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Excised Leaf Desiccation (ELD) as a Tool to Distinguish Desiccation Tolerance Among Almond Genotypes

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Excised Leaf Desiccation (ELD) as a Tool to Distinguish Desiccation Tolerance
Among Almond Genotypes

by

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THESIS

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ABSTRACT

Water scarcity is a major concern for almond growers worldwide. To achieve sustainable almond production, it is crucial to identify strategies for improved water use efficiency including new cultivars compatible with the improved irrigation strategies. Pulsed-irrigation strategies have been shown to provide improved water use efficiency but make orchards highly vulnerable to catastrophic irrigation failures because, while the tree-zone is continuously provided with sufficient water to meet current transpiration needs, insufficient surplus water is available in the root zone to supply tree needs if irrigation is interrupted for as little as a few hours under high heat stress (McClymont and Goodwin, 2016). Excised shoot desiccation is a method for quickly evaluating cultivar differences in rapid leaf water loss that involves exposing excised shoots to desiccation under dry environments to evaluate genetic differences in subsequent leaf tissue damage. This approach appears useful as a rapid screen for tolerance to catastrophic irrigation failures based on early field performance (Appendix A). However, the underlying physiological mechanism as well as the consequence on tree productivity are unknown. To better understand the physiological basis, leaf characteristics of twenty almond genotypes were measured to examine their relationship with plant responses to rapid and severe water stress. We hypothesize that differences in the rate of excised shoot desiccation will be determined by genetic differences in the rate of leaf water loss, primarily by water loss through stomata and secondarily by water loss through remaining epidermal tissue, and that these basic mechanisms will also determine differences in whole tree rate of water loss under stressful field conditions.

Excised shoots of the twenty genotypes were observed over 24 hours and excised shoot desiccation (ESD) scores were determined according to standardized wilting patterns ranging from 0 to 5 (0 for no desiccation, 5 for high desiccation). In subsequent experiments, excised leaves were used as a simplified model-system to evaluate differences in water retention under stress as previously described by McCaig and Romagosa (1991). Excised leaf desiccation (ELD) scores were determined using the same standardized wilting patterns used for ESD scoring. Relative water content (RWC) of sample leaves was recorded over time and the pattern of water loss with time was analyzed to identify underlying physiological mechanisms. This ELD experiment was then repeated with five genotypes selected for high productivity and kernel quality to provide more detailed data for genetic differences in excised leaf water loss patterns under more controlled laboratory environments. A moderate negative correlation was observed between ESD value and stomata density. The pattern of RWC decline differed among genotypes and ELD scores, demonstrating significant genetic differences. ELD score was significantly associated with water loss during the first 180 minutes when stomata closure response has been reported to be the major determinant of rate of water loss (Wang and Clarke, 1993; Darwish and Fahmy, 1997). It was found that the genotypes having lower ELD scores had reduced water loss in the first 180 minutes in the controlled environment. This suggests that genotypes showing low ELD scores were more effective in closing stomata following excision and so more effective in conserving leaf water. ELD scores were also significantly correlated with epidermal conductance (EC) for the five standard genotypes evaluated indicating that post stomata-closure differences in epidermal properties also play an important role in determining rate of water loss. The genotypes that had lower EC were also the genotypes that showed reduced water loss patterns during the later stages of leaf desiccation. Genotypes showing high yields and good quality kernels under

good commercial growing conditions expressed both high as well as low ESD scores, indicating that using ESD as a rapid screen for tolerance to catastrophic irrigation failures does not preclude selections for high yields and good kernel quality. While desiccation rates of excised shoots and excised leaves represent a relatively rapid test to identify inherent vulnerabilities in new as well as traditional breeding selections and cultivars, results need to be more fully assessed in both whole-plant as well as whole-orchard trials.

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INTRODUCTION

Almond (*Prunus dulcis* (Mill.) D.A. Webb) is one of the most economically important horticultural crops in California. Originating from central Asia and the Levant, almond is grown for its seed rather than fruit as with other *Prunus* species; therefore, horticulturally it is considered as a nut instead of a stone fruit (Socias i Company et al., 2017). The major producers of almond are the United States, Spain, Iran, Turkey and Australia, respectively (FAOstat, 2019) (Fig. 1). In the United States, almonds are mostly grown in the Central Valley of California, where 7,600 farmers produce 80% of world almond supply (ABC, 2019).

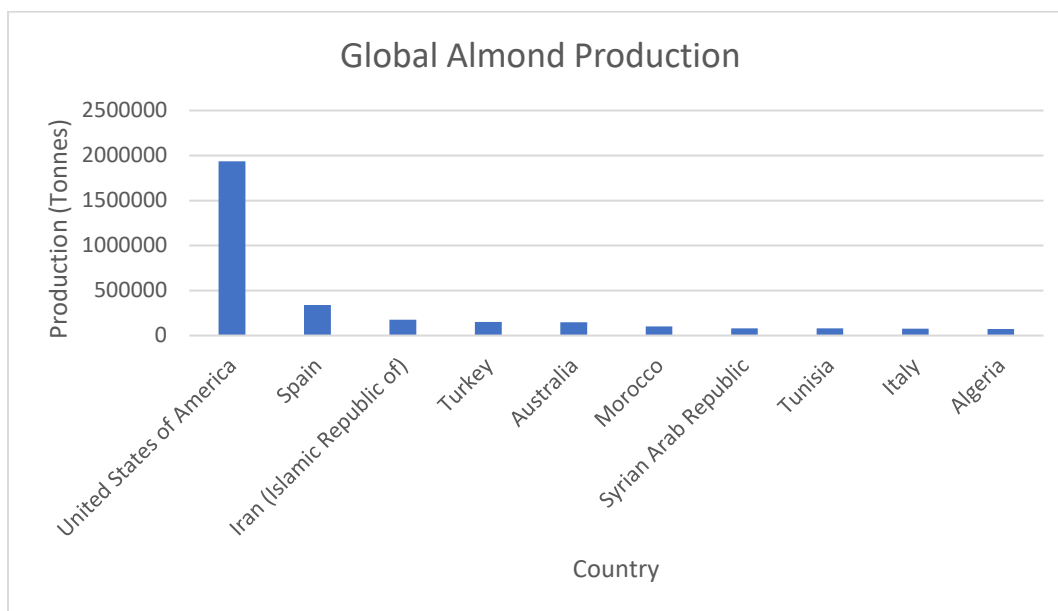


Figure 1: 2019 Global almond production.

Most agricultural production in the Central Valley is irrigated. In recent years, as the price of irrigation water has increased with increasing water shortages, a large proportion of row and field crops have been converted to almond, walnut and pistachio orchards because of their higher market price. The increasing popularity of tree nut production has resulted in a higher

water demand. Considering that approximately 1.1 gallon of water is required per almond kernel (ABC, 2018), and that California drought periods are expected to intensify with climate change (Mann and Gleick, 2015), increased water use efficiency (WUE) has become a major concern for almond growers.

In Australia, which has a long history of water scarcity, pulse-irrigation has become a common practice for improving water use efficiency. Pulse irrigation is a technique for irrigating the soil in cycles, which provides just enough water for the tree root zone while maintaining soil aeration and thus improving plant growth and water use efficiency (Rank and Vishnu, 2021). This technique was initiated by Karmeli and Peri (1974). They suggested that with pulse irrigation a very low application rate can be achieved with an irrigation system. These systems apply water on a regular and consistent basis for uniform water distribution to the root zone (Al Al-Baaj et al., 2019). In pulse irrigation, there are a series of irrigation time cycles with two phases in each: an active (irrigation) phase and inactive (resting) phase (Rank and Vishnu, 2021; Phogat et al., 2013). The advantages of pulse irrigation include more efficient water use, increased yield as well as improved crop quality (Abdelraouf et al., 2013). These benefits have led to a world-wide increase in usage of this irrigation strategy (Al Al-Baaj et al., 2019). However, this pulsed-irrigated strategy of only providing enough water for current transpiration needs results in orchards being particularly vulnerable to catastrophic irrigation failures because there are insufficient soil water reserves to supply tree needs if irrigation is interrupted (Rank and Vishnu, 2021; McClymont and Goodwin, 2016). While even limited drought can create a water deficit in plants (Torrecillas et al., 1996) that negatively affects yield, desiccation is the severest form of water loss from plant cells (Oliver, 1996). Due to an ongoing trend of sustained higher temperatures and lower precipitation, plant exposure to severe desiccation stress is expected to

increase (Nardini et al., 2013). Vegetative desiccation tolerance can occur, but only if plants lose water at a reduced rate (Oliver et al., 2000). The majority of research related to plant water stress reduction has been through examinations of leaf morphology and anatomy, including stomata characteristics, because it is believed that the effects of water stress are reflected by the leaf features more clearly than in the other plant organs such as stems and roots (Guerfel et al., 2009). For example, in wheat (*Triticum* spp.), improved adaptation to water deficit conditions was found to be associated with the rate of water loss from excised leaves (Wang and Clarke, 1993).

In the almond breeding program at the University of California at Davis, excised shoot desiccation (ESD) has shown promise as a rapid screen for determining genetic tolerance to catastrophic irrigation failures (Appendix A). In this screening method, sections of current shoot growth are excised and placed in a desiccation environment for 24 to 48 hours and the degree of desiccation is scored using visual standards, though the physiological basis as well as potential consequence and whole tree yield and kernel quality are unknown. Water loss through excised leaves or shoots can be through stomata openings within the leaf epidermis or through the epidermis itself (Lawson and Blatt, 2014; Wang et al., 2007). Stomata are located on the leaf epidermis and control water and carbon dioxide exchange between the leaf and the atmosphere; therefore, stomatal characteristics (stomatal behavior and density) of a plant are important factors determining plant productivity as well as water use efficiency (Wang et al., 2007; Torii, 2015).

