



The Combined Effects of Salinity and Drought on Young Almond Trees and Physiological Parameters

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ABSTRACT

In drought years, almond growers have to restrict fresh water application, either stressing the trees with inadequate water or adding saline water and reducing water stress but causing salt stress. Tree response to combined water and salt stress are critical consideration on management decisions but there is no appropriate information currently. That's why, it was investigated the water and salt stress and combined water-salt stress on two almond varieties in a two year (2015 and 2016) outdoor experiment with young trees. Trees were 1 year old at the beginning of the experiment. The experiment was conducted USDA (United States Department of Agriculture) Salinity Laboratory, Riverside, California, USA. Drought treatments consisted of 100%, 80% and 60% of tree evapotranspiration (ET) and salt treatments of Electrical Conductivity (EC= 0.55, 1.20, 2.40 and 3.0 dS m⁻¹), for a total of 120 trees in twelve treatments with two varieties and five replicates. We examined water use, trunk diameter and physiological parameters (leaf net photosynthetic rate, stomatal conductance and leaf water potential). Photosynthetic rate values

(Pn) ranged between 3.53 and 11.08 μmol CO₂ m⁻² s⁻¹ for Nonpareil and 4.58 and 9.48 μmol CO₂ m⁻² s⁻¹ for Aldridge. Stomatal conductance values ranged between 0.076 and 0.283 mol H₂O m⁻² s⁻¹ for Nonpareil and 0.097 and 0.302 for Aldridge. All parameters showed significant decline starting at 80% water application and EC 1.2 dS m⁻¹. In terms of growth rather than survival, almond was sensitive to water as well as salt stress. We evaluated combined stress using three stress response models: additive stress, dominant stress model and a multiplicative stress model where the predicted growth loss is obtained by multiplying the relative growth response for the individual stresses. Equation (2) for reduction in trunk growth were developed for treatments with either salinity only or water only stress. Both varieties grafted to Nemaguard rootstock were very sensitive to salinity with growth loss starting at EC 1.2 dS m⁻¹. The results indicate that the Nonpareil is more sensitive to drought and salt stress than Aldridge. Aldridge almond variety can be recommended for areas where water supplies are scarce and salinized.

Keywords: Almond, Combined stress, Photosynthesis, Stomatal conductance, Trunk growth

1. Introduction

Salinity is one of the most important problems threatening both arid and semi-arid agricultural lands. Throughout the world, more than twenty percent of the agricultural lands are irrigated and approximately 20% are under direct threat of salinity (Worldbank 2021). Increased salinity levels significantly limit crop quality and yields and when associated with increased sodicity, also deteriorate soil structure. In addition, salinity-induced stress influences plant growth through various physiological, biochemical and molecular changes exerted in plant internal mechanisms (Ashraf & Foolad 2007). Impacts of salinity on plant and soil mechanisms should clearly be identified in order to grow a crop with saline water or saline soil (Düzdemir et al. 2009). Salinity management in irrigation requires knowledge of the irrigation water amounts and salinity levels which avoid or minimize decrease in yield. Species and varieties of a genus of crops (plants) have wide variability in their resistance to salinity enabling growers to utilize this information when making crop or varietal crop selections.

Drought is another factor threatening agricultural production. The major part (approximately 70%) of water consumption in the world is used for agricultural production. However, as the ratio of water use increases due to rapidly increasing population and developing industry, water amounts used in agriculture decreases (Önder et al. 2005). Thus, there is a high likelihood that plant species cultivated in irrigated agricultural areas will face water deficit in coming years. In this case, researchers need to determine the resistance of different plant species to both drought and salt stress.

Both salinity and drought stress cause plants to limit water uptake and result in reduction of the growth rate associated with metabolic changes. When irrigating with saline water, reduced water application reduces salt leaching and increases salt

accumulation in the soil. Thus, not only water and salt stress needed to be examined but also plant response to combined salt and water stress.

Almond is considered sensitive to salt stress and its productivity rapidly reduces at salt concentrations above 1.5 dS m^{-1} , with a 50% yield reduction at a soil salinity concentration of 4 dS m^{-1} (where data are reported as EC_e , the salinity of a soil saturation extract), (Maas & Hoffman 1977; Ottman & Byrne 1988; Grieve et al. 2012). However almond response to salt stress has been shown to vary considerably as related to rootstock (El-Motaium et al. 1994; Zrig et al. 2016; Sandhu et al. 2020), as well as genotype of scion (Momenpour et al. 2018).

Contrary to the salt sensitivity of almonds, it is regarded as tolerant to water deficit conditions (Feres & Goldhamer 1990; Torrecillas et al. 1996; Yıldırım et al. 2021). Almond has always been traditionally considered a drought tolerant crop, grown in areas with limited water supply (Gispert et al. 2011). However, this consideration is based primarily on survival as related to seasonal drought. Evaluation and identification of the tolerant cultivars of fruit trees are very important for drought stress and their ability to grow under these conditions. Drought stress generally has significant effects on plant physiology of almonds. Plant physiological characteristics such as photosynthesis and transpiration rate are dependent on the severity and duration of drought stress (Ranjbar et al. 2019). It has also been noted that response to water stress is dependent on rootstock (Isaakidis et al. 2004). The spectacular vegetative and productive response of this crop to irrigation (Leon et al. 1985) justifies the interest in the knowledge of the plant water relations in almond trees under drip irrigation conditions.

According to production data of year 2018, USA with 1,872,500 tons of annual production is first in world almond production (58.8% of total world production) (FAO 2020), with almost all the production in California. The almond growing regions in California face periodic restrictions on fresh water supply (surface water) and in drought years will need to evaluate if it is best to just reduce water application causing drought stress or to supplement with saline ground water causing reduced drought stress but adding salt stress. Our objectives were thus to 1) examine the salt, drought and combined salt and drought response of two different almond scions grafted to a commonly used rootstock and 2) evaluate predictive models for the response to the combined stresses to enable decision making regarding optimal quantities of applied saline water when fresh water is inadequate to meet tree ET demand.

2. Material and Methods

This project was conducted consecutively for two years (2015 and 2016) at an experimental area in USDA Salinity Laboratory, Riverside, California, USA. Two very commonly used varieties of almond, Nonpareil and Aldridge were grafted on a widely used rootstock, Nemaguard. Recent information indicates that Nemaguard is a relatively salt sensitive rootstock (D. Sandhu, personal communication).

Almond trees were planted in March 2015 into pots having 100 L volume. Soil mixture was including soil from the westside of the San Joaquin Valley and peat moss (1:1 ratio), with soil analysis provided in Table 1. Each tree was irrigated with control water (Riverside Gage Canal water, $\text{EC}_w = 0.55 \text{ dS m}^{-1}$) until soil water level reached field capacity up to the beginning of June for each year. This simulates typical winter-spring conditions in Central California and Mediterranean climate where rain is sufficient during this period to avoid need for irrigation.

Table 1- Properties of soil mixture in pots

<i>Texture</i>	<i>Clay loam</i>
Saturation (%)	45.4
Salinity (dS m^{-1})	2.13
pH	7.94
Na ($\text{mmol}_c \text{ l}^{-1}$)	6.629
K ($\text{mmol}_c \text{ l}^{-1}$)	0.565
Ca ($\text{mmol}_c \text{ l}^{-1}$)	13.180
Mg ($\text{mmol}_c \text{ l}^{-1}$)	5.050

There were 12 different drought and salinity treatments in this experiment: Three different drought treatments for each irrigation salinity (D_0 ; full irrigation, 100% evapotranspiration of almond trees, no stress, D_1 ; 80% evapotranspiration of almond trees, 20% deficit irrigation, moderate stress, D_2 ; 60% evapotranspiration of almond trees, 40% deficit irrigation, severe stress). We had four different salinity treatments (S_0 ; $\text{EC} = 0.55 \text{ dS m}^{-1}$, S_1 ; 1.20 dS m^{-1} , S_2 ; 2.40 dS m^{-1} , S_3 ; 3.0 dS m^{-1}) for each of the drought treatments. There were five replications for each treatment and each replication had one trees. 60 trees were used for each variety, totally 120 trees were used in the study. Maximum air temperature of vegetation period in 2015 and 2016 are shown in Figure 1a and 1b, respectively.

