had an increase in leaf content of reducing sugars and significant differences in physiological parameters. Two of the rootstocks they used were also used in our experiment. This same study reported that St. George (SG) had an initially higher transpiration rate than most, reached the wilting point by day 21 with no photosynthetic activity while Salt Creek (SC) was in a group with intermediate transpiration rate. These differences were attributed to difference in the ability to maintain osmotic adjustment, with sensitive cultivars producing less osmolites [11]. In our long term experiment we found no significant rootstock differences in fruit yield, or trunk diameter associated with reduced water application either across the entire data set or even in examination of only the non-saline treatments with water stress. In a similar 25-day experiment with salinity of 2 and 4 Ds m^{-1}, based on their reported data, Salt Creek was not in the grouping with higher osmotic potential nor accumulation of reducing sugar [11].

Salt tolerance is generally expressed in terms of relative yield [7]. We calculated relative yield for each variety and each year at each salinity level using the non-saline non-water stress treatments as control. The ANOVA results indicated that increased salinity significantly adversely impacted relative yield and that there were significant rootstock differences and yearly differences. Based on the subset of treatments with salinity only, SG rootstock was the most salt tolerant, significantly more tolerant than SC but not significantly greater than Ru. This is due primarily to the low yield of SG under control conditions. As seen, the relative yield of SC declined with increasing salinity and with increasing number of years (Figure 11a). That the salt tolerance decreased with time, suggests that even for young vines, the long-term consequences of increased salinity are not known after four years of salt application. In a similar manner the salt tolerance of Ru rootstock also decreased as the experiment progressed, however SG did not, except at the highest salinity (Figure 11b,c).

Comparison to existing salt tolerance data for grapes is difficult because of the decreasing salt tolerance with time as vines accumulate salt. Grieve et al. [7] reported that 50% yield loss is expected for grapevines at EC$_e$ 4.5 dS m^{-1}, based on vegetative yield in one-year experiments. Zhang et al. [5] evaluated data from three field experiments with cv Colombard grafted on Ramsey and determined that the threshold of yield loss occurred at EC$_e$ = 3.3 dS m^{-1} with a 5.7% yield loss for each additional increase of 1.0 dS m^{-1} of the soil extract.
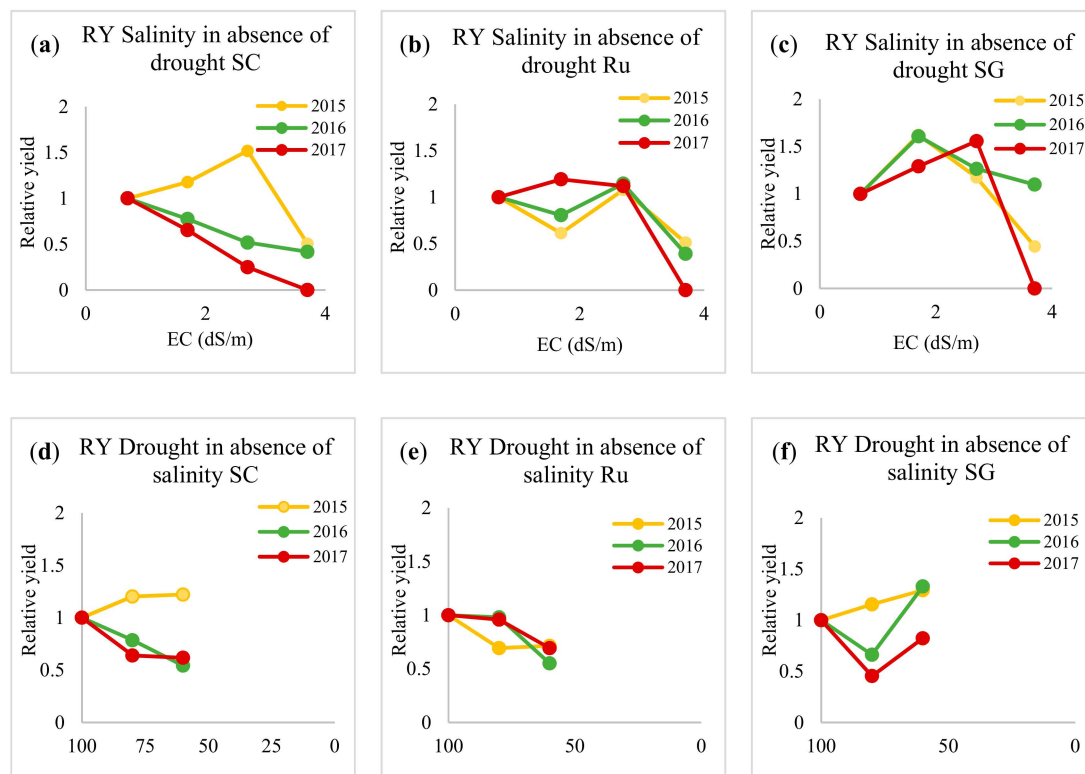
Figure 11. Relative yield as related to salinity in the absence of water stress for (**a**) SC (Salt Creek), (**b**) Ru (140 Ruggeri), (**c**) SG (St. George), rootstock and relative yield as related to drought in the absence of salinity stress for (**d**) SC, (**e**) Ru, and (**f**) SG rootstocks.