Water use efficiency (WUE) is defined as the amount of carbon gained as biomass, or alternatively, crop production per unit of water use (Howell, 2001; Hatfield and Dold, 2019). Considering that 95% of gas exchange between the leaf and the environment is controlled by the stomata, manipulating stomata to improve WUE of cultivated plants has been a frequent goal among researchers (Lawson and Blatt, 2014). Genetic selection is one of the most promising

approaches to achieve improved WUE (Ennajeh et al., 2010). In the 1970s and 1980s, breeding programs started to select crop plants by considering the differences in stomata behavior (closing response) and stomata density (SD) to improve WUE (Lawson and Blatt, 2014). According to Wang et al. (2007) manipulating SD to increase WUE is more achievable than manipulating stomatal behavior. It has been shown that reduced SD has led to improved WUE in *Arabidopsis thaliana* (Hepworth et al., 2015; Franks et al., 2015; Doheny-Adams et al., 2012) as well as in millet (Blum and Sullivan, 1986).

Stomata closure in response to water stress usually occurs within 60 minutes of stress initiation (Darwish and Fahmy, 1997). Incomplete closure of stomata has been reported by Muchow and Sinclair (1989) for different sorghum genotypes. When the stomata have minimal conductance, water loss still occurs through the remaining leaf epidermis tissue (Smith et al., 2006). Therefore, a plant's ability to minimize water loss through the epidermis also plays an important role for plant survival under water deficit conditions (Muchow and Sinclair, 1989). Although water loss from the epidermis can occur through both cuticular transpiration and stomatal transpiration due to incomplete closure of stomata (Sinclair and Ludlow, 1986), the term 'epidermal transpiration' includes both water loss pathways, and its rate is generally referred to as epidermal conductance (EC) (Muchow and Sinclair, 1989; Sinclair, 2000).

In this study, excised shoot desiccation (ESD) as well as excised leaf desiccation (ELD) tolerance in almond was evaluated to better understand the physiological basis as well as potential impact on tree productivity under commercial orchard conditions. It is hypothesized that stomata play a major role in the initial and rapid water loss from plant leaf surfaces that continues even after the stomata are triggered to close through water loss from incompletely closed stomata and cuticular transpiration. While cultivars demonstrating low ESD scores may

provide an effective strategy for minimizing catastrophic irrigation failures such as can readily occur under pulsed irrigation, ESD as well ELD scores may also provide useful insights into the relative importance of different leaf components (stomata closure rates and final level of closure, and water loss through intact epidermis) to overall WUE.

To test this hypothesis, the consistency or repeatability of excised shoot desiccation (ESD) responses among twenty diverse almond genotypes grown under commercial conditions was evaluated. In addition, differences in stomata and epidermal characteristics among these genotypes were analyzed by comparing their leaf weight loss patterns observed under controlled conditions. Finally, the relationship between stomata and epidermal characteristics as well as between these traits and tree productivity under commercial orchard conditions was evaluated.

MATERIALS & METHODS

ESD Measurements

Twenty almond genotypes were selected from the UC Davis Regional Variety Trial (RVT) in Salida, CA which is being grown in a replicated block design under standard commercial conditions (Table 1). Trees were planted in the winter of 2014 on Nemaguard rootstocks. The Salida RVT consists of alternating rows of the Nonpareil cultivar to ensure adequate crop cross-pollination, with at least 10 trees per row for each of the 20 genotypes. On 10 July 2019 one tree per genotype was chosen randomly from each of the four replicated rows. One shoot was excised from south-west side of each selected tree to avoid inconsistencies resulting from direct sunlight and heat during the early morning collection period. The cuttings were made from current season growth from each selected branch and immediately placed in an

insulated cooler containing ice where the shoot samples were separated from the ice by layers of perforated foam to minimize desiccation and plant deterioration. When samples arrived at the lab, one fully expanded and undamaged leaf at the base of the shoot was harvested for stomata density analysis. The excised shoots were then placed horizontally on two layers of 0.28 mm diameter gray fiberglass screening on a lab bench. After 24 hours of desiccation under moderate vapor pressure deficit (1.96 kPa) the shoots were evaluated based upon visible wilting. The wilt stages (ESD scores) were determined according to the modified version of the methods of Tyree et al. (2002) (Fig. 2). The descriptions for ESD scores were as follows: (0) Not wilted: no sign of wilting, (1) Slightly- wilted: leaves green but leaf angled slightly towards the midrib, (2) Wilted: leaves green , limited necrosis on leaf blades that angled inward parallel to midrib, (3) Severely-wilted: leaves olive-green, extensive curling of leaves, leaves still bendable, (4) Nearly-desiccated: most leaves necrotic, more extensive curling, leaves still olive-green to brown-green and somewhat soft near the midrib, (5) Desiccated: necrosis on all leaves, extensive curling, leaf blades brittle.



Figure 2: Excised shoot desiccation (ESD) scores of excised shoots after 24 hours. 0: Not Wilted, 1: Slightly-wilted, 2: Wilted, 3: Severely-wilted, 4: Nearly-desiccated, 5: Desiccated.



Figure 3: Excised leaf desiccation (ELD) scores of excised leaves after 24 hours. 0: Not Wilted, 1: Slightly-wilted, 2: Wilted, 3: Severely-wilted, 4: Nearly-desiccated, 5: Desiccated.

Table 1: Selected genotypes and the location of collected samples at UC Davis Regional Variety Trial in Salida

Item	Rep-Lane	Row	Rep-Lane	Row	Rep-Lane	Row	Rep-Lane	Row
UCD3-40	1	2	2	36	3	56	4	16
Winters	1	8	2	30	3	42	4	52
UCD8-27	1	10	2	28	3	36	4	56
Capitola	1	14	2	44	3	32	4	12
UCD1-271	1	20	2	6	3	50	4	38
Folsom	1	22	2	42	3	2	4	20
Sterling	1	26	2	50	3	6	4	26
Jenette	1	28	2	14	3	58	4	4
Sweetheart	1	30	2	10	3	48	4	58
Kester	1	32	2	58	3	28	4	44
UCD1-232	1	34	2	56	3	24	4	6
Supareil	1	38	2	24	3	14	4	46
UCD8-160	1	42	2	48	3	20	4	2
UCD18-20	1	44	2	32	3	12	4	54
UCD8-201	1	46	2	26	3	60	4	30
Booth	1	48	2	34	3	4	4	14
Nonpareil	1	49	2	11	3	39	4	49
Aldrich	1	54	2	4	3	38	4	18
UCD7-159	1	56	2	46	3	18	4	34
UCD1-16	1	60	2	54	3	34	4	8

Stomata Density

Leaves that were detached from the excised shoots were analyzed. First, a thin layer of clear nail polish was applied to the abaxial epidermis. Once the leaves were dry, the nail polish was carefully peeled off and fixed on a microscope slide and examined under a compound microscope (Zeiss STEMI SV8 at 10X-100X, Carl Zeiss AG, Oberkochen, Germany) equipped with a camera (AmScope model MT1000, United Scope LLC, Irvine, California) interfaced to a computer. To assess stomatal density, a magnification of 40-100X were used. The images were obtained by using IS Capture software (United Scope LLC, Irvine California). The number of stomata were measured using two separate counts for each leaf. The area of each image was 0.4 by 0.6 mm.

Comparison of Shoot Desiccation to Individual Leaf Desiccation

Shoot samples of seven genotypes were collected from south-west side of each selected tree with four replicates from the UC Davis Tree Architecture Evaluation Block (TAEB) located at the Wolfskill Experimental Orchards in Winters, CA (Table 2). The cuttings were made from the current season growth of each selected branch and were placed in an insulated cooler containing ice where the shoot samples were separated from the ice by layers of perforated foam to minimize desiccation and plant deterioration. When samples arrived at the lab, one fully expanded and undamaged leaf at the base of the shoot was harvested. Both the selected leaf and remaining shoot were placed horizontally on fiberglass mesh as previously described. After 24 hours, excised shoot desiccation (ESD) and excised leaf desiccation (ELD) scores were determined with the modified version of the study of Tyree et al. (2002) (Fig. 2 and Fig. 3).

Table 2: Selected genotypes for the comparison experiment in UC Davis Wolfskill Experimental Orchards in Winters, CA

Variety/selection	Rootstock	Source	Field	Planted
Monterey	Nemaguard	Wolfskill	TAEB	2017
Nonpareil	Nemaguard	Wolfskill	TAEB	2017
Pyrenees	Cornerstone	Wolfskill	TAEB	2017
Shasta	Nemaguard	Wolfskill	TAEB	2017
UCD 1-232	Cornerstone	Wolfskill	TAEB	2017
UCD 8-201	Cornerstone	Wolfskill	TAEB	2017
Wood Colony	Nemaguard	Wolfskill	TAEB	2017

Relative Water Content (RWC) and Epidermal Conductance (EC) of Excised Leaves

Twenty genotypes were selected at UC Davis Regional Variety Trial (RVT) in Salida, CA. All samples were collected from the most northern block (Rep-Line 1 in Table 1). Four trees were randomly selected per genotype, and one shoot was excised from south-west side of each selected tree on 14th August 2020 in the early morning. The cuttings were made from the current season growth of each selected shoot. The excised shoots were placed in individual bags and then were placed in an insulated cooler containing ice where the shoot samples were separated from the ice by layers of perforated foam to minimize desiccation and plant deterioration. When samples were brought to the lab, each shoot was recut with the base of the stem underwater to eliminate possible stem embolisms and was then rehydrated with distilled water overnight in upright position in the dark. When the shoots were rehydrated, the first fully expanded, undamaged and healthy leaf was detached from all shoots to be used for the experiment. The leaves were weighed to obtain the turgid weight (TW). This value was used to measure the relative water content (RWC) at 0 minute. Then petioles were dipped into the paraffin to prevent water loss from the cut section. Then the leaves were hung petiole-side up in a room with a controlled environment (30°C, 30% humidity). The leaves were weighed over time (0, 115, 200,

285, 360, 430, 510, 600, 660, 720, 1520 minutes). After 24 hours, their ELD scores were determined according to the modified version of the methods of Tyree et al. (2002) (Fig. 3).

