Title

Leaf Thickness and Relative Water Content: Evaluating Water Relations in Pistacia vera Under Reduced Irrigation

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Leaf Thickness and Relative Water Content: Evaluating Water Relations in *Pistacia vera* Under Reduced Irrigation

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Abstract

Water use efficiency is of extreme importance in California agriculture, especially in woody species such as *Pistachio*, *Almond*, and *Grape*. This study evaluates the relationship of leaf relative water content (RWC) with stem water potential ($\mathbf{\Psi}_{\text{stem}}$), leaf water potential ($\mathbf{\Psi}_{\text{leaf}}$), stomatal conductance (g_{sw}), and leaf thickness under different irrigation treatments. The main objective was to evaluate the potential of using leaf thickness and relative water content as proxies for $\mathbf{\Psi}_{\text{stem}}$, $\mathbf{\Psi}_{\text{leaf}}$ or g_{sw}, for use in irrigation scheduling. The experiment was conducted in a pistachio orchard in Woodland, CA, with trees irrigated at 100% and 50% of their ETc values.

Results showed minor treatment effects on $\mathbf{\Psi}_{\text{stem}}$ and $\mathbf{\Psi}_{\text{leaf}}$ overall and on select measurement days throughout the summer. Relationships were inconsistent and weak between RWC and $\mathbf{\Psi}_{\text{stem}}$, $\mathbf{\Psi}_{\text{leaf}}$, and g_{sw}. The relationship between leaf thickness and relative water content was also inconsistent, with significance found only in the drought treatment, and in most cases leaf thickness decreased following leaf rehydration. Thus, leaf thickness measured with a micrometer with a torque clutch was found to not be a reliable proxy for relative water content across treatments; speculatively, this may have resulted from the mechanical influence of prior measurements in the same leaf spot.

Leaf relative water content provides valuable information on leaf water status, but it may not capture the water potential dynamics or stomatal behavior in *Pistachio*. Despite there being some meaningful variation in the data, most of the data were too variable to draw any conclusions regarding using RWC as a proxy for plant water status rather than $\mathbf{\Psi}_{\text{stem}}$.

Introduction

Pistachio (*Pistacia vera* L.), is a tree in the *Anacardiaceae* family. The tree is believed to have originated in modern day Iran and is widely cultivated in warm to temperate climates such as Afghanistan and California (*Pistachio | Description, Uses, & Nutrition | Britannica*, 2025). The fruits of *Pistachio* are botanically classified as drupes, similar to cashews. Leaves are large and paripinnately compound with 3-5 leaflets with a single terminal leaflet at the leaf apex, all usually 4-6 cm wide and 4-7 cm long (Parfitt et al., 2007). *Pistachio* is a dioecious species so trees have either male or female flowers, this leads to a ratio of male to female trees in commercial orchards, usually around 1:19-24, male to female (Kallsen, 2022). *Pistacia* is a xerophytic genus with many adaptations to arid climates such as extended development of mesophyll tissue, deep root growth, and isobilateral leaf anatomy (Al-Saghir et al., 2006). *Pistachio* roots are classified as a phreatophytic meaning they have extensive root systems that are always in touch with water, this adaptation allows them to survive and maintain long periods of drought (Ferguson et al., 2005).

Regulated deficit irrigation is an irrigation strategy that is used commonly in various deciduous perennial tree crops. The genus *Prunus* has some of the first examples of this strategy put into practice. Chalmers et al. (1981) was one of the first experiments suggesting that yield from *Peaches* could be maintained or even improved by withholding water at specific stages of growth. Further research on plant water status from McCutchan & Shackel (1992) showed that stem water potential ($\boldsymbol{\Psi}_{\text{stem}}$) could be used as a viable plant-based measure of water stress in *Prunus*; this study provided the groundwork for the measurement of plant water status as a means to check tree water stress and maintain yields while saving on irrigation costs. This method of measuring plant water status by stem water potential is now industry standard in most tree crops; however, $\boldsymbol{\Psi}_{\text{stem}}$ may not be the most relevant measurement of water status.

The utility of using leaf thickness as a measure of when to irrigate plants has been researched recently, and with some success. Seelig et al. (2012) made continuous leaf thickness measurements under greenhouse conditions in *Vigna unguiculata* and found that using leaf thickness as timer of when to irrigate could conserve between 25 and 45% of irrigation water compared to a normal irrigation cycle. Another study from Afzal et al. (2017) using a magnetic sensor to measure leaf thickness and infer plant water status in *Zea mays*, *Sorghum bicolor*, *Glycine max*, and *Vicia faba* found that leaf thickness was correlated with leaf water content. The piecewise model used in the Afzal et al. (2017) study used a parameter for the salt tolerance of the species which could be a useful factor when considering salt tolerant species such as *Pistachio*. Those authors also mentioned that it is possible to use leaf thickness as a proxy for relative water content, but noted that models need to be adjusted for species, leaf location, and variety (Afzal et al. 2017).