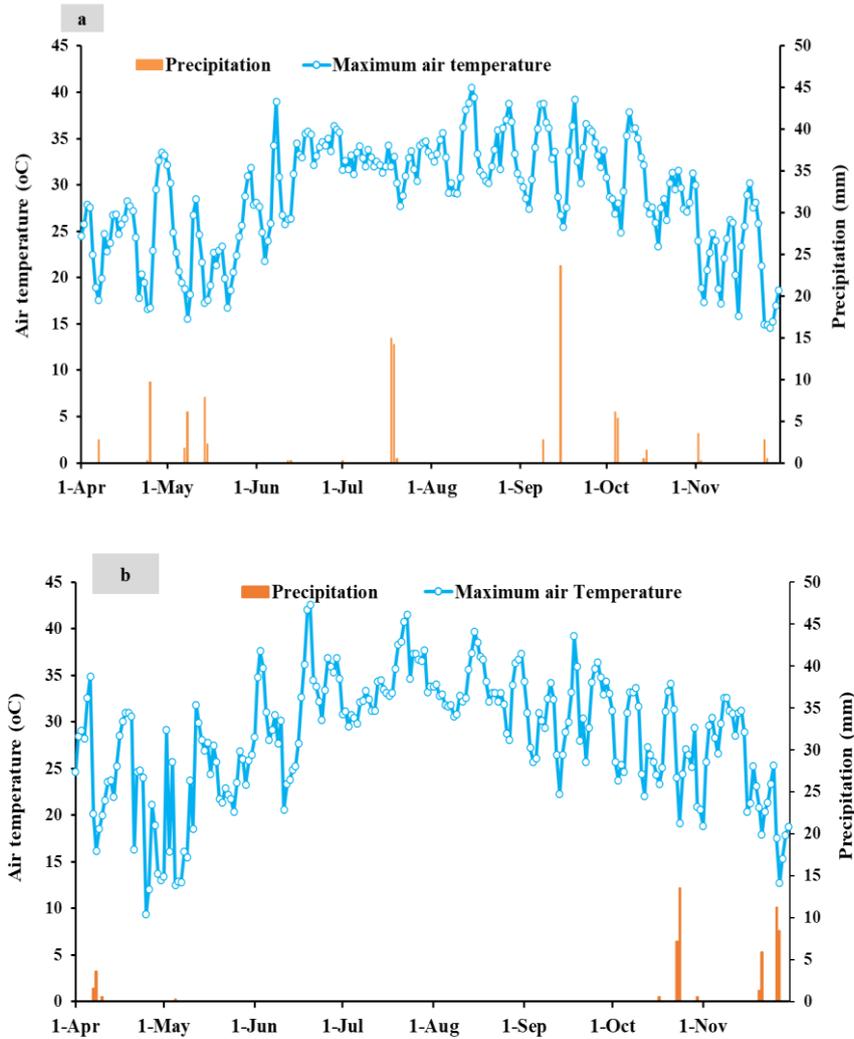


Figure 1- Maximum daily air temperature and precipitation values of experimental area in a) year 2015 and b) year 2016

2.1. Salt composition

In order to obtain the saline irrigation waters, KNO_3 , KCl , $MgCl_2$, $CaCl_2$, $NaCl$, and Na_2SO_4 salts were added to the canal water during study years (2015 and 2016). During the preparation of saline waters, sodium adsorption ratio (SAR) values of each treatment were maintained less than 5.0 in order to avoid the adverse effect of increasing SAR on soil structure and water gas movement. There were four tanks for different salt treatments, each tank had a 1400 L volume. All the salts were mixed and added to tanks located near the experimental area (Table 2). The canal water ($EC=0.55 \text{ dS m}^{-1}$) was used as control treatment (S_0). All trees were fertilized with NPK and micronutrients per agronomic recommendations.

Table 2- Salt amounts used for preparing of saline water

Treatments	Salts ($g L^{-1}$)					
	KNO_3	KCl	$MgCl_2$	$CaCl_2$	$NaCl$	Na_2SO_4
S_1 (1.20 dS m^{-1})	0.051	0.037	0.212	0.154	0.112	0.217
S_2 (2.40 dS m^{-1})	0.051	0.037	0.470	0.344	0.262	0.471
S_3 (3.0 dS m^{-1})	0.051	0.037	0.600	0.438	0.339	0.595

2.2. Measurements

2.2.1 Soil water measurement

Soil water content was measured using soil moisture sensors (Decagon 5TE Soil Moisture Corp.). One sensor was used for each replication. The soil field capacity and wilting point were 25.36 g g⁻¹ and 17.20 g g⁻¹, respectively. Irrigation interval was 3-4 days (two irrigations in a week). Soil water was measured before each irrigation and amounts of irrigation water was calculated as liter per pot. We started to apply drought and salinity treatments as of July in 2015 and June in 2016.

Evapotranspiration volume (ET) between two consecutive irrigations was calculated by using the water balance Equation (1) as follows:

$$ET = [(W_n - W_{n+1}) / P_w] + (I - R) \quad (\text{Eq. 1})$$

are the pot weights before the n^{th} and $n + 1^{\text{th}}$ irrigation (kg), P_w is water bulk density (1 kg dm³ or 1 kg l⁻¹), I and R are amounts of applied and drainage water (litres).

2.2.2. Leaf net photosynthetic rate and stomatal conductance

The Leaf Net Photosynthetic rate and leaf stomatal conductance (Li-Cor 6400 instrument) were measured before irrigations at 11⁰⁰-14⁰⁰ PM. The measurements were made four times on July 29, August 12, August 29 and September 17 in 2015 and three times in 2016, June 16, July 14 and August 11 (there were no leaves on some treatments in September 2016).

2.2.3. Leaf water potential

Leaf water potential (LWP) was made by pressure chamber (Soil Moisture Company) before irrigations at pre-dawn (05⁰⁰-06³⁰ AM) during the years of study. LWP was measured four times in 2015 and two times in 2016 because there were no leaves on some treatments in 2016.

2.2.4. Trunk diameter

Trunk diameter were measured two times each year using digital caliper. Trunk diameter was measured on east-west and north-south orientation at 10 cm above the graft point and the average of the two values was calculated and taken as trunk diameter. Covariance analysis was made for trunk diameter.

2.2.5. Experimental design and statistical analysis

The experiment was designed as a split plot. Main plots were drought treatments (D) and sub plots were saline water (S). There were five replications for each treatment and each replication had one trees. All the trees were pruned in February 2016.

3. Results and Discussion

3.1. Plant water consumption (Evapotranspiration, ET)

ET of Nonpareil variety varied from 99.7 L to 218.3 L in 2015 and 70.6 and 248.5 L in 2016 (Table 3). ET decreased as drought and salinity stress levels increased for both years. High salt content of irrigation water increases osmotic potential around root zone. Due to high osmotic potential, roots cannot use water efficiently (Suarez 2012). Excessive amounts of soluble salts in the soil are known to reduce plant water use (Yang et al. 2002). Many researchers have established that plant water consumption was affected by water salinity and water consumption decreased with increasing water salinity (Germana et al. 2000; Murkute et al. 2005). In addition to salinity stress, drought stress also affected plant water consumption. ET decreased with decreased amounts of irrigation water.

In the absence of salinity, the measured ET progressively decreased with water deficit (20% and 40%), as shown in Table 3. Drought stress thus affected ET of young almond trees even at 20% deficit. Salinity decreased tree ET starting at the lowest salinity level EC=1.2 dS m⁻¹. These data confirm the high sensitivity of almond to salt stress. The ET increased for second year of the study in S₀ and S₁ treatments (for D₀ and D₁ drought levels), not unexpected as the trees developed in second year of the experiment. Despite increasing salinity, almond trees continued growth but the S₂ and S₃ salinity treatments caused decrease in ET of almond trees compared to the first year of the experiment (Table 3).

Amounts of daily ET in all treatments were close to each other up to beginning of August in 2015 (Figure 2), suggesting that trees endured one month of reduced water before adverse effects commenced. After that date, daily ET changed depending on drought and salinity levels. Water and salinity stress affected after beginning of August for the first year. But when considered

second year, daily ET of all treatments were very different (Figure 3). ET was affected more in 2016 than in 2015 due to cumulative stress effects.