Relative Water Content (RWC): After rehydration, the leaves were weighed to obtain the turgid weight (TW). During the experiment, the weights of the leaves were recorded over time and described as fresh weight (FW). When ELD scores were determined, the dry weight (DW) was measured after oven drying at 60 °C for 72 hours and RWC was calculated as (Jensen et al., 2000):

$$RWC = \frac{FW - DW}{TW - DW}$$

Epidermal Conductance (EC): Based on the results from the previous experiment, five genotypes were chosen based on the differences in ELD, as well as demonstrated productivity under commercial conditions, for further analysis on the mechanism of water loss and its correlation with ELD scores. The chosen genotypes were Capitola, UCD7-159, Winters, UCD18-20 and Nonpareil. All samples were collected from the most northern block (Rep-Line 1 in Table 1) at UC Davis RVT in Salina, California. Eight trees were randomly selected per genotype, and one shoot was excised from south-west side of each selected tree on 9 September 2020 in the early morning. The same protocol was followed as the previous experiment except that before hanging the leaves in the controlled room the leaf surface areas of each leaf was measured. For the leaf area measurement, the leaves were imaged using a smartphone and analyzed using Easy Leaf Area Free (Easlon and Bloom, 2014). The leaves were weighed at 0, 100, 180, 295, 380, 440, 500, 560, 620, 680 and 1550 minutes.

Epidermal conductance (EC, $\text{mmol m}^{-2} \text{s}^{-1}$) was calculated from the formula that used by Muchow and Sinclair (1989). The time interval between 440 minutes and 680 minutes was used for this calculation.

$$g_e = \frac{\Delta W}{t} * \frac{1}{A} * \frac{1}{\Delta E} * 2.31 \times 10^6$$

ΔW = The change in leaf mass (g) over time

t= Time(s)

A= Leaf area (m^2)

ΔE = The absolute humidity (mmol m^{-3}) difference between the leaf and the air

2.31×10^6 is a coefficient with the units $\text{mmol water} * \text{mmol air} * \text{g}^{-1} \text{water} * \text{m}^{-3} \text{air}$.

ΔE was assumed to be equal to the absolute humidity of water vapor in the leaf which was calculated from the leaf temperature. It was assumed that leaf and air temperatures were the same.

Data Analysis

Differences among treatment means were determined by analysis of variance (ANOVA), using R studio software, version 1.2.5033 (R studio, Inc., Vienna, Austria). Comparison of means was performed using Tukey's comparison test, with a 5% significance level. For the correlation between variables, the Spearman correlation test was used.

RESULTS & DISCUSSION

Variation for ESD Among and Within Genotypes

A wide range of ESD responses was observed in the 20 genotypes evaluated (Table 3). ANOVA demonstrated that ESD scores were also significantly different among genotypes (Table 4). The lowest ESD scores were observed for Capitola, Sterling, and UCD7-159, with ratings of 1.3, 2 and 2.3, respectively. Shoots of these genotypes showed only low levels of desiccation following the treatment. In contrast, the most severely affected genotypes included Aldrich, UCD8-160, Sweetheart, Jenette and Winters with ESD scores of 3.8, 4.3, 3.8, 3.5 and 3.8, respectively. Severe desiccation was observed to the extent that shoot recovery probably would not occur when the desiccation stress was removed. The remaining 11 genotypes had moderate ESD scores ranging from 2.5 to 3.3.

Nonpareil, which is the dominant commercial cultivar in California because of its good kernel quality, wide-adaptability and high productivity, showed a moderate ESD value of 3. In contrast, the cultivars Aldrich and Winters (Gradziel et al., 2007) which are also commercially important cultivars with demonstrated potential for high productivity, showed high ESD scores of 3.8. Winters, however, has a grower reputation for needing more water than Nonpareil in order to achieve consistent, high yields. The cultivar Kester, while also showing an intermediate ESD value of 3, has also proven capable of high tree yields as well as good kernel quality.

Table 3: Excised shoot desiccation (ESD) scores and stomata density (SD) of 20 genotypes. ESD Scores: 0= Not wilted, 1= Slightly- wilted, 2= Wilted, 3= Severely-wilted, 4= Nearly-desiccated, 5=Desiccated.

Genotype	ESD Scores	SD (n/mm ²)
Aldrich	3.8 ± 1	216.7 ± 12.6
Booth	3.3 ± 0.5	235.4 ± 42.2
Capitola	1.3 ± 0.5	260.4 ± 29.5
Folsom	3.3 ± 0.5	243.8 ± 28.1
Jenette	3.5 ± 0.6	197.9 ± 16.5
Kester	3 ± 0	206.3 ± 19.8
Nonpareil	3 ± 0.8	258.3 ± 23.6
Sterling	2 ± 0.8	318.8 ± 39.3
Supareil	3.3 ± 1	225 ± 42.7
Sweetheart	3.8 ± 0.5	193.8 ± 30.8
UCD1-16	3.3 ± 0.5	275 ± 44.5
UCD1-232	3 ± 0.8	254.2 ± 58.9
UCD1-271	3 ± 0.8	266.7 ± 47.1
UCD18-20	3 ± 0.8	227.1 ± 36.7
UCD3-40	3 ± 0	272.9 ± 59.7
UCD7-159	2.3 ± 0.5	239.6 ± 28.1
UCD8-160	4.3 ± 0.5	231.3 ± 36.1
UCD8-201	3.3 ± 1	243.8 ± 12.4
UCD8-27	2.5 ± 0.6	227.1 ± 23.5
Winters	3.8 ± 1.3	277.1 ± 36.7

Table 4: Analysis of variance for excised shoot desiccation (ESD) response of genotypes. The model uses ESD as the response variable.

Analysis of Variance Table						
Response: ESD						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Genotype	19	36.949	1.94470	3.7012	5.832e-05	***
Residuals	59	31.000	0.52542			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Differences in Leaf Stomata Density Among Genotypes

Stomata density means also varied widely among the genotypes tested, ranging from a high value of 318.8 stomata/mm² in the relatively new and so commercially untested cultivar Sterling, to a low value of 193.8/mm² in the cultivar Sweetheart (Table 3). Both Aldrich and Kester also had relatively low values of 206.3 and 216.7/mm², respectively, while Nonpareil had a moderate value of 258.3/mm² with the Winters cultivar also showing a high value of 277.1 /mm². As with ESD ratings, a wide variation also existed for stoma density and no strong correlation (Spearman's coefficient of -0.3) was apparent between these traits (Fig. 4). A previous report by Hepworth et al. (2015) working with *Arabidopsis thaliana* (L.) Heynh stomatal development mutants showed that reduced SD led to improved WUE, which was also the finding of Doheny-Adams et al. (2012) with traditional *Arabidopsis* and Blum and Sullivan (1986) for millet. In the almond cultivar Boniva, Oliveira et al. (2018) recently reported adaptations that reduce water loss included reduced SD. These findings indicate that while SD can differ among cultivars, the final effect on desiccation rate is more complicated and affected by other shoot and leaf traits. Similarly, when yield data was plotted against stoma density no clear trends were observed.

These findings suggest that SD by itself may not be the best parameter for evaluating almond for either WUE or yield potential. This was somewhat unexpected because stomata regulate photosynthesis through CO₂ uptake (Lawson and Vialet-Chabrand, 2019) but reinforces the general view that reproductive and vegetative development are also major determinants of yield (Xu et al., 2017), confounding any direct relationships. For example, the effect of SD on photosynthesis was previously reported to be inconsistent with a positive association reported in *Leymus chinensis*, but negative correlation for wheat and various plants adapted to a more

Mediterranean climate (Zhao et al., 2015). Stomatal development is also affected by the leaf environment (Casson and Gray, 2008) resulting in difficulties in making generalizations about any relationship between stomata density, water loss and even productivity. Some researchers also believe that it may not be wise to use stoma density as a marker for water stress tolerance (Yadollahi et al., 2011) as resistant cultivars often have unique adaptations to water stress (Ennajeh et al., 2010). Manipulating the stomatal characteristics may still prove a promising approach to improve WUE and photosynthetic activity because WUE and carbon uptake are affected by the rapidity of the stomatal response to changing conditions (Lawson and Blatt, 2014).