Leaf relative water content (RWC) fluctuates with leaf and stem water potentials, and evidence (discussed below) suggests that stomatal closure during water stress is driven by a sensory cascade that begins with changes in tissue water content, rather than water potential. Moreover, it may be more inexpensive and practical to measure RWC than $\boldsymbol{\Psi}_{\text{stem}}$ in orchards, given low-cost, leaf-mounted sensors of RWC and leaf thickness currently under development (Momeni et al, unpublished). To investigate the idea of using RWC and/or leaf thickness as proxies for $\boldsymbol{\Psi}_{\text{stem}}$ (and for leaf water potential [$\boldsymbol{\Psi}_{\text{leaf}}$] and stomatal conductance [g_{sw}], both of which are sensitive measures of leaf water stress), diurnal and mid-day water relations parameters were measured in a mature *Pistachio* orchard under reduced irrigation treatments in Woodland, CA from June through August 2024 with little to no rainfall. The research question that led to this experiment was,

Can leaf relative water content (RWC) be a useful measure of plant water status, as an alternative to stem water potential ($\boldsymbol{\Psi}_{\text{stem}}$), for guiding irrigation in tree crops?

If the goal of irrigation is to keep stomata open for photosynthesis, then irrigation should target the measure of water status that stomata actually respond to in drought. Evidence suggests that measure is leaf relative water content (Sack et al., 2018), not stem water potential. Therefore, I hypothesized that leaf relative water content should predict irrigation requirements at least as accurately as stem water potential. This could become a practical tool, given the current development of new sensor tools that can measure RWC in-situ. Moreover, because such sensors would also measure leaf thickness, and that measurement would likely be more precise than for RWC, there is value in testing the utility of leaf thickness for predicting irrigation requirements as well. A low-cost non-invasive sensor that measures leaf thickness and relative water content per unit bulk leaf volume would be a practical approach, enabling tree crop growers to irrigate as effectively as using stem water potential but for lower labor costs and with better temporal and spatial resolution.

Some non-destructive proximal leaf mounted sensors have been developed in recent years deploying varying technologies. LPCP or ZIM probes utilize magnets and give an estimate of leaf turgor pressure. These probes have been used successfully in a variety of crops, especially in olive where they have been validated against $\boldsymbol{\Psi}_{\text{stem}}$ as a promising tool for continuous monitoring of plant water status to improve irrigation scheduling (Marino et al., 2016, 2021). There are some problems with these sensors though, as noted by Marino et al. (2016), where the sensors could not detect mild water stress in trees as the parameter only allows for 3 distinct levels of tree water status with Stage 1 being -1.5 to -2.5 MPa. Not being able to detect water stress until a $\mathbf{\Psi}_{\text{stem}}$ of -2.5 MPa would most likely cause a decrease in g_{sw} for most species, thus decreasing photosynthesis and productivity.

Why might RWC be useful for scheduling irrigation?

Stomata are what give plants the ability to photosynthesize, transpire, and respire. This ability has an enormous impact on carbon dioxide and water exchange in earth's atmosphere. Soil drought causes stomata to reduce their apertures, presumably an adaptive response to conserve moisture; irrigation is needed to prevent excessive stomatal closure so that photosynthesis can continue. Crop productivity thus relies on stomatal responses to various factors that affect plant water status but, there is still a lack of knowledge as to how these responses function mechanically (Buckley, 2019). Water status of a given plant tissue is determined by three factors: soil water potential, transport resistance, and transpiration rate (Buckley, 2019).

The precise mechanism by which reduced water availability leads to stomatal closure remains somewhat elusive, although it is widely accepted that the biological process involves the plant hormone abscisic acid (ABA), which is synthesized in response to tissue dehydration. Earlier studies suggested that roots were the main site for initiating the signaling cascade for ABA in plants (Zhang & Davies, 1991). However, more recent findings suggest that leaves are the most likely site for ABA synthesis, with roots playing an amplifying role (Rodriguez-Dominguez et al., 2016). Supporting this conclusion, (Holbrook et al., 2002) demonstrated that

ABA-deficient tomato mutants still exhibited stomatal closure under soil drought, which would indicate that root-derived ABA is not essential. This reinforces the idea that ABA is synthesized primarily in the leaf tissue in response to drought conditions.