Shani and Ben-Gal [22] examined yield in lysimeters irrigated with water at EC 0.5, 3.0, 5.0 7.0, 9.0, and 12 dS m^{-1}. They did not observe a threshold, as yield of cv Sugraone decreased from the first salinity level EC$_e$ of 2.0 dS m^{-1} to the next value of EC$_e$ of 3.5 dS m^{-1}. The estimated 50% yield loss occurred at EC 3.7 dS m^{-1}. In our experiment, the S3 treatment with an irrigation water salinity of 3.7 dS m^{-1} had a calculated average rootzone EC$_e$ of 3.0 dS m^{-1}. Our data suggests that the average four years of irrigation with this salinity with resultant EC$_e$ = 3.0 dS m^{-1} can cause a 50% yield loss in the short term, not too different than [22], but continued use results in vine mortality.

Averaging the yield responses over time for each rootstock and fitting the data to the stepwise piecemeal regression equation [7] we obtained the relations shown in Figure S4, indicating somewhat more sensitivity to salinity than the generalized value previously obtained for biomass over one year experiments [7]. Construction of the salt tolerance model consisted of averaging all relative yield values until there was a significant difference between control and yield at a given salinity level. In this instance, variability was increased because the yield data was averaged across years. Thus, although the relative yield relations are similar for all rootstocks, we see that Ru had no yield loss until EC 3.7 dS m^{-1} while SC had decreasing yield at all values below the control and SC had decreasing relative yield above EC 1.7 dS m^{-1} (Figure S4). Based on Figure S4 the relative salt tolerance ranking would be Ru > SG > SC.

Similar to salt stress, we calculated yearly rootstock yield response to different water application levels to obtain relative drought tolerance in the absence of salt stress, (yield under reduced water application divided by yield at 100% water application) (Figure 11d–f for SC, Ru, and SG respectively). The ANOVA results indicated that there were no significant differences in rootstock tolerance to water stress in the absence of salt stress nor did the relative drought tolerance differ across the two water application levels. As shown in Figure 11d–f. The relative yield of SC decreased with decreased water application in 2016 and 2017 for SC and for Ru in all years but results for SC showed first decreased and then increased relative yield with decreasing water application.

Salinity response models were constructed for each variety using the D0 (no water stress) treatments for each year using the data shown in Figure 11. In a manner similar to the salinity response model, we developed a water response model from the non-saline treatments (S0D0) and the optimal yield and relative yield calculated as the ratio of D1/D0 and D2/D0 yield. The response of each variety was calculated for each year and then averaged over the individual years to obtain results shown in Figure S5. We averaged the yearly data because in contrast to relative yield and salt stress, there were no significant differences in tolerance to water stress across years.

Using the individual response models we next predicted the response to combined salt and water stress using the multiplicative stress response model from the UNSATCHEM [23] and SWS chemical transport and water flow model [24]. The models use a response function where the product of the response to water and salt stress is calculated, using different response functions for matric and osmotic stress (hψ50, and hφ50 respectively). The combined response function follows:

$$RY\ (h\psi, h\varphi) = (\alpha\psi(h\psi))(\alpha\varphi(h\varphi)) \tag{4}$$

where RY is relative yield, hψ and hφ are the dimensionless stress response function (relative yield) for osmotic and matric stress ($\alpha\psi$ and $\alpha\varphi$ respectively). In this instance we use the relative yield relations given above for salt (Figure 11) and water stress (Figure S5). This multiplicative relationship has been shown to more accurately predict combined water and salt stress than other models such as dominant stress, additive stress, and additive response to stress [21].