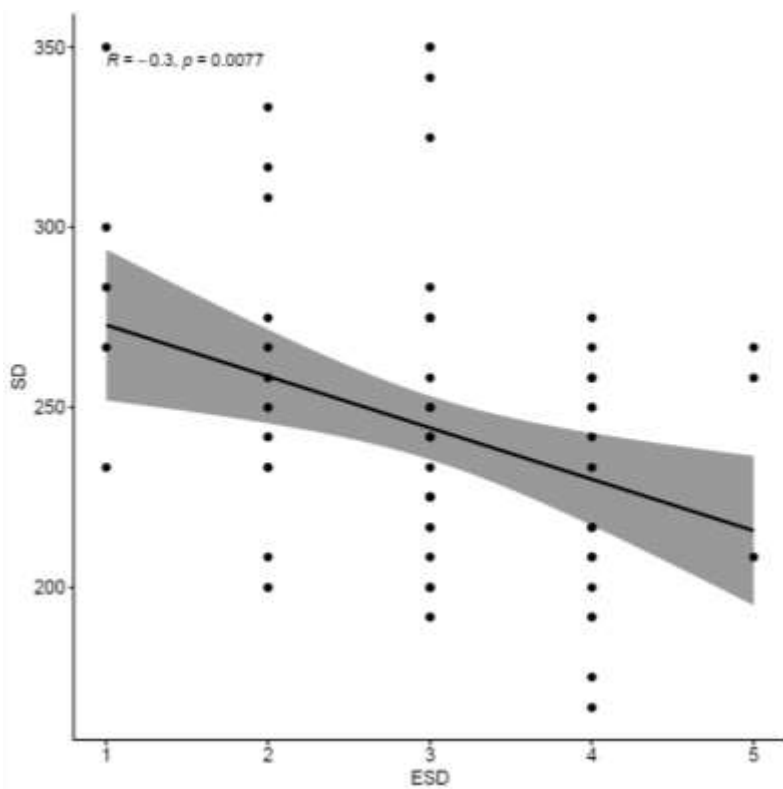


Figure 4: Spearman correlation between stomata density (SD, n/mm²) and excised shoot desiccation (ESD) scores.

Relation of Shoot to Leaf Desiccation Rating Within and Among Genotypes

Although the shoots used for the ESD experiment were uniformly selected from current season growth of selected genotypes, differences in shoot architecture introduced difficulties in standardizing sample collection. The number of leaves that each shoot possessed also differed among samples. Using individual leaves for desiccation experiments was seen as an approach to reduce this variability and so improve standardization. To test this possibility, the desiccation scores of shoots and leaves were compared among seven genotypes grown in a young commercial orchard being evaluated for tree architecture effect on productivity. Very good to excellent agreements were observed for leaf versus shoot samples analyzed (Table 5). Consequently, for subsequent experiments individual leaf samples rather than whole shoot samples were evaluated to reduce within-sample variation.

Table 5: The comparison between excised shoot desiccation (ESD) scores and excised leaf desiccation (ELD) scores for seven almond genotypes.

Genotype	ESD Score	ELD Score
Monterey	3.8 ± 0.5	3.8 ± 0.5
Nonpareil	4.3 ± 0.6	4 ± 0
Pyrennes	4.3 ± 0.5	4 ± 0
Shasta	4.6 ± 0.6	4 ± 0
UCD 1-232	5 ± 0	4.8 ± 0.5
UCD 8-201	5 ± 0	5 ± 0
Wood Colony	4.6 ± 0.6	4.6 ± 0.6

Dynamics of Relative Water Content (RWC) Loss under Desiccation Conditions and Relation to ELD

Leaf relative water content (RWC) differed significantly among genotypes and over time (Tables 6 and 7). Pronounced differences in RWC loss occurred between 0 and 180 minutes (Table 8). High ELD-scoring genotypes showed steeper declines in RWC during this time. This is the period when stomata would be responding to water stress (Sinclair and Ludlow, 1986). However, because stoma closure is a rapid response, typically occurring in the first 60 minutes after stress (Darwish and Fahmy, 1997) the variability observed appears more likely to be due to differences in final degree of stoma closure. Alternatively, the relatively lower rate of water loss in the first 60 minutes may be the result of the shoots previously being kept in the dark while being rehydrated. After leaves were excised, the stomata may have started to open while they were being placed to the controlled room. It was found that the genotypes having a higher ELD scores of 4 and 5 generally had steeper water loss in the first 180 minutes in the controlled environment when compared to lower ELD scores (Fig. 5). Results suggests that genotypes with low ELD scores, which were the genotypes demonstrating the lowest leaf desiccation damage, were able to achieve fuller closure in shorter time and so effectively conserve leaf water. In contrast, genotypes with high ELD scores, which were selections that showed high levels of leaf desiccation, lost water at a much higher rate during this time period. This would suggest these stomata were not as responsive but remained at least partially open during this early critical stage of desiccation allowing excessive water loss. A notable exception was seen in UCD18-20 where, despite showing a high ELD score, the RWC loss patterns were similar to genotypes having low ELD scores (Fig. 5). UCD18-20 has been one of the most productive genotypes in the regional variety trials with a notably denser foliage. Closer examination of the shoot structure showed an

unusual growth pattern where the normal expansion of primary leaves preformed during the previous winter was followed by a second atypical flush of growth occurring in midsummer (Fig. 6). While this atypical binary shoot structure appears to contribute to the higher overall productivity owing to the resulting higher leaf and flower density, it might also explain its deviation from the more typical leaf response to water stress patterns seen in the remaining more traditional genotypes.

To further evaluate the mechanism behind these observed patterns, we retested later-season leaf samples from UCD18-20 along with the other genotypes that consistently produced high yields of good commercial quality kernels but differed in ELD response. These selections included Nonpareil, Winters and UCD18-20 having high ELD scores and so high vulnerability to water loss, and Capitola and UCD7-159 which showed the lowest ELD score of 2.3, indicating resistance to water loss. Large differences were again observed for ELD response during the critical early desiccation period of 0-180 minutes but not 0 to 60 minutes (Fig. 7 & Table 9). The high ELD genotypes of Nonpareil and Winters showed significantly larger RWC declines during this critical early desiccation period than low ELD genotypes Capitola and UCD7-159 (Fig. 7). As in previous tests, UCD18-20 was intermediate to both groups despite having a high ELD score of 5. While this relatively early yet delayed response supports the presence of cultivar and so genetic differences in final level of stomata closure, additional interacting factors appear to also affect the rate of water loss from excised leaves and shoots. Roark et al. (1975) presented the RWC curve of detached cotton leaves as two phases: stomatal and cuticular. After shoot detachment, stomata closure rapidly occurs, then rate of water loss becomes linear for several hours (McCaig and Romagosa, 1991). During this linear phase, water loss might occur due to incomplete stomata closure because there is not sufficient proof that stomata of detached leaves

had fully closed (Sinclair and Ludlow, 1986; Dhanda and Sethi, 1998). Therefore, although this later phase is often described as epidermal leakage (Muchow and Sinclair, 1989), it may be more useful to separate this post stomata-closure period into two responses: water loss through incompletely close stomata and water loss through otherwise intact epidermis.

A uniform and rapid initial stomata closure response among different almond genotypes might be expected because almond has evolved a high level of drought tolerance owing to the distinctly arid environments within its center of origin (Sorkheh et al., 2009). This study demonstrated differences in the subsequent (0-180 minutes) response as well as the later response which appear to represent a different general pathway for leaf water loss controlled by final levels of stomata closure as well as subsequent epidermis integrity. As might be expected, these responses also appear to be somewhat interdependent. Extensive water loss early in the evaluation period, presumably from incomplete stomata closure, is generally associated with the smaller response during the final general epidermis failure phase. However, this may be simply due to the already low relative water content in these genotypes, precluding opportunities for large water losses associated with the final epidermis collapse. Interestingly, several genotypes initially selected for their high early orchard yield potential, including the recently released cultivar Kester (Gradziel and Lampinen, 2019) and the advanced breeding lines UCD 3-40 and UCD 8-27, appear more vulnerable to leaf desiccation, particularly during the early stomata related phases (Table 6 and Fig. 5). These results raise the possibility that selection for high productivity could inherently select for traits such as high stomata density and prolonged gas exchange durations as these might improve the crucial photosynthetic efficiency. However, the same traits could also make these genotypes more vulnerable to the type of leaf desiccation pressures anticipated in future of warming climates. The atypical results of UCD 18-20,

however, also offer the possibility that such inherent productivity barriers can be overcome with new genetic and/or cultural management strategies.

Table 6: Relative water content (RWC) of twenty almond genotypes over time under a controlled environment. ELD= Excised leaf desiccation, min.=minutes.