Table 3- Total plant water consumption values in 2015 and 2016

Treatments		Plant water consumption (Liters per tree)	
Drought levels	Salinity levels	2015	2016
D ₀	S ₀	218.3	248.5
	S ₁	193.1	205.8
	S ₂	174.4	155.7
	S ₃	159.8	92.4
D ₁	S ₀	172.3	212.3
	S ₁	154.3	164.6
	S ₂	142.6	119.8
	S ₃	130.2	75.4
D ₂	S ₀	132.2	156.7
	S ₁	122.3	120.1
	S ₂	111.6	93.7
	S ₃	99.7	70.6

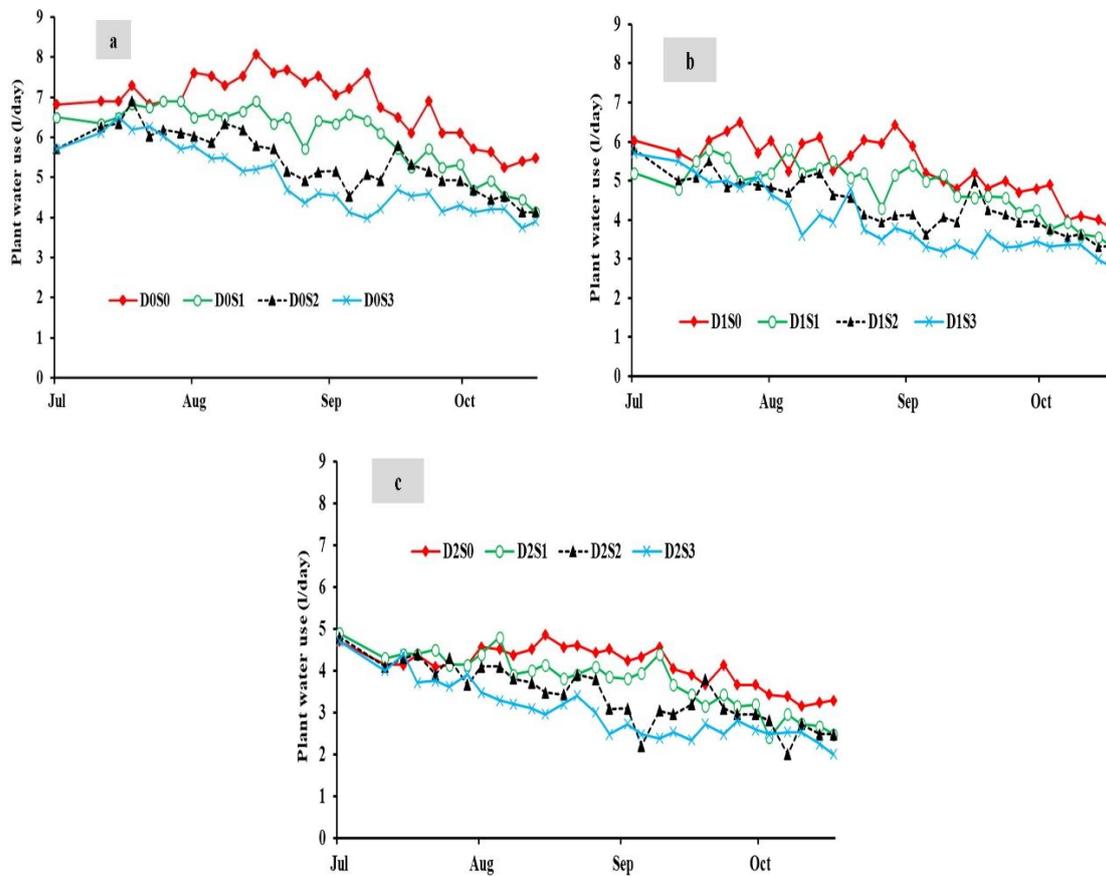


Figure 2- Daily ET of treatments after applying drought and salinity treatments in 2015 (D₀; full irrigation, D₁; moderate stress, D₂; severe stress, S₀; EC= 0.55 dS m⁻¹, S₁; 1.20 dS m⁻¹, S₂; 2.40 dS m⁻¹, S₃; 3.0 dS m⁻¹)

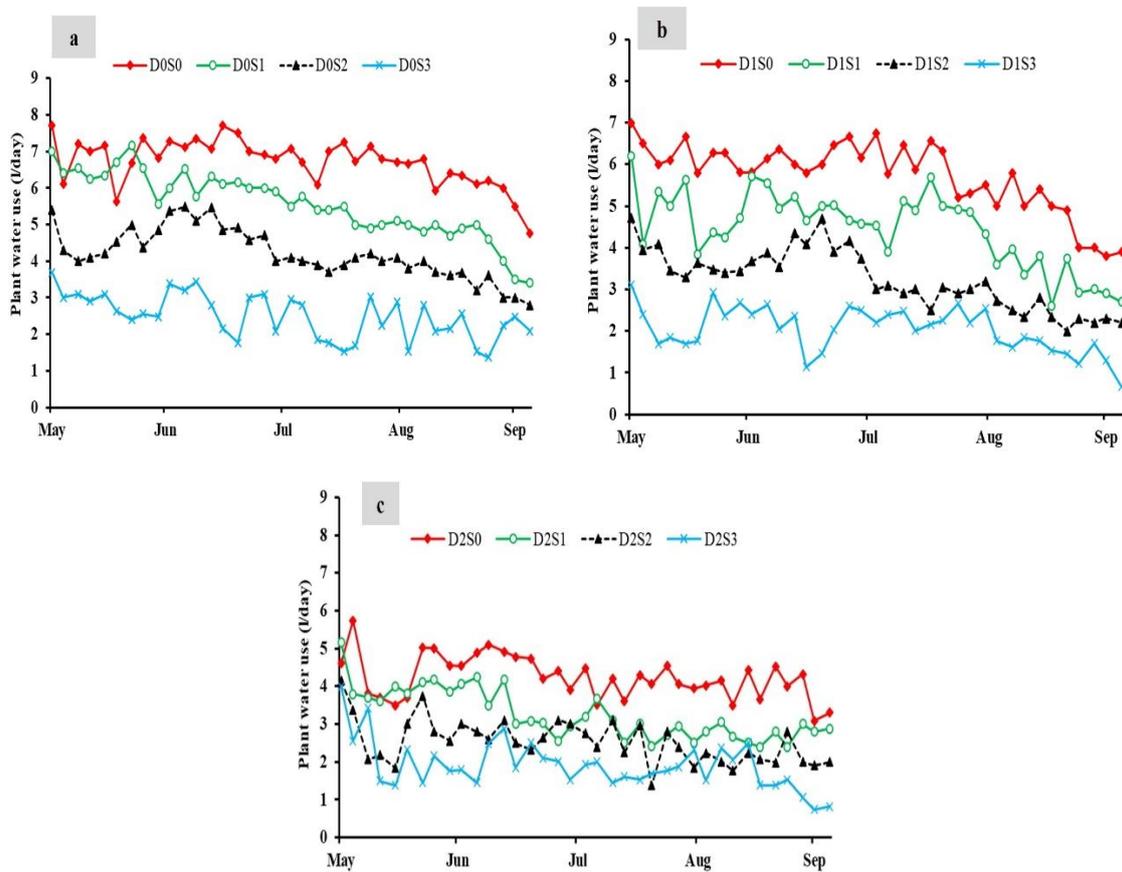


Figure 3- Daily ET of D₂ treatments after applying drought and salinity treatments in 2016

3.2. Photosynthetic rate (P_n)

Drought, salinity and drought salinity interaction had significantly adverse effects on photosynthetic rates (P_n) of Nonpareil and Aldridge almond in 2015 ($P < 0.01$). The effects of drought and salinity on P_n were separate in 2016 ($P < 0.01$) (Table 4 and Table 5).

P_n results were generally similar for both varieties in 2015 but they were different in 2016. This reason may be that cumulative effect of drought and salinity stress was more pronounced in second year. The values of P_n decreased continuously after the first measurement for each year. The response to drought and salinity stress became more severe with time.

Salt stress causes decreasing photosynthetic effectiveness (Sayed 2003). Increasing salt stress has earlier been reported a higher salinity level to decrease P_n values in almond variety and rootstocks (Zrig et al. 2015; Zrig et al. 2016). They found no significant effect until $EC = 9.95 \text{ dS m}^{-1}$ treatment. We attribute our reported sensitivity at much lower salinity to our longer-term application of salt (2 year versus 4 weeks in Zrig et al. 2016).

When 80% of ET was applied as irrigation water, drought stress also negatively affects P_n (Anjum et al. 2011). Romero et al. (2004) reported that long-term water stress led to a progressive decline in a with significant reductions after 21 days in the RDI (regulated deficit irrigation) treatment.