The aspects of leaf dehydration regulating ABA synthesis & release is still not completely understood. A study from McAdam & Brodribb (2016) proposed that decreases in leaf turgor pressure triggers ABA biosynthesis within the leaf. This led to a subsequent analysis from (Sack et al., 2018) which challenged the idea that ABA is accumulated in leaves due to a loss in leaf turgor and suggested that it is more closely tied with a reduction in relative water content (RWC) or cell volume. Overall, the evidence suggests that stomatal responses to drought are driven by active feedback mechanisms from changes in leaf tissue hydration, mediated by ABA biosynthesis within the leaf itself (Buckley, 2019).

It stands to reason that, since farmers irrigate orchards partly to keep stomata open for photosynthesis, they should choose the amount of irrigation based on what is needed to keep stomata open and keep plant stress to a minimum. Irrigation is often applied at a rate to keep stem water potential ($\mathbf{\Psi}_{\text{stem}}$) at or above some target value depending on the season, species, and rainfall (McCutchan & Shackel, 1992). Often, stem and leaf water potentials have been measured using a Scholander Pressure Chamber (Scholander et al., 1965; Tyree & Hammel, 1972). Osmotic adjustment, which is a well understood plant physiological mechanism to maintain turgor pressure by the influx of solutes into cells to maintain water potentials during drought conditions (Blum, 2017; Sanders & Arndt, 2012), can disrupt the connection between leaf water potential and leaf relative water content (Turner et al., 1978). However, given that stomata apparently sense water content rather than water potential (Sack et al., 2018), as discussed above, and that osmotic adjustment tends to help sustain stomatal opening during drought (Turner et al., 1978), the relationship between stomatal conductance and water content should be more stable than that between stomatal conductance and leaf water potential, if osmotic adjustment is occurring.

If relative water content and stem water potential were to be decoupled, there are two possible applied predictions. One would be that, if keeping stomata open depends upon sustaining relative water content and not stem water potential, then you might irrigate more than necessary if you based irrigation on stem water potential rather than water content. The second would be that, if stomata close very sensitively in response to small fluctuations in relative water content, then RWC might be highly conserved, which would mean relative water content might *not* be a useful measurement for irrigation. The evidence for these predictions is mixed and somewhat contradictory, perhaps due to differences in stomatal and hydraulic behavior between environments and species such as annual herbaceous crops and perennial woody crops. For example, osmotic adjustment keeps stomata open in some cases, such as in experiments by Turner et al. (1978) which measured sunflower and sorghum in irrigated and drought conditions; this supports the first conclusion above. However, another similar experiment with sorghum grown in harsh conditions by Flower et al. (1990) suggests the opposite: osmotic adjustment did *not* keep stomata open, and g_{sw} was poorly related to RWC.

Other evidence points to a strong negative feedback regulation of relative water content during soil drying. Bennett et al. (1987) found that relative water content was unrelated to the soil drying treatment except in the most extreme case, suggesting the stomata closed to keep relative water content from changing measurably. Additionally, a drought experiment on *Pistachio* cultivars from Esmaeilpour et al. (2016) found that relative water content was unaffected by soil drought, with differences between cultivars, and increases in water use efficiency in cultivars under osmotic stress. However, the opposite was found in experiments involving regulated deficit-irrigated Almond by Romero and Botía (2006), in which relative water content was lower in July (during periods of water stress) than in May (before stress), with minimum daily RWC being closely related to midday g_{sw}. The discrepancies between these contradictory lines of evidence lead to several questions:

1) *In California tree crops such as pistachio, how are stem water potential, leaf relative water content, and stomatal conductance related to each other, both in relation to sustained differences in water status caused by differences in irrigation, and in relation to fluctuations in plant water status caused by environmental conditions?*

2) *Does osmotic adjustment (increase in osmotic pressure, measured at full turgor) occur during soil drought in Pistachio, do stem water potential and leaf relative water content diverge during drought as a consequence, and is stomatal conductance better correlated with water potential or with water content?*

3) *Which measure – stem water potential or relative water content – is a better proxy for the degree of plant water stress, defined as suppression of stomatal conductance during mild to moderate soil drought?*

4) *If relative water content is a potentially viable substitute for stem water potential, what are the measurement requirements to make this possible? For example, how large of a sample size is needed, how precise of a measurement of relative water content is needed, and can leaf thickness be used as a high-throughput proxy for water content?*

In this study, I set out to address these questions by measuring RWC and leaf thickness in relation to $\mathbf{\Psi}_{stem}$, $\mathbf{\Psi}_{leaf}$, and stomatal conductance, in a pistachio orchard with fully irrigated and 50% deficit irrigated treatments.