Genotype	ELD Scores	Relative Water Content(RWC)													
		0 min.	15 min.	200 min.	285 min.	360 min.	430 min.	510 min.	600 min.	660 min.	720 min.	1520 min.			
Aldrich	3 ± 0.8	1 ± 0	0.95 ± 0.01	0.88 ± 0.04	0.7 ± 0.02	0.62 ± 0.03	0.56 ± 0.05	0.5 ± 0.06	0.44 ± 0.07	0.41 ± 0.07	0.38 ± 0.07	0.15 ± 0.07			
Booth	3.5 ± 1	1 ± 0	0.97 ± 0.00	0.88 ± 0.05	0.76 ± 0.07	0.66 ± 0.09	0.6 ± 0.1	0.55 ± 0.11	0.49 ± 0.12	0.46 ± 0.12	0.42 ± 0.12	0.15 ± 0.08			
Capitola	2.3 ± 0.5	1 ± 0	0.97 ± 0.01	0.9 ± 0.04	0.74 ± 0.04	0.66 ± 0.04	0.61 ± 0.04	0.56 ± 0.06	0.52 ± 0.06	0.49 ± 0.07	0.45 ± 0.07	0.17 ± 0.07			
Folsom	3.5 ± 0.6	1 ± 0	0.93 ± 0.01	0.81 ± 0.03	0.7 ± 0.02	0.61 ± 0.03	0.53 ± 0.05	0.45 ± 0.07	0.37 ± 0.08	0.32 ± 0.09	0.28 ± 0.09	0.06 ± 0.03			
Jenette	4.5 ± 0.6	1 ± 0	0.91 ± 0.03	0.77 ± 0.07	0.69 ± 0.1	0.59 ± 0.15	0.52 ± 0.17	0.44 ± 0.17	0.38 ± 0.17	0.34 ± 0.16	0.3 ± 0.15	0.07 ± 0.05			
Kester	5 ± 0	1 ± 0	0.83 ± 0.06	0.61 ± 0.08	0.41 ± 0.14	0.28 ± 0.14	0.2 ± 0.12	0.15 ± 0.1	0.1 ± 0.07	0.08 ± 0.05	0.06 ± 0.04	0 ± 0			
Nonpareil	3 ± 0.8	1 ± 0	0.94 ± 0.02	0.87 ± 0.06	0.69 ± 0.04	0.64 ± 0.07	0.58 ± 0.09	0.52 ± 0.11	0.46 ± 0.12	0.42 ± 0.13	0.38 ± 0.12	0.12 ± 0.08			
Sterling	3.8 ± 0.5	1 ± 0	0.94 ± 0.02	0.74 ± 0.02	0.66 ± 0.02	0.59 ± 0.04	0.54 ± 0.06	0.48 ± 0.07	0.42 ± 0.08	0.39 ± 0.08	0.35 ± 0.09	0.12 ± 0.05			
Supareil	2 ± 0	1 ± 0	0.96 ± 0.01	0.91 ± 0.04	0.85 ± 0.02	0.75 ± 0.02	0.71 ± 0.02	0.67 ± 0.02	0.63 ± 0.02	0.6 ± 0.02	0.58 ± 0.03	0.31 ± 0.06			
Sweetheart	3.8 ± 1	1 ± 0	0.91 ± 0.03	0.72 ± 0.05	0.63 ± 0.05	0.54 ± 0.05	0.48 ± 0.06	0.41 ± 0.06	0.35 ± 0.07	0.31 ± 0.07	0.27 ± 0.07	0.05 ± 0.04			
UCD1-16	3.5 ± 0.6	1 ± 0	0.93 ± 0.02	0.84 ± 0.03	0.64 ± 0.03	0.58 ± 0.02	0.53 ± 0.02	0.47 ± 0.02	0.42 ± 0.03	0.38 ± 0.03	0.35 ± 0.04	0.1 ± 0.04			
UCD1-232	4 ± 1.4	1 ± 0	0.96 ± 0.01	0.81 ± 0.08	0.66 ± 0.04	0.57 ± 0.07	0.5 ± 0.08	0.43 ± 0.1	0.36 ± 0.13	0.32 ± 0.13	0.28 ± 0.14	0.08 ± 0.11			
UCD1-271	3.8 ± 1	1 ± 0	0.91 ± 0.03	0.77 ± 0.06	0.67 ± 0.07	0.57 ± 0.1	0.51 ± 0.11	0.44 ± 0.12	0.37 ± 0.12	0.33 ± 0.11	0.29 ± 0.11	0.06 ± 0.03			
UCD18-20	4.8 ± 0.5	1 ± 0	0.92 ± 0.01	0.85 ± 0.05	0.72 ± 0.04	0.61 ± 0.05	0.54 ± 0.06	0.46 ± 0.07	0.39 ± 0.08	0.33 ± 0.09	0.29 ± 0.09	0.05 ± 0.04			
UCD3-40	5 ± 0	1 ± 0	0.83 ± 0.03	0.62 ± 0.09	0.45 ± 0.12	0.32 ± 0.1	0.24 ± 0.08	0.18 ± 0.07	0.14 ± 0.05	0.12 ± 0.04	0.1 ± 0.04	0.02 ± 0.01			
UCD7-159	2.3 ± 0.5	1 ± 0	0.97 ± 0.01	0.92 ± 0.04	0.76 ± 0.06	0.67 ± 0.06	0.63 ± 0.08	0.59 ± 0.1	0.55 ± 0.11	0.53 ± 0.12	0.5 ± 0.12	0.29 ± 0.13			
UCD8-160	3.3 ± 0.5	1 ± 0	0.93 ± 0.02	0.8 ± 0.03	0.69 ± 0.05	0.62 ± 0.04	0.56 ± 0.05	0.49 ± 0.07	0.43 ± 0.08	0.39 ± 0.08	0.35 ± 0.09	0.09 ± 0.07			
UCD8-201	4.3 ± 1.5	1 ± 0	0.92 ± 0.03	0.74 ± 0.06	0.64 ± 0.07	0.55 ± 0.1	0.48 ± 0.11	0.41 ± 0.13	0.35 ± 0.13	0.32 ± 0.14	0.28 ± 0.13	0.08 ± 0.1			
UCD8-27	5 ± 0	1 ± 0	0.83 ± 0.04	0.6 ± 0.05	0.47 ± 0.08	0.35 ± 0.1	0.28 ± 0.09	0.22 ± 0.07	0.17 ± 0.06	0.14 ± 0.05	0.11 ± 0.04	0 ± 0			
Winters	4.3 ± 1	1 ± 0	0.93 ± 0.03	0.79 ± 0.1	0.66 ± 0.14	0.55 ± 0.19	0.48 ± 0.21	0.4 ± 0.21	0.34 ± 0.21	0.3 ± 0.2	0.27 ± 0.2	0.07 ± 0.08			