On the other hand, P_n was slightly higher in 2016 than that in 2015 in D₀S₀ treatments (no stress) for both varieties (Figure A1 and Figure A2). The reason may be that almond trees growth at second year of the study. Considered the last measurements of D₂S₃ treatments, P_n decreased in 2016. Drought and salinity stress had a significant impact in 2016 as compared to 2015 due to cumulative effects. For example, there were no leaves on the trees in D₂S₂ and D₂S₃ treatments of Nonpareil variety at the last measurement (August 11) in 2016, so P_n could not be measured in those treatments.

Figure 4a and 4b show the rates of P_n results according to D₀S₀ treatments (100%) in 2015 and 2016 respectively. The rates of P_n decreased as drought and water salinity level increased. The rates of P_n were close in similar treatments for both varieties in the first year. They were very different from each other in the second year. the results of P_n measurements of Aldridge were higher than P_n measurements of Nonpareil. Nonpareil did not resist to drought and salinity stress (D₂S₂ and D₂S₃ stress levels) conditions after beginning of August so no leaves on the trees were seen in that treatments. The results indicate that the Nonpareil is more sensitive to drought and salt stress than Aldridge.

Table 4- Photosynthetic rate of Nonpareil at last measurement ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

Drought levels	Salinity levels			
	S ₀	S ₁	S ₂	S ₃
2015				
D ₀	9.85 a**	7.82 b	6.81 c	6.20 def
D ₁	7.71 b	6.35 cde	5.78 f	5.28 g
D ₂	6.56 cd	5.92 ef	5.07 gh	4.60 h
2016				
Drought levels	Salinity levels			
D ₀	10.93 a**	S ₀	11.08 a**	
D ₁	7.85 b	S ₁	8.69 b	
D ₂	3.53 c	S ₂	5.96 c	
		S ₃	4.02 d	

** : P<0.01 Values with common letters do not differ significantly

Table 5- Photosynthetic rate (Pn) of Aldridge at last measurement ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

Drought levels	Salinity levels			
	S ₀	S ₁	S ₂	S ₃
2015				
D ₀	9.90 a**	7.82 b	6.78 c	6.18 de
D ₁	7.83 b	6.23 cde	5.61 fg	5.30 gh
D ₂	6.65 c	5.90 fg	4.95 hi	4.58 i
2016				
Drought levels	Pn	Salinity levels		Pn
D ₀	9.33 a**	S ₀	9.48 a**	
D ₁	8.00 a	S ₁	8.59 ab	
D ₂	5.64 b	S ₂	6.88 bc	
		S ₃	5.68 c	

** : P<0.01 Values with common letters do not differ significantly

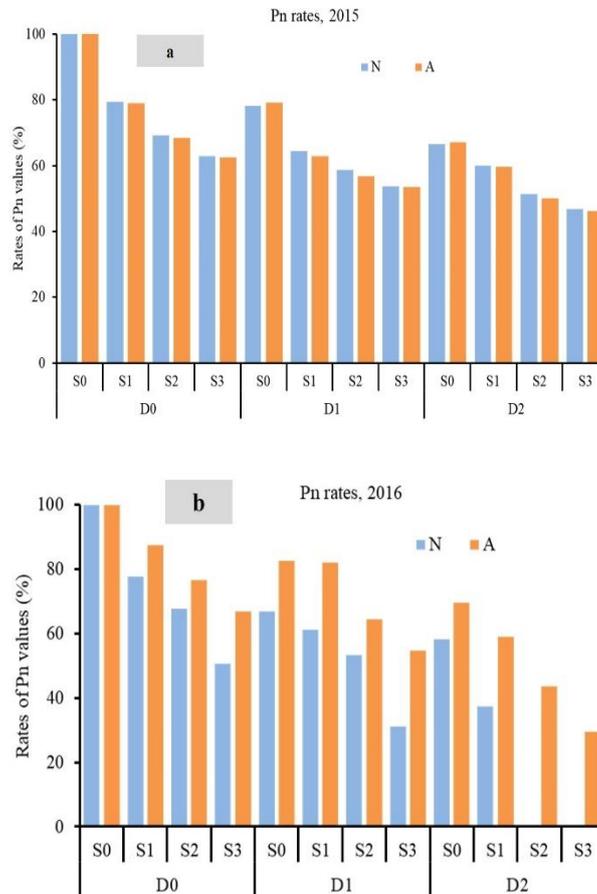


Figure 4- The Pn rates relative to control treatment for Nonpareil (N) and Aldridge (A) at the last measurement

3.3. Stomatal conductance (gsw)

The stomatal conductance (gsw) results varied between 0.135 and 0.350 mol H₂O m⁻² s⁻¹ for Nonpareil and between 0.120 and 0.330 mol H₂O m⁻² s⁻¹ for Aldridge in 2015 (Figure A3). There was no interaction effects of salinity and drought on gsw, according to variance analysis of Nonpareil for both years (P<0.01). There are 3 different groups for drought treatments (Table 6). The lowest value was obtained from D₂ treatments. Salinity treatments were divided into three different groups. S₂ and S₃ treatments had the lowest gsw results. Drought and salinity treatments had separate effects for the first year of Aldridge (P<0.01). There were 3 different statistical groups for both drought and salinity stress treatments. In the second year drought and salinity stress and interaction was statistically significant (P<0.01).

The results of different treatments for both varieties were similar in the first year of the study. The gsw was higher in second year than first year in no-stress treatment. But gsw were less in second year than first year for other treatments. The decrease in gsw was significantly different from the control even at the lowest salinity level and at the 20% reduction in water for Nonpareil (Table 6) as well as Aldridge (Table 7) in 2015 and 2016. The effects of stress applications on gsw were more evident in second year. The gsw values decreased continuously after the first measurement for each year. As with the photosynthesis data discussed earlier, the impact of drought and salinity stress became more severe in year two but was significant in both years and varieties at the lowest salt stress applied.

As the treatment drought and salinity stress level increased, gsw decreased. Nonpareil and Aldridge varieties had similar results for gsw. As salinity level increases, decreasing of photosynthesis is in relationship with closure of stomata (Sibole et al. 1998). Zrig et al. (2015) stated that gsw was affected negatively by increasing salt stress in almonds. When plants are exposed to salinity stress, they close their stomata firstly, in prevent water loss. Stomatal closure gives rise to decreased gsw (Ashraf 2004; Munns & Tester 2008).

The gsw was not measured at the last measurement in 2016 due to lack of leaves on Nonpareil almond trees in S₂ and S₃ salinity levels of D₂ treatments but it was measured in Aldridge (Figure A4).

Table 6- Stomatal conductance (gsw) of Nonpareil at the last measurement (mol H₂O m⁻² s⁻¹)

Drought treatments	gsw	Salinity treatments	gsw
2015			
D ₀	0.243 a**	S ₀	0.249 a**
D ₁	0.203 b	S ₁	0.213 b
D ₂	0.163 c	S ₂	0.183 bc
		S ₃	0.168 c
2016			
D ₀	0.283 a**	S ₀	0.279 a**
D ₁	0.182 b	S ₁	0.202 b
D ₂	0.076 c	S ₂	0.144 c
		S ₃	0.096 c

** : P<0.01 Values with common letters do not differ significantly

Table 7- Stomatal conductance (gsw) of Aldridge at the last measurement (mol H₂O m⁻² s⁻¹)

Level	gsw	Level	gsw
2015			
D ₀	0.237 a**	S ₀	0.248 a**
D ₁	0.204 b	S ₁	0.210 b
D ₂	0.163 c	S ₂	0.180 bc
		S ₃	0.170 c
2016			
	S ₀	S ₁	S ₂
D ₀	0.302 a**	0.226 b	0.157 cd
D ₁	0.217 b	0.269 c	0.140 cde
D ₂	0.159 cd	0.128 def	0.116 ef
			S ₃
			0.132 cdef
			0.134 cdef
			0.097 f

** : P<0.01 Values with common letters do not differ significantly

Figure 5 and Figure 6 show the gsw results final measurements in 2015 and 2016, respectively, relative to the control (D₀S₀ treatments =100%). The gsw rates were also similar to each other at the end of the first year (Figure 5). The gsw rates which had the highest drought and salinity stress were very close. The rates were 47.7% and 48.4% for Nonpareil and Aldridge almond varieties, respectively. The rates obtained in 2016 were significantly different for the two varieties (Figure 6). Aldridge was affected less than Nonpareil by drought and salinity stress according to gsw results, consistent with Pn results discussed above.

If water applications through either irrigation or rainfall are not adequate to meet water requirements, stomatal closure will be initiated, reducing gas exchange and rate of photosynthesis (Doll & Shackel 2015). Gsw decreased in non-watered almonds as compared to watered almonds (Gomes-Laranjo et al. 2006). Due to drought and salinity stress in our experiment, gsw and Pn results were negatively affected for both almond varieties.