The results shown in Figure 12 indicate relative yield from combined stresses and do not use the data used to develop the relations shown in Figure 11 and Figure S5. The prediction of yield under combined stress based on relationships of separate stress is marginally useful for Ru and SC but very poor for SG. We note that the 1:1 predicted: Observed line is very similar to the regression line of predicted: Observed for Ru and SC, (but not SG, as shown in Figure 12). Increasing salinity resulted in increased soil water content and increased leaching, thus in the presence of salt stress the reductions in water application of 20% and 40% of estimated requirements, still provided adequate water. This is consistent with model predictions where water consumption is not fixed but related to yield [24]. The adverse effect of reduced water application in treatments with combined stresses is related in part to the increased soil salinity when less water is applied. This information suggests that water requirements including leaching requirements, will be overestimated based on ET$_c$, calculations, consistent with SWS model predictions [24].
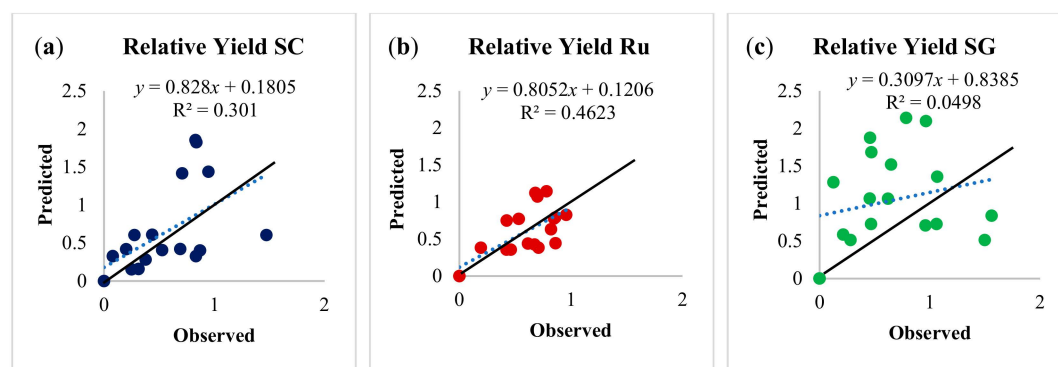
Figure 12. Predicted and observed relative fruit yield under combined water and salt stress for (**a**) SC (Salt Creek), (**b**) Ru (140 Ruggeri), and (**c**) SG (St. George) rootstocks.

4.2. Relative Pruning Weight

Examining only the salinity data in the absence of water stress, the ANOVA indicated highly significant differences in rootstock and salinity with no significant interactions. Ru was significantly greater than SG and SC. In terms of salinity, S3 was significantly different than S0, S1, and S2. Thus,

for both fruit yield and vegetative growth (relative pruning weight), RU was more salt tolerant and there were no differences in terms of tolerance to water stress.

In a manner similar to fruit yield we calculated the relative response of pruning weight to salinity, water stress, and rootstock. Across years, salt and water treatments there were no yearly significant differences but there were significant differences based on water application ($p < 0.05$) while salinity was significant only at $p < 0.11$ and rootstock differences at $p < 0.07$. Relative pruning weight decreased with time 2015 > 2016 > 2017, consistent with the earlier finding that for relative fruit yield, the vines became more sensitive to salinity with time. Similarly, relative pruning weight was in the order S1 > S2 > S3 as expected and D1 was significantly greater than D2. Based on the ANOVA Duncan grouping, Ru had significantly greater relative pruning weight than SC. Relative pruning weight was in the order Ru > SG > SC.

The relationships between relative pruning weight and salinity for each rootstock and relative pruning weight and water application are all given in Figure S6. From these relationships the predicted effects of combined water and salt stress were calculated and compared to the observed data. As with relative fruit yield, the model predictions (Figure S7) were not satisfactory.

In contrast to the data of [6], we did not find that pruning weight was a more sensitive indicator of salt stress. Our results are in accordance with [16], who found pruning weight to be less sensitive to salinity than fruit yield.

5. Conclusions

Based on the overall data, Ru (140 Ruggeri) was the top producer under all treatments and was also the highest ranked with Salt Creek in terms of salt tolerance. Ru was also ranked first in terms of other growth parameters trunk diameter and pruning weight. Absolute values for salt tolerance were difficult to quantify due to decreasing salt tolerance with time, suggesting that we underestimate salt tolerance when we conduct short term experiments. We found no significant rootstock differences related to water stress. The reductions in water consumption under high salinity were sufficient such that water stress did not exist under high salinity, in contrast to calculated stress under non-saline conditions. This suggests that less water is required under saline conditions to meet vine ET. Nonetheless we observed reductions in yield under reduced water applications. Under non-saline conditions we had water stress, under saline conditions the reduced water applications result in reduced leaching and thus higher soil salinity. Thus, we recommend that water application should not be reduced under salinity conditions despite the reduced water consumption. We were not able to accurately predict the effects of combined stress based on the quantification of salt stress in the absence of water stress and water stress in the absence of salt stress. This failure may result from the different processes limiting yield associated with reduced water application. Under low salinity reduced water application results in water stress, under high salinity it causes increased soil salinity (and salt stress) relative to full ET water applications.

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