Table 7: Analysis of variance table showing that leaf relative water content (RWC) differed significantly among genotypes over time, and analysis of variance table showing that excised leaf desiccation (ELD) scores significantly differ among genotypes.

```

Analysis of Variance Table

Response: RWC
      Df Sum Sq Mean Sq  F value    Pr(>F)
Genotype  19  7.825   0.412   21.517 < 2.2e-16 ***
Time       1 49.692  49.692 2596.189 < 2.2e-16 ***
Residuals 859 16.442   0.019
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

Analysis of Variance Table

Response: ELD
      Df Sum Sq Mean Sq  F value    Pr(>F)
genotype  19 63.637   3.3493   5.783 8.185e-08 ***
Residuals 60 34.750   0.5792
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

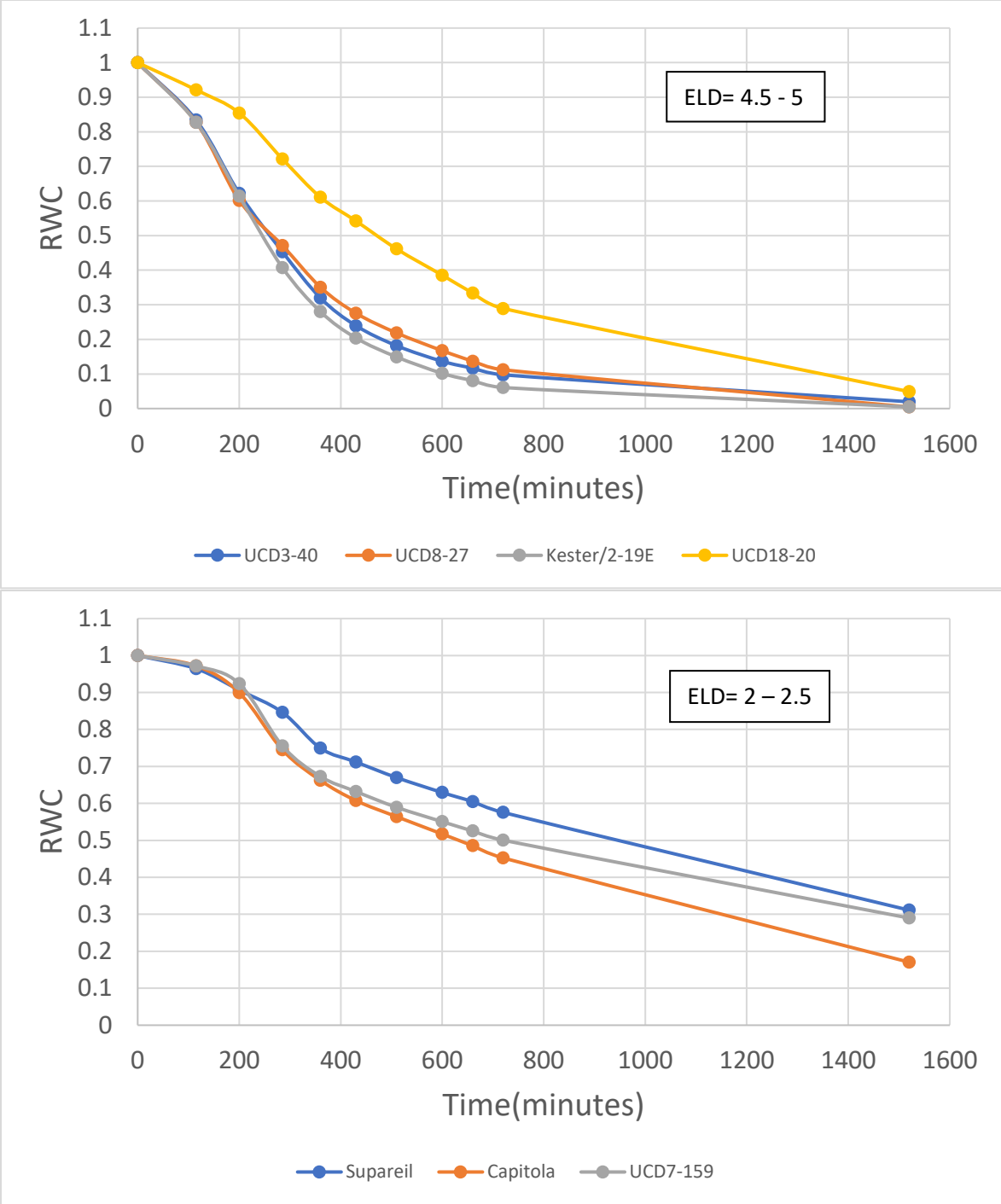


Figure 5: Relative water content (RWC) curve for genotypes demonstrating more extreme ELD scores. Steeper water loss in the first 180 minutes is observed for genotype having a high ELD score. ELD= Excised leaf desiccation.

Table 8: The relationship between relative water content (RWC) differences and excised leaf desiccation (ELD). (Example: 0-100 means RWC difference between 0 and 100 minutes. The most significant RWC difference relative to ELD scores was between 0 and 180 minutes.)

Analysis of Variance Table						
Response: ELD						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
0-100	1	8.8818	8.8818	35.6743	3.643e-06	***
0-180	1	15.4558	15.4558	62.0790	4.129e-08	***
0-295	1	11.9555	11.9555	48.0199	3.634e-07	***
0-380	1	4.1954	4.1954	16.8510	0.000404	***
0-440	1	3.4779	3.4779	13.9690	0.001020	**
0-500	1	2.2819	2.2819	9.1654	0.005814	**
0-560	1	1.9941	1.9941	8.0096	0.009257	**
0-620	1	0.2577	0.2577	1.0351	0.319116	
0-680	1	0.2434	0.2434	0.9778	0.332619	
0-1550	1	0.8216	0.8216	3.2999	0.081797	.
180-1550	1	1.1718	1.1718	4.7067	0.040173	*
295-1550	1	0.7181	0.7181	2.8843	0.102377	
380-1550	1	0.2192	0.2192	0.8805	0.357420	
440-1550	1	1.0176	1.0176	4.0873	0.054493	.
500-1550	1	0.3078	0.3078	1.2364	0.277188	
Residuals	24	5.9753	0.2490			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Table 9: Relative water content loss between 0-180 minutes significantly differed between the genotypes with high excised leaf desiccation scores (Winters and Nonpareil) and the genotypes with low ELD scores (UCD7-159 and Capitola). UCD18-20 is a developmental outlier as explained in the text.

Genotype	emmean	SE	df	lower.CL	upper.CL	.group
UCD7-159	0.194	0.022	35	0.149	0.239	a
Capitola	0.195	0.022	35	0.150	0.240	a
UCD18-20	0.277	0.022	35	0.232	0.322	ab
Winters	0.288	0.022	35	0.243	0.332	b
Nonpareil	0.297	0.022	35	0.252	0.341	b

Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 5 estimates
significance level used: alpha = 0.05



Figure 6: Unique growth habit of UCD18-20 showing a second distinctive midsummer growth spurt following the normal expansion and maturation of the preformed apical shoot.

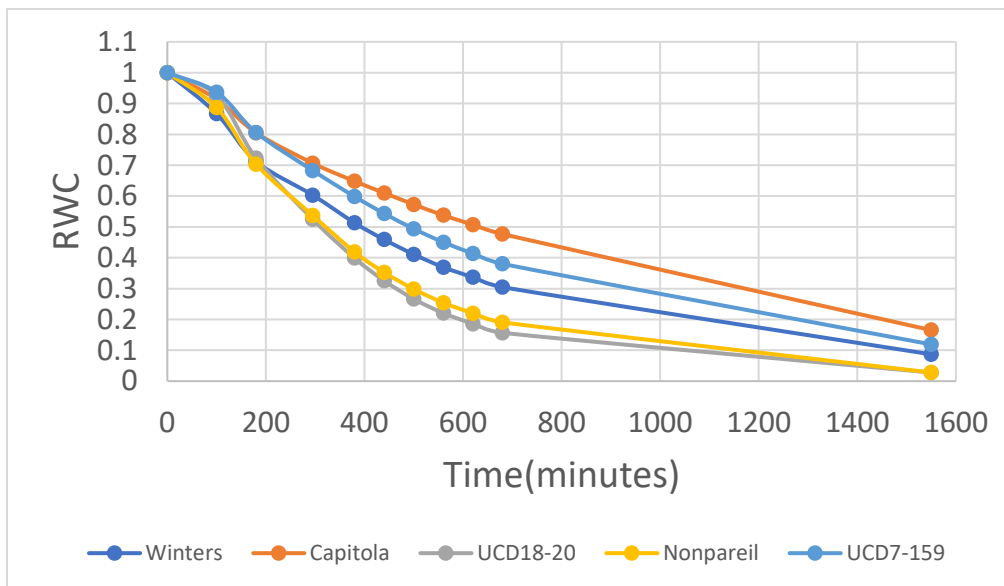


Figure 7: Relative water content (RWC) curve of 5 chosen genotypes.

Differences in Epidermal Conductance (EC) of Excised Leaves Among Tested Genotypes

Increasing ELD scores were associated with increasing epidermal conductance (EC) for the five standard genotypes evaluated (Table 10 and Table 11). Spearman analysis also showed a positive correlation between ELD and EC ($R=0.56$) (Fig. 8). EC was the lowest for Capitola ($33.4 \text{ mmol m}^{-2} \text{ s}^{-1}$), which also had the lowest ELD score (2.3). UCD7-159 had the second lowest EC ($40.2 \text{ mmol m}^{-2} \text{ s}^{-1}$) with an ELD score of 2.6. The EC of Winters was $42.4 \text{ mmol m}^{-2} \text{ s}^{-1}$, and its ELD score was 4.3. UCD18-20 and Nonpareil had the highest ELD score. UCD18-20 also showed the highest EC ($49.1 \text{ mmol m}^{-2} \text{ s}^{-1}$) value (Table 10). In contrast, no significant correlation was observed between EC and the change in RWC between 0 and 180 minutes. This suggests that water loss within first 180 minutes appears to be primarily due to differing degrees of final stomata closure and to a lesser degree the differences in epidermal integrity, though multiple interacting (cuticular wax quality and quantity, cellular integrity, leaf age, etc.) factors would likely be involved. Leaf maturation appears to influence EC as it has been suggested that younger leaves could have higher EC than older leaves (Smith et al., 2006). When leaves reached full expansion, EC reportedly decreased in *Pyrus* species (Schönherr, 1976; Smith et al., 2006). In our experiment, the first fully expanded leaves on the current season growth were sampled, which should have minimized the effect of leaf maturity. When whole shoots were tested to determine excised shoot desiccation (ESD) scores, however, the more basal, fully developed leaves were often the first to show desiccation symptoms. Other, unrelated factors may also have contributed. For example, unlike ELD scoring, the cut surface of stems used for ESD scoring were usually not sealed to prevent desiccation at the cut. Genotype UCD18-20 also demonstrated a unique growth habit in that the shoots showed a second phase of growth later in the summer and so the pattern of leaf development from tip to base might be expected to be distinctly

different. Consequently, EC may have been differentially affected by the leaf maturation for this genotype, which also showed the highest EC.

Muchow and Sinclair (1989) concluded that the amount of water loss via EC was correlated with plant's survival under drought and that low EC might be useful for the identification of genotypes with enhanced ability to tolerate water stress. Other researchers also reported that when water stress increases, EC decreases (Dube et al., 1975; Bengston et al., 1978). This is consistent with our observations that the rate of water loss decreased with increasing time in the desiccation treatment (Fig. 7). Regardless of mechanism, results from this study clearly document the existence of significant genetic variation in the observed water stress response among the different genotypes evaluated.

Table 10: RWC, epidermal conductance (EC, $\text{mmol m}^{-2} \text{s}^{-1}$) and excised leaf desiccation (ELD) scores of five almond genotypes over time. (min=minutes)

Genotype	ELD	EC($\text{mmol m}^{-2} \text{s}^{-1}$)	Relative Water Content(RWC)										
			0(min)	100(min)	180(min)	295(min)	380(min)	440(min)	500(min)	560(min)	620(min)	680(min)	1550(min)
Capitola	2.3 ± 0.5	33.4 ± 7.3	1 ± 0	0.91 ± 0.02	0.81 ± 0.05	0.71 ± 0.03	0.65 ± 0.04	0.61 ± 0.04	0.57 ± 0.05	0.54 ± 0.06	0.51 ± 0.07	0.48 ± 0.07	0.17 ± 0.1