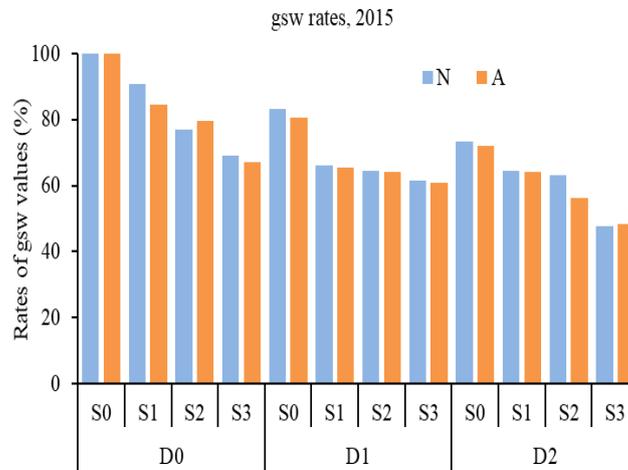


Figure 5- The gsw rates relative to control treatment rates for Nonpareil (N) and Aldridge (A) varieties at the last measurement in 2015

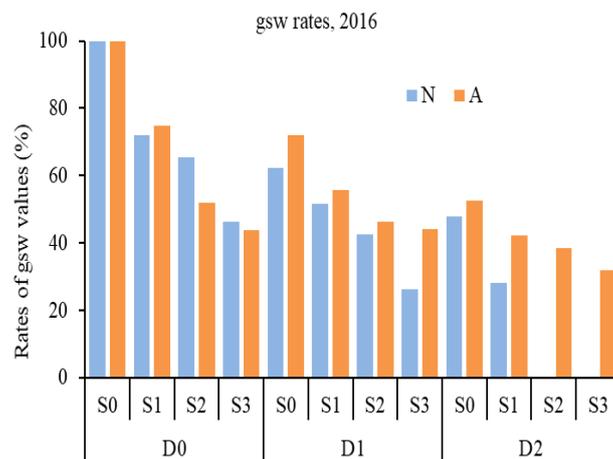


Figure 6- The gsw rates relative to control treatment rates for Nonpareil (N) and Aldridge (A) varieties at the last measurement in 2016

3.4. Leaf water potential (LWP)

There were three different statistical groups for LWP in salinity treatments of Nonpareil and Aldridge varieties in 2015. The 1.2 dS m^{-1} and 2.4 dS m^{-1} salinity levels did not have significantly different LWP but the LWP was greater with increased salinity in the order $S_3 > S_2 > S_1 > S_0$. The 2.4 dS m^{-1} and 3.0 dS m^{-1} salinity levels had almost similar LWP results in Aldridge in 2016. Cumulative salt effects may explain the shift of the 2.4 dS m^{-1} treatment in the second year of experiment.

Drought and salinity stress had significant effects on almond trees ($P < 0.01$). There were no significant interactions between drought and salinity stress interaction on Nonpareil and Aldridge varieties. According to results of data for drought stress, there were three different groups for drought treatments in 2015 (Table 8 and Table 9). Each water deficit level had a significantly different increasing adverse effect on LWP of Nonpareil and Aldridge trees. In 2016, 20% (D_1) and 40% (D_2) water deficit levels were in the same group for Nonpareil, no-stress (D_0) and 20% (D_1) water deficit levels were in the same group for Aldridge. In 2016 a 20% water deficit did not affect LWP on Aldridge trees, likely due to trees adjusting growth to decreased water for two years.

Table 8- LWP of Nonpareil at the last measurement

Level	Least Sq Mean	Level	Least Sq Mean
2015			
D ₀	1.13 c**	S ₀	1.23 c**
D ₁	1.36 b	S ₁	1.40 b
D ₂	1.73 a	S ₂	1.42 b
		S ₃	1.57 a
2016			
D ₀	0.96 b**	S ₀	1.05 c**
D ₁	1.21 a	S ₁	1.12 bc
D ₂	1.25 a	S ₂	1.16 ab
		S ₃	1.23 a

** : P<0.01 Values with common letters do not differ significantly

Table 9- LWP of Aldridge at the last measurement

Level	Least Sq Mean	Level	Least Sq Mean
2015			
D ₀	1.16 c**	S ₀	1.23 c**
D ₁	1.31 b	S ₁	1.38 b
D ₂	1.68 a	S ₂	1.40 b
		S ₃	1.52 a
2016			
D ₀	1.23 b**	S ₀	1.21 b**
D ₁	1.27 b	S ₁	1.29 ab
D ₂	1.41 a	S ₂	1.35 a
		S ₃	1.38 a

** : P<0.01 Values with common letters do not differ significantly

LWP values varied from -0.75 to -1.85 MPa and from -0.75 to -1.75 MPa for Nonpareil and Aldridge varieties in 2015, respectively (Figure 7). LWP measurements in 2016 varied from -0.9 to -1.3 MPa and from -0.7 to -1.4 MPa for Nonpareil and Aldridge varieties, respectively (Figure 8). Because there were no leaves on some trees after early August 2016, only two measurements were taken that year.

LWP values decreased towards the end of the season for all treatments. LWP values were affected negatively by increasing drought and irrigation water salinity. Decrease in LWP for almond with water stress (Karimi et al. 2015,) and salt stress (Shibli et al. 2003) has been reported earlier, however their decreases in LWP were relatively small in contract to our large negative values. LWP decreased in none-watered almond trees (Gomes-Laranjo et al. 2006). Our study included combined drought and salinity stress at levels that eventually killed some trees, thus the treatments affected almond trees more negatively than earlier studies.

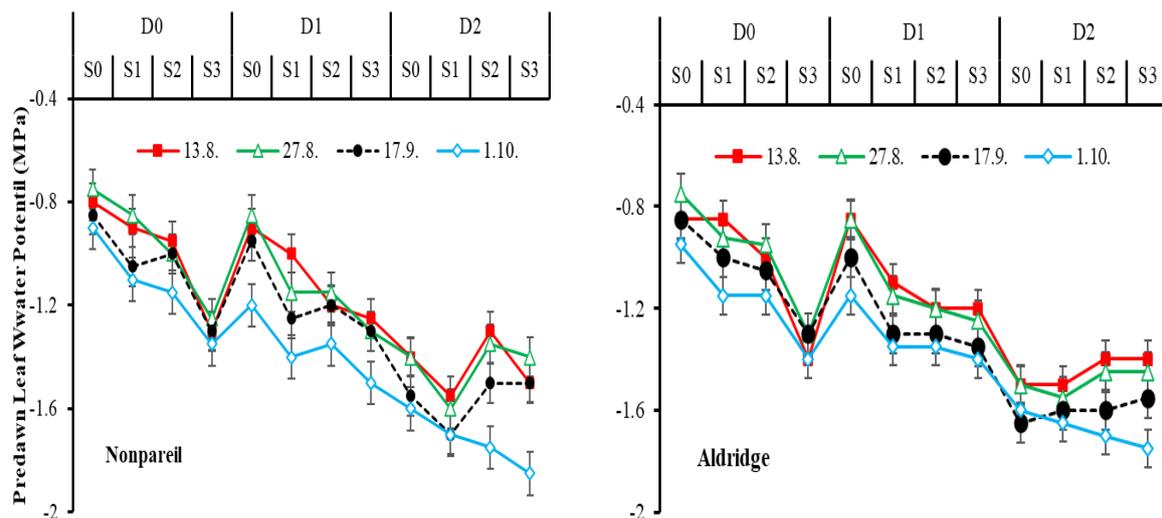


Figure 7- LWP of almond trees during growing period in 2015. (Error bars indicate standard errors of the means)