Methods & Materials

Experimental Site. An experiment using different irrigation treatments was managed in a commercial Pistachio orchard of 'Golden Hills' grafted on UCB1' seedling rootstock in Woodland, CA on a deep, well drained silty clay loam soil 152 cm deep (Soil Web). Three randomly selected rows were used in the orchard site and trees were spaced 5 m apart in each row and 6m between rows (5 m x 6 m) m apart. The experimental site was irrigated with two, side-by-side drip lines with 1.89 liter/hr in-line drippers spaced 40 cm apart along each line down the row. The coordinates for the site are 38° 37'35.1" N, 121° 43'33.1" W with a very dry climate from June-August having an average high temperature of 33°C and an average precipitation of 2.1 mm. During the winter months (October-April) the area receives about 70 mm of precipitation on average each month.

Experimental Design & Plots. For this experiment, there were three irrigation treatments that were replicated four times using a randomized complete block design utilizing three orchard rows with 68 trees total in each row and each row spaced x-1-x-x-2-x-x-x-x-3-x (x=border row, number=experimental row). Male pollenizer trees were interspersed throughout the orchard site at a ratio of ~1:20. The experimental rows were split into four blocks horizontally, each block had 1 treatment randomly assigned for each of the 3 experimental rows, for a total of 3 plots in each block and 12 plots total, representing one of three treatments. In each plot there were 17 trees but only two adjacent, randomly selected, female, healthy trees per plot were measured on measurement days.

<u>*Irrigation Treatments.*</u> A homogenous irrigation treatment was applied to match the site's crop ET_c values from 04/05/2024 to 05/30/2024. Control and deficit irrigation treatments started on 05/31/2024 and K_c values for water application were calculated from Goldhammer (2005). Irrigation was performed based on estimated ET_c values using local CIMIS data.
The irrigation treatments given throughout the experiment are listed below:

- Control: irrigation was applied at 100% of ETc values.
- Reduced Irrigation: irrigation was applied at 50% of ETc values.

Mid-day Measurement Days. Mid-day leaf sample collection occurred on eight separate occasions from June 18th, 2024 - August 28th, 2024. On each mid-day sample collection day, the same two trees were sampled per plot with a total of 48 g_{sw} measurements, 24 stem-water potential samples, 24 leaf-water potential samples, and 24 RWC samples (2 measurements of $\boldsymbol{\Psi}_{\text{stem}}$, $\boldsymbol{\Psi}_{\text{leaf}}$ and RWC, and 4 measurements of g_{sw}, per tree). Measurement variables g_{sw}, $\boldsymbol{\Psi}_{\text{stem}}$, $\boldsymbol{\Psi}_{\text{leaf}}$, and RWC were all measured within the same tree; for each measurement of $\boldsymbol{\Psi}_{\text{leaf}}$, g_{sw} was measured in the sample leaf before harvest for $\boldsymbol{\Psi}_{\text{leaf}}$ measurement (and likewise for leaves sampled for RWC).

Diurnal Measurement Days. Diurnal measurement days occurred on July 9th & 16th, 2024. Three separate time points were measured; pre-dawn, mid-morning (10-11 am), & late afternoon (3-4 pm). During pre-dawn measurements, only g_{sw} and $\boldsymbol{\Psi}_{\text{leaf}}$ were measured. For the mid-morning and late-afternoon samplings, all response variables were measured with the same protocol as the mid-day measurements.

Repeated Leaf Thickness and g_{sw} in the same leaves. In addition to mid-day & diurnal measurements, paired measurements of g_{sw} and leaf thickness were taken at mid-day once a week for four weeks. One leaf for every tree was tagged and marked with a permanent marker to make repeated measurements of g_{sw} and thickness on the same spot of the marked leaf.

Measurements. Climate data was taken from CIMIS station #6 in Davis, CA, which is 11 km from the experimental site. This data was used to gather ET_o values to estimate ET_c for irrigation requirements. Average daily values of VPD, ET_o, wind speed, and max air temperature were recorded (Fig. 1).

Abaxial stomatal conductance (g_{sw}) was measured with a LI-600 porometer (LI-COR Inc., Lincoln, NE).