Nonpareil	4.8 ± 0.5	42.7 ± 11.4	1 ± 0	0.89 ± 0.04	0.70 ± 0.09	0.54 ± 0.12	0.42 ± 0.14	0.35 ± 0.14	0.30 ± 0.14	0.25 ± 0.13	0.22 ± 0.12	0.19 ± 0.11	0.03 ± 0.01
UCD18-20	5 ± 0	49.1 ± 10.2	1 ± 0	0.93 ± 0.03	0.72 ± 0.08	0.53 ± 0.08	0.40 ± 0.1	0.33 ± 0.1	0.27 ± 0.09	0.22 ± 0.09	0.19 ± 0.08	0.16 ± 0.07	0.03 ± 0.01
UCD7-159	2.6 ± 0.5	40.2 ± 7.3	1 ± 0	0.94 ± 0.02	0.81 ± 0.05	0.68 ± 0.04	0.60 ± 0.06	0.54 ± 0.07	0.49 ± 0.08	0.45 ± 0.08	0.41 ± 0.09	0.38 ± 0.09	0.12 ± 0.06
Winters	4.3 ± 0.7	42.4 ± 4.3	1 ± 0	0.87 ± 0.01	0.71 ± 0.03	0.60 ± 0.03	0.51 ± 0.03	0.46 ± 0.03	0.41 ± 0.03	0.37 ± 0.04	0.34 ± 0.04	0.31 ± 0.04	0.09 ± 0.02

Table 11: Analysis of variance table showing that epidermal conductance (EC, $\text{mmol m}^{-2} \text{s}^{-1}$) significantly differs among excised leaf desiccation (ELD) scores.

Analysis of Variance Table							
Response: EC							
	Df	Sum Sq	Mean Sq	F value	Pr(>F)		
ELD	1	942.66	942.66	13.868	0.0006345	***	
Residuals	38	2583.02	67.97				

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1							

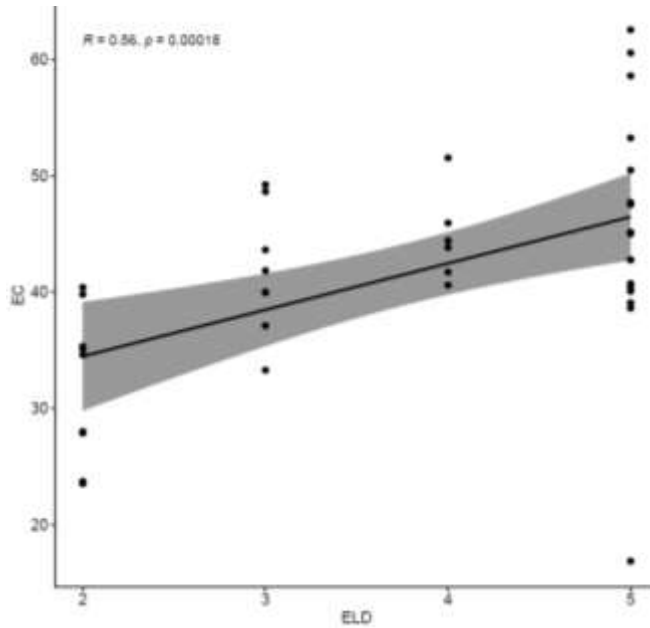


Figure 8: Spearman correlation between excised leaf desiccation (ELD) scores and epidermal conductance (EC, $\text{mmol m}^{-2} \text{s}^{-1}$). (R=0.56)

ELD Associations with General Performance

The trees grown in the UC Davis RVT in Salida were not managed with pulsed irrigation. Instead, they were managed to minimize water stress using conventional micro-sprinkler irrigation. Thus, potential yield differences of genotypes under pulsed irrigation strategies would not be reflected in the yield data involved in these trials. Under the conventional orchard management used, a wide range was observed for tree productivity as well as excised shoot and excised leaf desiccation among genotypes tested (Table 12). ELD and 2020 yield was found to be significantly related and negatively correlated (Fig. 9). The highest 2020 yield (3520.9 kernel lbs/ac) was seen in cultivar Nonpareil having a moderate ELD score of 3. This was followed by Aldrich (3097.7 kernel lbs/ac) and Capitola (3062.4 kernel lbs/ac) with ELD score of 3 and 2.3, respectively. Showing the highest ELD score of 5, UCD8-27 had the lowest 2020 yield with 1489.4 kernel lbs/ac. Somewhat similar findings were reported by Clarke et al. (1989) for wheat

genotypes. They used excised wheat leaves to measure water loss rates and identified genotypes with low rate of water loss yet relatively high yields. However, they also noted that before recommending this technique, the same experiment should be conducted in different environments in order to better establish the relationship between tolerance to leaf water loss and final yield potential.

No strong association with cumulative yield was observed when ESD and ELD scores were analyzed against cumulative yields (Table 13 & Fig. 10). Within any desiccation score it was possible to find genotypes demonstrating high as well as medium and low productivity. Results support opportunities to employ ESD and ELD scores to select genotypes having tolerance to catastrophic water loss as would occur with sudden irrigation line failure, without necessarily sacrificing long-term tree productivity.

These results, as well as previous findings concerning the mechanisms involved in excised shoot as well as excised leaf desiccation, further emphasize the complexity of factors controlling both leaf desiccation as well as final tree yield. Consequently, while factors that encourage high gas exchange through stomata would seem to favor both high susceptibilities to leaf desiccation as well as higher photosynthesis-driven yields, the involvement of multiple other factors and their interactions also appear to be important contributors to final performance. Consequently, while ESD as well as ELD scores are relatively good predictors of tolerance to catastrophic irrigation failures, they remain poor predictors of final tree productivity demonstrating the importance of other contributing factors. It should be noted that the germplasm evaluated contains an unusually wide genetic diversity since many of the breeding selections have incorporated the self-fruitful gene from peach or wild almond resulting in a high likelihood that other exotic though undetected genes are also present. Clear developmental

differences are also observed in this germplasm such as the previously described bimodal shoot growth habit of UCD18-20 as described in the previous section. (Not surprisingly, UCD 18-20 was frequently an outlier from general data trends as demonstrated in figure 9 where it is identified with a star symbol.)

Low water stress is a major contributor to maximum crop yield depending on timing, intensity, and duration of stress (Chaves and Oliveira, 2004). Slower vegetative growth may also increase the chances of plant survival against stress because stressed plants may be induced to produce molecules for effectively coping with stress rather than directing all of the resources to new growth (Zhu, 2002). Additionally, plants can eliminate the effects of stress by shortening their growth cycle when water deficits are gradually encountered; however, rapid dehydration can severely damage the photosynthesis cycle (Chaves and Oliveira, 2004) resulting in plants that cannot respond quickly to compensate for water loss. Plants can respond to water stress by reducing carbon uptake, cell division and expansion (Chaves and Oliveira, 2004; Zhu, 2001) as well as other growth and development processes. Because these physiological responses are genetically controlled, a recommended pathway for improved dehydration tolerance is the incorporation of novel stress tolerance genes/mechanisms from related germplasm (Sullivan and Eastin, 1974).

Table 12: Excised shoot desiccation (ESD) and excised leaf desiccation (ELD) with yearly and cumulative yield from UC Davis Regional Variety Trial in Salida.

Genotype	ESD Scores	ELD Scores	2016 Yield (lbs/ac)	2017 Yield (lbs/ac)	2018 Yield (lbs/ac)	2019 Yield (lbs/ac)	2020 Yield (lbs/ac)	Cum. Yield (lbs/ac)
Aldrich	3.8 ± 1	3 ± 0.8	161.8 ± 80.2	1674.8 ± 395.1	2330.7 ± 212.8	1479.5 ± 133.2	3097.7 ± 332.8	8744.5 ± 1154.1
Booth	3.3 ± 0.5	3.5 ± 1	127.7 ± 102.2	1550.0 ± 459.0	2226.3 ± 303.7	1497.6 ± 288.8	2701.4 ± 364.7	8102.9 ± 1518.3
Capitola	1.3 ± 0.5	2.3 ± 0.5	123.0 ± 81.5	1364.7 ± 422.6	2261.9 ± 212.8	1284.3 ± 157.6	3062.4 ± 189.8	8096.3 ± 1064.2
Folsom	3.3 ± 0.5	3.5 ± 0.6	280.8 ± 148.1	1241.4 ± 107.7	1316.2 ± 360.7	1572.7 ± 247.7	2282.3 ± 207.4	6693.5 ± 1071.6
Jenette	3.5 ± 0.6	4.5 ± 0.6	119.8 ± 41.6	1395.6 ± 137.1	1458.4 ± 145.9	1322.2 ± 200.9	1895.3 ± 455.4	6191.4 ± 981
Kester	3 ± 0	5 ± 0	320.7 ± 145.2	1648.4 ± 520.2	1817.9 ± 148.5	1617.9 ± 120.3	2374.6 ± 95.0	7779.5 ± 1029.2