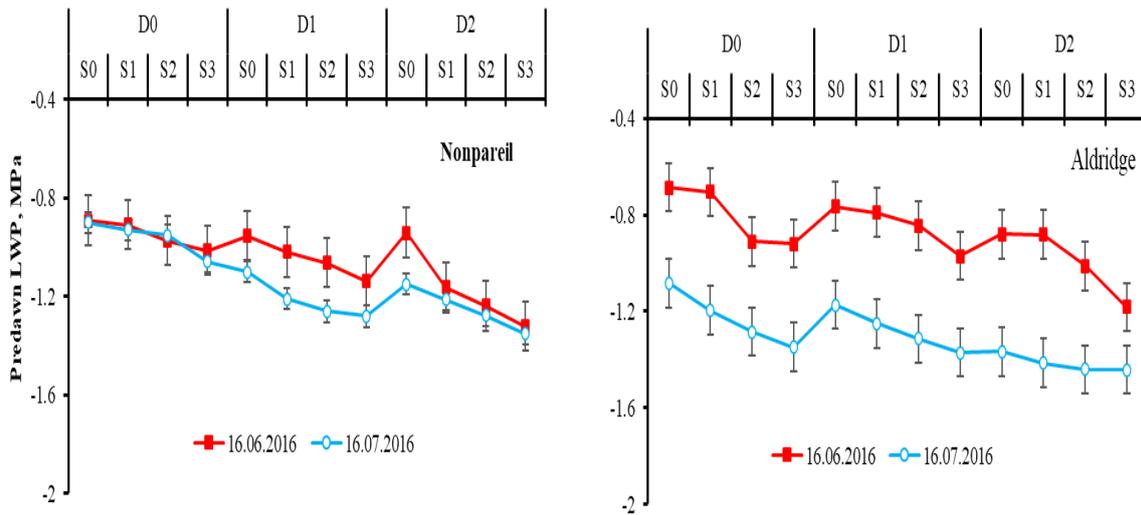


Figure 8- LWP of almond trees during growing period in 2016. Error bars indicate standard errors of the means

3.5. Trunk diameter

Drought and salinity stress interaction had no significant effects on Nonpareil and Aldridge varieties in first year of the study (Table 10 and Table 11). Only drought stress affected Nonpareil variety in 2015 ($P < 0.01$). The D_0 levels (no water deficit) had the highest trunk diameter with 20-35 mm. Drought and salinity interaction had a significant effect on trunk diameter in 2016 ($P < 0.01$). Combining the highest water deficit (D_2 , 40%) and salinity levels (S_2 , 2.4 dS m^{-1} and S_3 , 3.0 dS m^{-1}) resulted in the lowest trunk diameters in 2016 (Table 10).

Drought and salinity stress had adverse effects on trunk diameter of Aldridge separately in the first year of the study ($P < 0.01$ and $P < 0.05$, respectively). There were two different statistical groups (Table 11). The D_1 (20% water deficit) and D_2 (40% water deficit) were in the same statistical group. The S_0 (0.50 dS m^{-1}) and S_1 (1.2 dS m^{-1}) treatments had the same effects on trunk diameter for the Aldridge.

Drought and salinity interaction was significant ($P < 0.01$) in 2016. Combining the highest water deficit (D_2 , 40%) and salinity levels (S_2 , 2.4 dS m^{-1} and S_3 , 3.0 dS m^{-1}) had the lowest trunk diameters in 2016 (Table 11). Trunk of almond trees continued to grow even under drought and salinity stress conditions for the first year of the study (Figure 9). Drought and salinity stress decreased growth of trunk diameter for both varieties. The lowest increasing rates (%) were obtained in D_2 and S_3 treatments for all almond trees. Water deficit and salinity affected almond trees after planting date (Figure 10). Considering increasing rates of trunk diameter, trunk shrinkage can be seen considered in drought and salinity stress treatments at the second year. The highest trunk shrinkage was determined in S_3 treatments for all drought treatments.

Prediction of the impact of multiple stresses on biomass yield or crop yield has been made with various response models (Jin et al. 2020; Shahhosseini et al. 2021). The most commonly utilized are the dominant stress models, where the response is considered to be impacted only by the most dominant stress, the additive response model where the stresses are taken to be the sum of the individual stresses and the multiplicative model where the combined stress is taken as the product of the response of the individual stresses.

Earlier Dudley & Shani (2003) found that corn yield in the presence of salinity and water stress could be satisfactorily predicted using the UNSATCHEM (Suarez & Simunek, 1996) model, which considers the biomass response to osmotic and matric stress to be multiplicative. Similarly, Örs & Suarez (2017) found the multiplicative model to have better predictive capability than the additive or dominant stress models for spinach response to water and salt stress. While there is no theoretical basis for the model, it reflects the observation that response to one stress is generally less severe in the presence of another stress, for example the influence of salinity on boron toxicity of broccoli (Smith et al. 2010), elevated pH effect on response to EC (Huang et al. 2017).

Evaluation of the ability to predict the effect of combined stress based on the individual stress response functions was made by using the values of the changes in trunk diameter over the total time of the experiment (from the initial to final measurements) for each individual tree. This approach provided a more sensitive response to the stresses as it considered a longer time frame (than analysis of individual years) and was not impacted by initial variations in trunk diameter among plants.

Shown in Figure 11a is the response of trunk diameter to salinity, expressed as increase in diameter relative to the non-saline control, in the absence of water stress. These data indicate that trunk diameter changes were linear with salinity and that

substantial decrease in growth occurred even at the lowest salinity treatment. In a similar manner the response to water stress in the absence of salinity stress showed a decrease in growth with reduced water application, which was represented by the linear relationship (Figure 11b). A 25% decrease in water application resulted in a 37% reduction in growth (Figure 11b). These data indicate that the combinations of rootstock and scion were very sensitive to both salinity and water stress.

Table 10- Trunk diameter (mm) of Nonpareil

Drought treatments	Trunk diameter	Salinity treatments	
		2015	
D ₀	20.35 a**	S ₀	20.21 ns
D ₁	19.66 ab	S ₁	19.70
D ₂	19.05 b	S ₂	19.64
		S ₃	19.19
2016			
	S ₀	S ₁	S ₂
D ₀	23.62 a**	19.97 d	20.10 d
D ₁	21.92 c	20.00 d	23.34 ab
D ₂	20.12 d	18.43 e	17.7 e
			S ₃
			22.63 bc
			18.52 e
			15.30 f

** : P<0.01 Values with common letters do not differ significantly; Ns: no-significant

Table 11- Trunk diameter (mm) of Aldridge

Drought treatments	Trunk diameter	Salinity treatments	
		2015	
D ₀	22.54 a**	S ₀	22.17 a*
D ₁	21.40 b	S ₁	21.95 a
D ₂	21.04 b	S ₂	21.5 ab
		S ₃	21.02 b
2016			
	S ₀	S ₁	S ₂
D ₀	26.90 a**	24.87 b	21.82 c
D ₁	19.97 def	20.07 cde	21.67 cd
D ₂	19.93 def	21.49 cd	18.00 gh
			S ₃
			19.37 efg
			17.27 h
			18.22 fgh