Nonpareil	3 ± 0.8	3 ± 0.8	175.1 ± 34.4	1407.6 ± 264.6	2042.7 ± 609.4	1377.4 ± 74.6	3520.9 ± 313.5	8523.6 ± 1296.5
Sterling	2 ± 0.8	3.8 ± 0.5	53.8 ± 44.5	1465.3 ± 281.9	2002.6 ± 574.1	1447.2 ± 173.6	2393.1 ± 444.7	7361.9 ± 1518.7
Supareil	3.3 ± 1	2 ± 0	52.9 ± 53.7	1042.3 ± 163.0	1130.0 ± 276.1	1967.6 ± 334.9	2732.5 ± 573.7	6925.3 ± 1401.4
Sweethea	3.8 ± 0.5	3.8 ± 1	178.5 ± 55.7	936.3 ± 182.0	1612.2 ± 185.5	1554.2 ± 221.2	2534.3 ± 352.9	6815.5 ± 997.3
UCD1-16	3.3 ± 0.5	3.5 ± 0.6	356.5 ± 154.4	1223.2 ± 123.6	1353.9 ± 653.9	1294.7 ± 294.1	2271.1 ± 523.8	6499.4 ± 1749.7
UCD1-232	3 ± 0.8	4 ± 1.4	225.2 ± 57.2	1403.8 ± 73.8	1498.0 ± 269.2	1646.0 ± 144.6	2111.9 ± 579.3	6884.9 ± 1124.1
UCD1-271	3 ± 0.8	3.8 ± 1	85.6 ± 20.8	1233.5 ± 216.6	1612.5 ± 241.0	1630.4 ± 241.5	2007.2 ± 68.4	6569.2 ± 788.3
UCD18-20	3 ± 0.8	4.8 ± 0.5	262.0 ± 133.9	1970.9 ± 420.6	2367.8 ± 291.7	2121.2 ± 180.9	2601.4 ± 427.0	9323.3 ± 1454.3
UCD3-40	3 ± 0	5 ± 0	133.5 ± 116.8	1015.9 ± 257.7	1365.2 ± 306.1	1340.8 ± 53.0	2016.7 ± 238.4	5872.2 ± 972.1
UCD7-159	2.3 ± 0.5	2.3 ± 0.5	39.9 ± 18.4	1416.5 ± 302.4	2246.4 ± 560.4	1780.0 ± 361.7	2657.3 ± 315.0	8140.1 ± 1557.9
UCD8-160	4.3 ± 0.5	3.3 ± 0.5	224.2 ± 88.7	2058.0 ± 412.5	2005.7 ± 360.8	1991.8 ± 179.4	2089.2 ± 349.4	8369 ± 1390.8
UCD8-201	3.3 ± 1	4.3 ± 1.5	123.0 ± 66.2	1568.5 ± 401.8	1549.4 ± 329.3	1659.5 ± 235.7	2064.2 ± 244.1	6964.6 ± 1277
UCD8-27	2.5 ± 0.6	5 ± 0	178.1 ± 106.0	907.2 ± 99.8	1600.5 ± 123.7	1062.1 ± 124.6	1489.4 ± 359.4	5237.3 ± 813.5
Winters	3.8 ± 1.3	4.3 ± 1	195.3 ± 43.2	1544.0 ± 253.9	2135.9 ± 50.6	1340.6 ± 108.9	2688.0 ± 258.8	7903.7 ± 715.5

Table 13: Analysis of variance tables showing the relationship between excised leaf desiccation (ELD) scores as well as excised shoot desiccation (ESD) scores and cumulative yield.

Analysis of Variance Table						
Response: ESD						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Cumulative_Yield	1	0.0188	0.01882	0.0387	0.8463	
Residuals	18	8.7592	0.48662			
Analysis of Variance Table						
Response: ELD						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Cumulative_Yield	1	2.0161	2.01610	2.6317	0.1221	
Residuals	18	13.7894	0.76608			

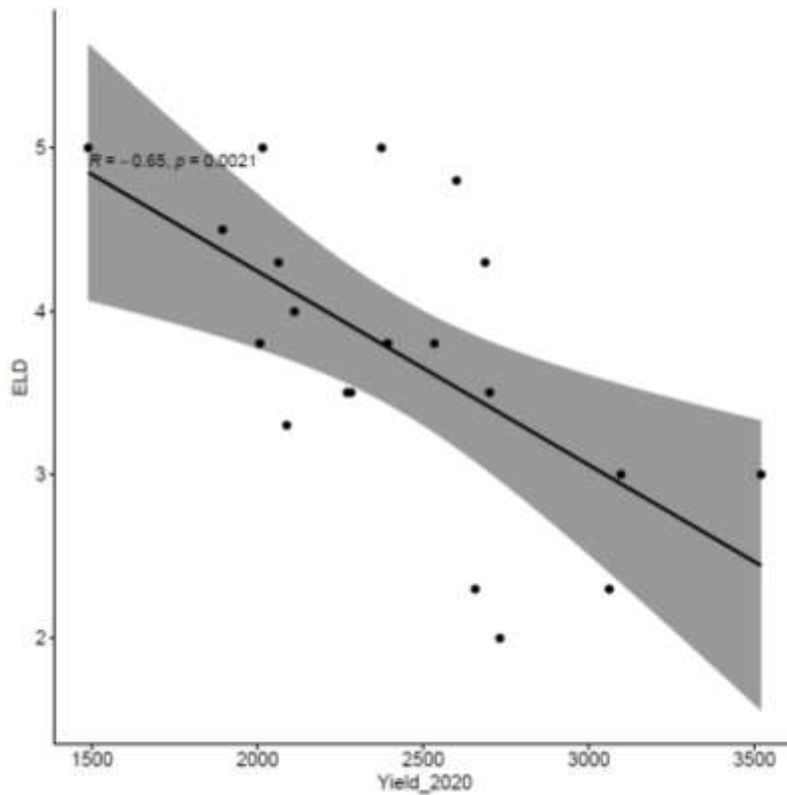


Figure 9: Spearman correlation between excised leaf desiccation (ELD) and 2020 yield (kernel lbs/ac). (R=-0.65)

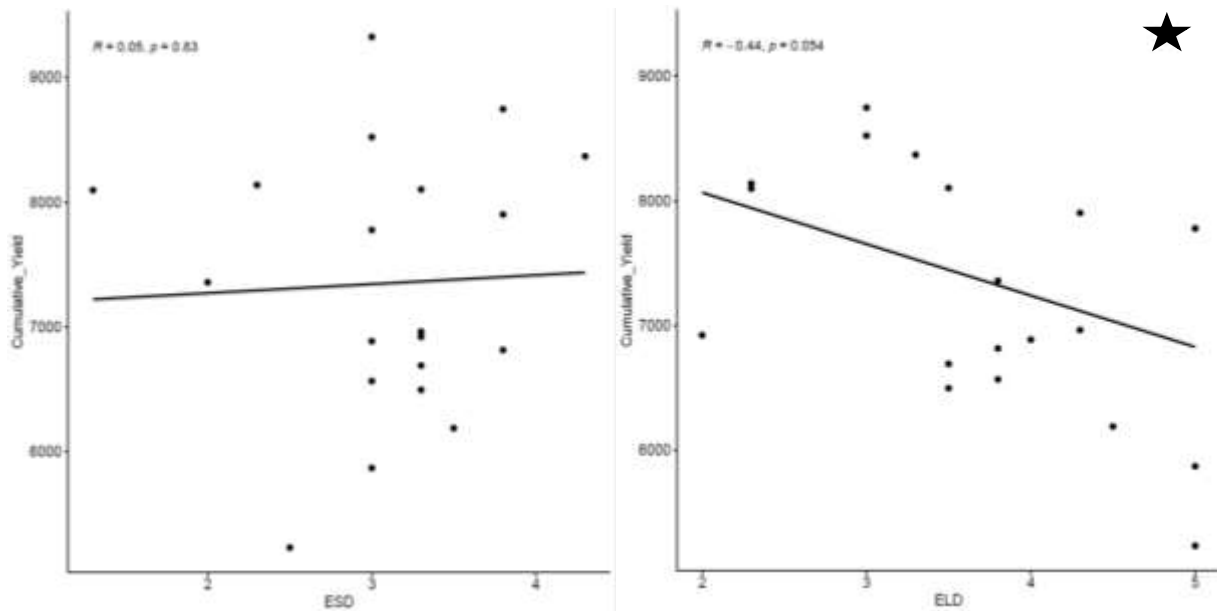


Figure 10: Spearman correlation between cumulative yield (kernel lbs/ac) from 2016 to 2020, and ELD & ESD scores. A negative correlation was observed with a Spearman correlation of -0.44 for ELD, and no correlation was observed with a Spearman correlation of 0.05 for ESD. (ELD= Excised leaf desiccation, ESD= Excised shoot desiccation) UCD 18-20 was frequently an outlier from general data trends as demonstrated with a star symbol.

CONCLUSION

The concerns related to future water availability have led to more research on how plants can successfully respond to water stress (Petit et al., 1999; Chaves et al., 2003). This study evaluated the use of excised shoot and leaf desiccation to predict stress tolerance for several diverse almond genotypes along with the relationship of this water stress response with stomata density (SD), water loss patterns, and epidermal conductance as determined under controlled environments.

Our findings show that SD by itself may not be the best parameter for evaluating plants for either desiccation tolerance or yield potential. The most significant genetic differences in rate of RWC loss occurred within the first 180 minutes when evaluated under controlled

environments. Excised leaf desiccation (ELD) was significantly associated with water loss during the first 180 minutes but not the first 60 minutes when stomata would be rapidly closing in response to water stress (Sinclair and Ludlow, 1986), suggesting that post stomata-closure responses were important determinants of rate of water loss during this time period. Two prominent responses identified in this study occurred early (0-180 minutes) and later (200-1520 minutes) and appear to represent different general pathways for leaf water loss controlled by final degree of stomata closure and general epidermis integrity, respectively. A positive relationship was also found between ELD score and epidermal conductance (EC). Genotypes that showed low rate of water loss also had the lowest EC. These findings agree with an earlier report by Muchow and Sinclair (1989) who concluded that EC is associated with plant survival under water deficit conditions and that low EC might be useful for the identification of genotypes with improved capacity to tolerate water stress.

These results only partially support our initial hypothesis that stomata play a pivotal role in the initial and rapid water loss of the plant leaf surface because no clear differences were identified during the first 60 minutes of water stress when stomata would be closing. Clear differences were identified during the first 180 minutes which is considered too late to represent differences in rates of stomata closure but too early to represent differences in water loss due to differences in general epidermis structure. Results support our modified hypothesis that the early differences in rates of water loss may be due to genetic differences in the final level or degree of stoma closure. Clear differences in both early and overall water loss patterns were observed among tested genotypes. Significantly, ELD provided an effective predictor of relative water loss vulnerability despite continuing uncertainties concerning the specific mechanisms involved.

Finally, it should be emphasized that water loss responses of excised organs such as leaves, and shoots do not necessarily represent responses of intact plants. Additionally, using ESD or ELD scores to select for tolerance to catastrophic irrigation failures does not necessarily lead to limitations in final tree productivity. While desiccation rates of excised shoots and excised leaves represent a relatively rapid test to identify inherent vulnerabilities in new as well as traditional breeding selections and cultivars, results need to be more fully assessed in both whole-plant as well as whole-orchard scenarios.

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Appendix

Appendix A: Relationship between ESD score on July 27, 2018 to final tree desiccation score on August 30, 2018 following the elimination of irrigation starting August 1, 2018 for 46 trees from a six-year-old almond seedling population, demonstrating a general increase in total tree vulnerability to wilting with increasing ESD score [Pearson correlation coefficient (R) is 0.66].