** : P<0.01; * : P<0.05 Values with common letters do not differ significantly

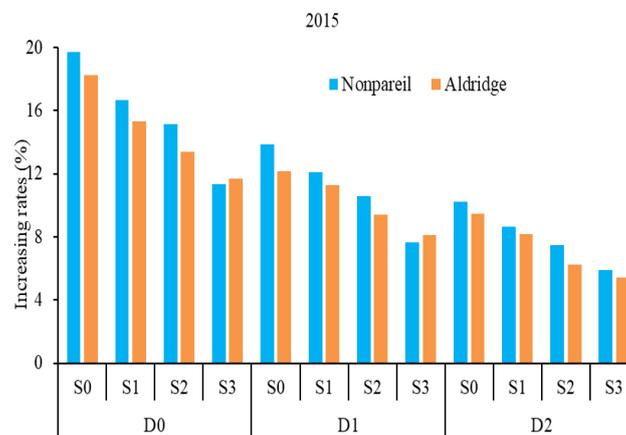


Figure 9- Increasing rates of trunk diameter measurements in 2015

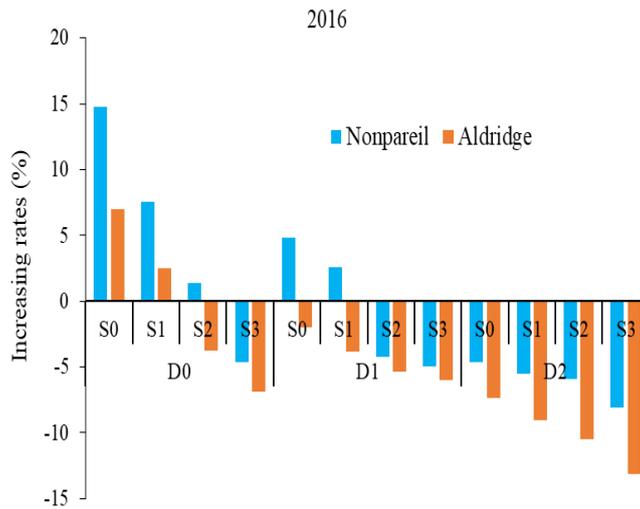


Figure 10- Increasing rates of trunk diameter measurements in 2016

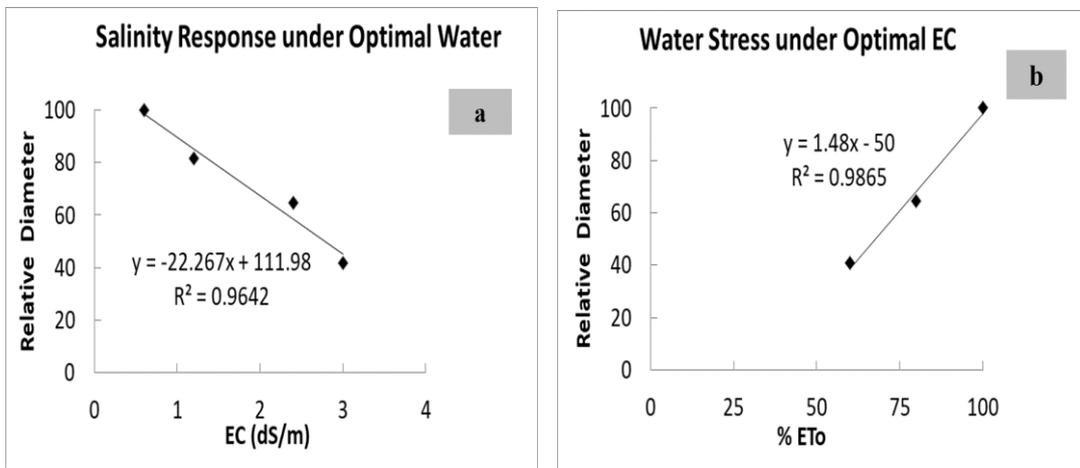


Figure 11- Relative trunk diameter as related to a) salt and b) water stress

Using the results of Figure 11 (a and b) we compared the predictions of the three stress response models to measurements in the change in trunk diameter for the treatments with combined stress. In each instance we used only data from the combined stress treatments, so no experimental data used to develop the predictive equations for water and salt stress (shown in Figure 11a, b) were used in the evaluation of the combined stress models. For the additive stress model, we added the predicted decreases from the separate stresses to predict the response to combined stress. For the major stress model, we evaluate the decrease in growth expected from the water and salt stress and selected the response from the stress with the greater response. For the multiplicative model we calculated the response expected from water and salt stress and multiplied them.

As shown in Figure 12a, the additive model predicted lower growth than observed, consistently overpredicting loss of growth, indicating that the presence of one stress reduces the impact of another stress. Predictions from the major stress model overestimated growth (Figure 12b) as might be expected since presence of a major stress did not eliminate the growth reduction due to other stresses. The multiplicative model predictions shown in Figure 12c also overestimated growth (underestimated the impact of combined stress) but appeared to be the most satisfactory model. As shown in Table 12 we evaluated the model fit to experimental data using a number of statistical tests. In all instances the multiplicative model provided the best fit. Perhaps the statistic of most interest to those interested in predicting growth is the value for mean absolute deviation. The multiplicative model had a low value of 5.4 versus 10.1 for the additive model and 21.4 for the major stress model. The overall predictive equation is

$$RG = 32.96 EC (V_a/V_c) + 165.7(V_a/V_c) - 1113.4EC - 5599 \quad (\text{Eq. 2})$$

Where: RG is relative growth; V_a is volume of water applied (mm); V_c ; is volume needed to meet crop demand under no stress condition (mm); EC; is irrigation water electrical conductivity (dS m^{-1}).

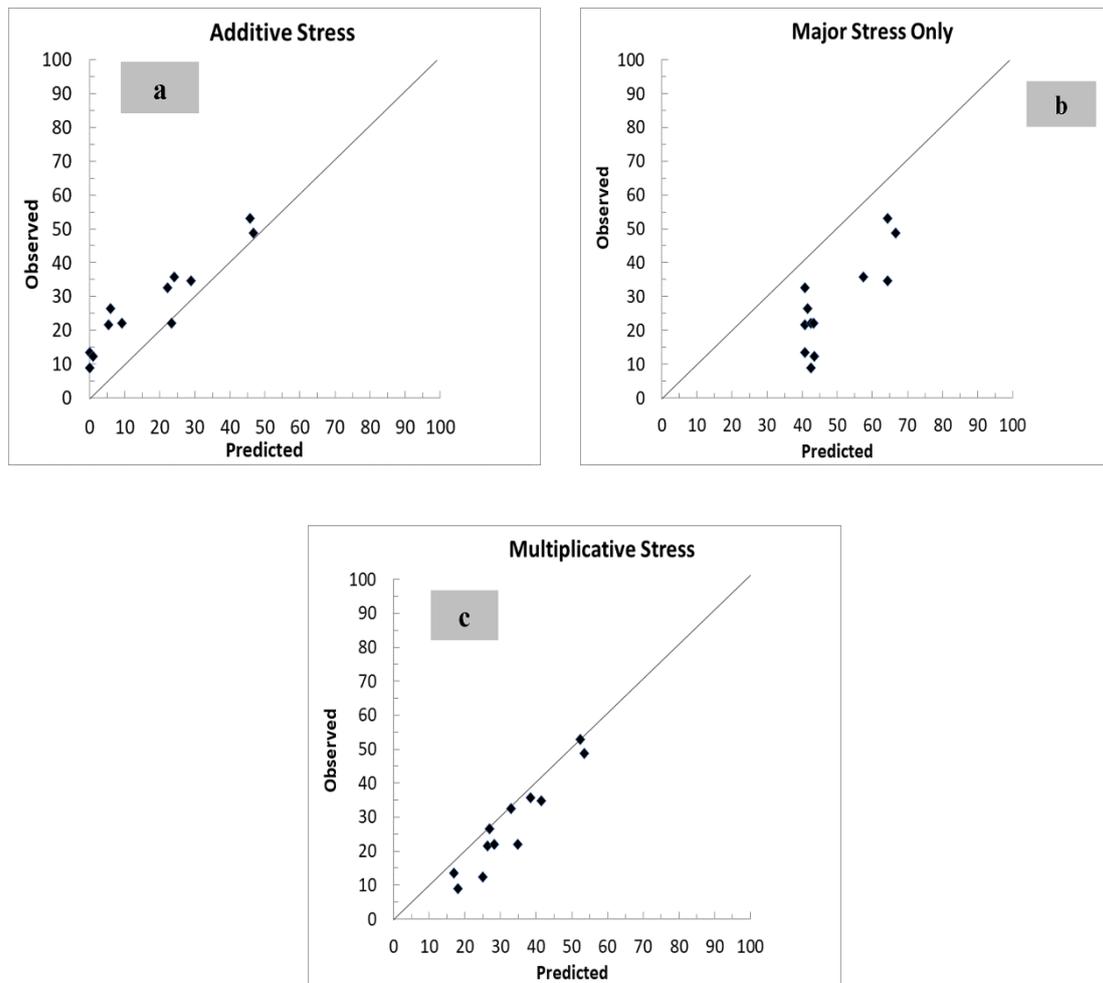


Figure 12- Measured relative trunk growth versus predicted values using a) additive stress model, b) major stress only model, and c) multiplicative stress model.

Table 12- Statistical evaluation of model predictions

Statistical parameter	Additive stress	Major stress	Multiplicative stress
Mean absolute deviation	10.1	21.4	5.4
Mean square error deviation	131	515	46.5
RMSD	11.4	22.7	6.82
Mean error	-9.9	21.4	5.3
Correlation	0.94	0.83	0.95

4. Conclusions

Drought and salinity stress levels affected plant water consumption, growth, and physiological parameters of Nonpareil and Aldridge almond varieties. The results suggest that almond may not be as drought tolerant as currently considered. Trunk growth significantly decreased with a 20% decrease in applied water. The data indicated that even for very young trees the adverse effects increased in year two as compared to the first year of stress, suggesting that one year studies are not sufficient to characterize tree response to water or salt stress. The growth data was consistent with results from the physiological measurements. The data for both varieties were similar in first year but it was established that scion varieties not just rootstock are relevant to improved stress tolerance. Nonpareil was more sensitive than Aldridge in the second year of stress. For example Nonpareil almond trees had mortality in August of year two at and above EC 2.4 dS m⁻¹ in 40% water deficit treatment. Both varieties grafted to Nemaguard rootstock were very sensitive to salinity with growth loss starting at EC 1.2 dS m⁻¹.

All parameters showed significant decline starting at 80% water application and EC 1.2 dS m⁻¹. In terms of growth rather than survival, almond was sensitive to water as well as salt stress. Trunk growth under combined water and salt stress treatments was well predicted only when using a multiplicative stress response function. Equation (2) for reduction in trunk growth were developed for treatments with either salinity only or water only stress. The results indicate that the Nonpareil is more sensitive to drought and salt stress than Aldridge. Aldridge almond variety can be recommended for areas where water supplies are scarce and salinized.

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Appendix

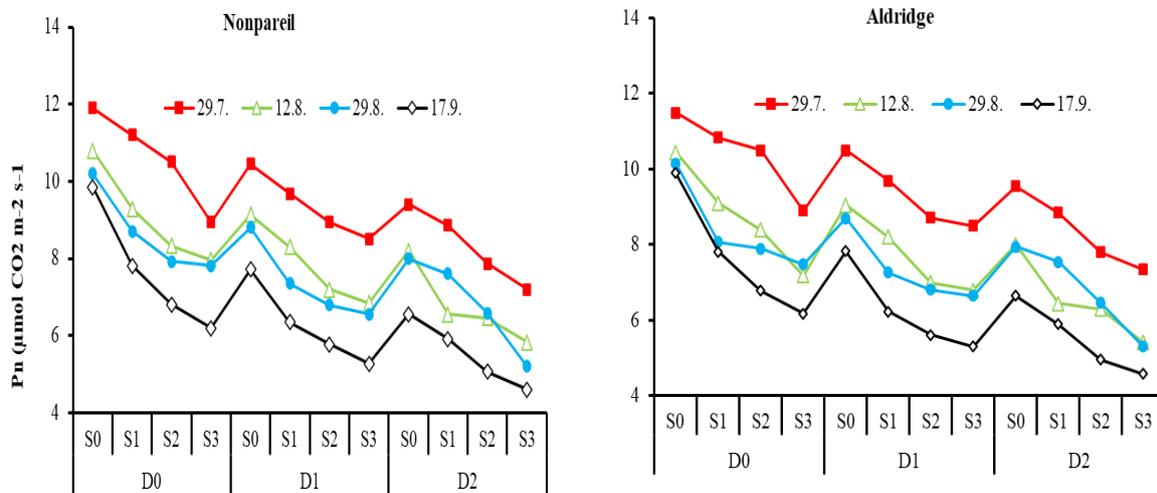


Figure A1. Seasonal fluctuation of Pn for Nonpareil and Aldridge in 2015

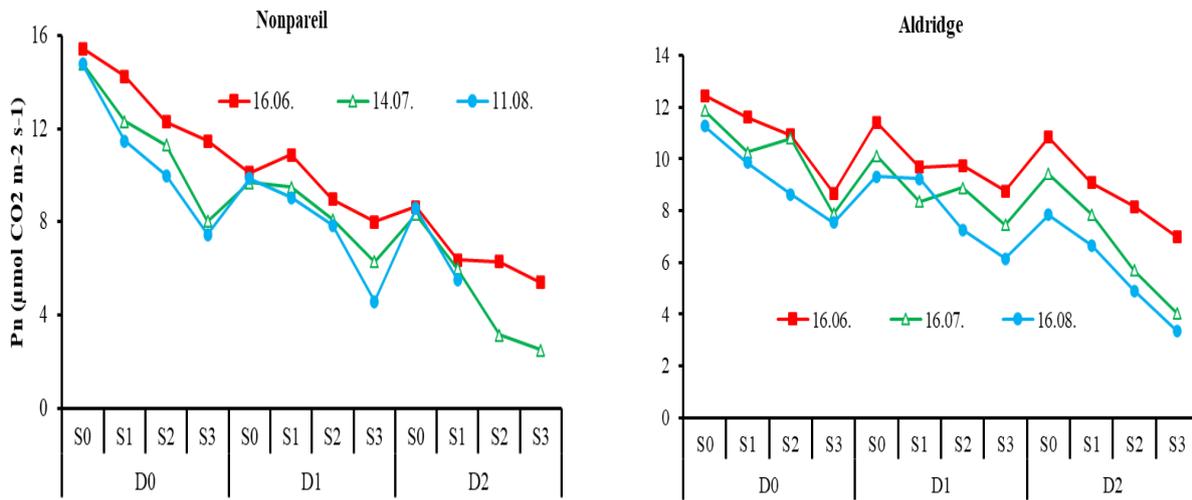


Figure A2. Seasonal fluctuation of Pn for Nonpareil and Aldridge in 2016

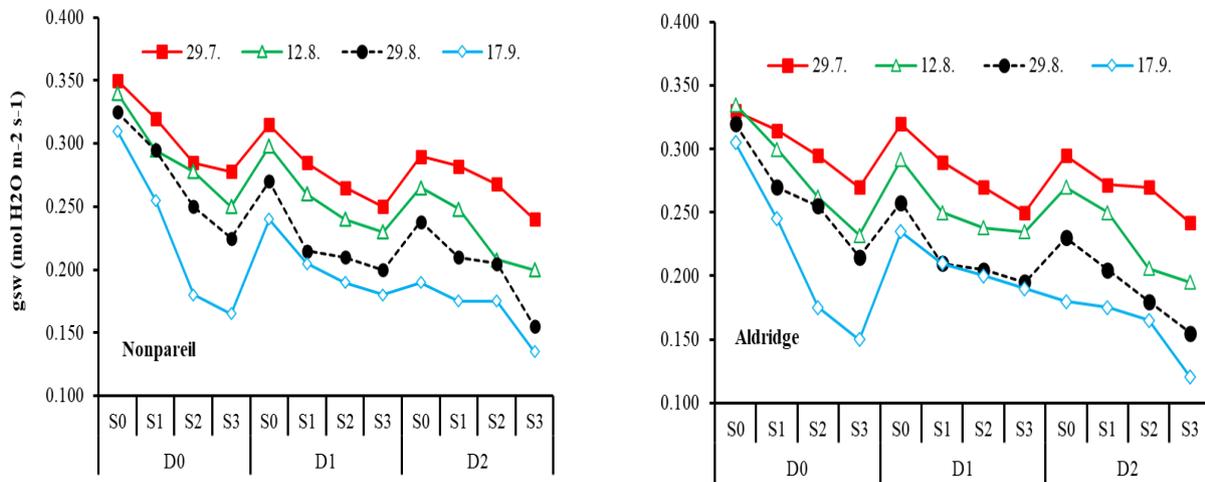


Figure A3- Seasonal fluctuation of gsw for Nonpareil (a) and Aldridge (b) in 2015

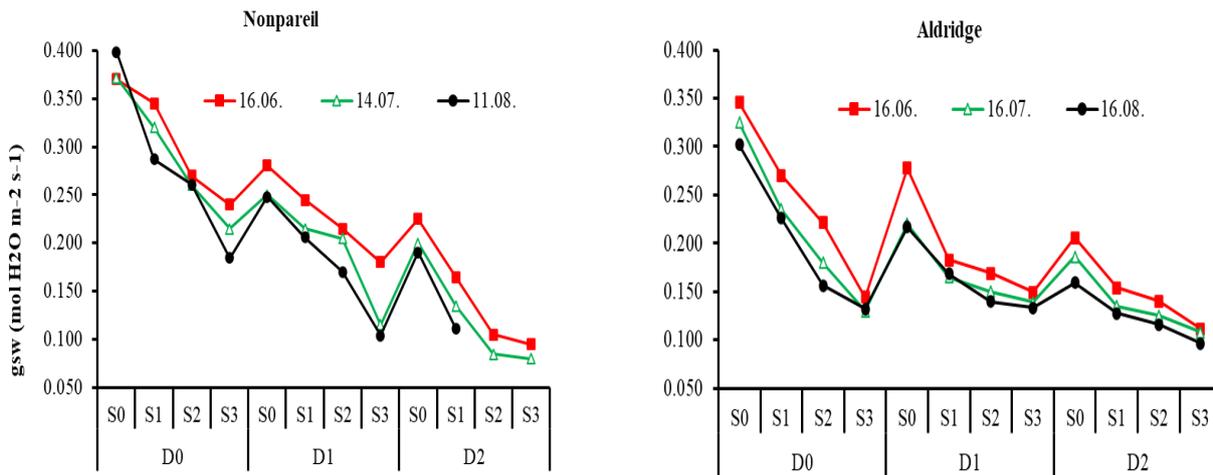


Figure A4- Seasonally fluctuation of gsw for Nonpareil (a) and Aldridge (b) in 2016