Stem water potential ($\mathbf{\Psi}_{\text{stem}}$) was sampled and measured each measurement day by placing large Whirl-Pak bags covered in aluminum foil on fully expanded healthy leaves closest to the base of each subsample (Tree) within each plot (Treatment: Block) and allowing them to equilibrate for 30 minutes. Once the leaves equilibrated, they were quickly excised with shears at the base of the petiole, the cut petiole was enclosed in the bag delicately to minimize damage, the bag was rolled up quickly and placed in another zip-loc bag with a wet paper towel inside and finally placed in a dark cooler on ice. $\mathbf{\Psi}_{\text{stem}}$ was then measured in the lab on the same day with a Scholander pressure chamber.

Leaf water potential ($\mathbf{\Psi}_{\text{leaf}}$) was measured each measurement day by first tagging a healthy fully expanded leaf in the sun, taking g_{sw} measurements of that leaf, then excising the leaf with shears at the base of the petiole; subsequent procedures were identical to those for $\mathbf{\Psi}_{\text{stem}}$ measurement.

Relative Water Content (RWC) & Thickness of Leaf, Before & After Rehydration (t_{after} & t_{before}) were measured each measurement day by first tagging a healthy fully expanded leaf in the sun, taking g_{sw} measurements of that leaf, excising the leaf with shears at the base of the petiole, and then bagging the leaf as described earlier for $\mathbf{\Psi}_{\text{stem}}$ and $\mathbf{\Psi}_{\text{leaf}}$ measurements. To measure RWC, fresh weight (FW) was measured using a 0.01-mg balance (model #Mettler-Toledo), and leaves were then rehydrated by recutting the petiole under water and leaving the petiole in deionized water for 2 hours, before measuring saturated weight (SW). Finally, leaves were dried in a drying oven at 90°C for 24 hours before measuring dry weight (DW). The petiole fragment created by recutting under water for rehydration was also allowed to rehydrate, and its saturated and dried weight were added to those of the bulk leaf. Leaf thickness was also measured prior to rehydration. RWC, relative water loss (RWL), and relative Thickness Loss (RTL) were calculated using Eqns. 1-3, respectively (t_{aft} and t_{bef} in Eqn. 3 are leaf thickness after and before rehydration, respectively).

$$Eq.1\text{: RWC \%} = \left(\frac{\text{FW} - \text{DW}}{\text{SW} - \text{DW}}\right) * 100$$

$$Eq.2\text{: RWL} = (100 - \text{RWC})$$

$$Eq.3\text{: RTL} = \left(\frac{t_{aft} - t_{bef}}{t_{aft}}\right) * 100$$

<u>Statistical Analysis.</u> Linear mixed models and treatment vs. control comparisons were used to investigate treatment differences and significance of variation in water status variables (RStudio,

2024.12.0+467). Treatment and response variable differences were considered significant at $P<0.05$.

Results

Environmental conditions. Environmental conditions during the experiment, measured by CA CIMIS Station 6, are shown in Figure 1. Wind speed was typically between 2 and 4 m/s, daily maximum air temperature ranged from 30-41 ºC, daily mean VPD ranged from 0.9 to 1.7 kPa (> 1.25 kPa after the first measurement day, 6/18/2024), and the reference evapotranspiration rate ranged between 6.25 and 9.5 mm/day.

Differences in water status parameters between treatments. I found negligible differences in mid-day $\mathbf{\Psi}_{\text{stem}}$, $\mathbf{\Psi}_{\text{leaf}}$, RWC or g_{sw} between irrigation treatments. Figure 2 shows the overall distribution of each variable for all measurement days combined, but grouped within treatments. In the control treatment (green in Figure 2), $\mathbf{\Psi}_{\text{stem}}$ was -1.43 ± 0.03 MPa (mean ± SE), $\mathbf{\Psi}_{\text{leaf}}$ was -2.12 ± 0.03 MPa, g_{sw} was 0.218 ± 0.007 mol m^{-2} s^{-1}, and RWC was 91.6 ± 0.2 %. In the 50% irrigation deficit treatment (pink in Figure 2), $\mathbf{\Psi}_{\text{stem}}$ was -1.59 ± 0.04 MPa (mean ± SE), $\mathbf{\Psi}_{\text{leaf}}$ was -2.25 ± 0.03 MPa, g_{sw} was 0.215 ± 0.01 mol m^{-2} s^{-1}, and RWC was 91.1 ± 0.4 %. These treatment differences were significant for $\mathbf{\Psi}_{\text{stem}}$ ($p = 0.0129$) and $\mathbf{\Psi}_{\text{leaf}}$ ($p = 0.0274$), but not for either RWC or g_{sw}.

Mean $\mathbf{\Psi}_{\text{stem}}$ and $\mathbf{\Psi}_{\text{leaf}}$ were also lower in the deficit irrigation treatment on most individual days, particularly near the end of the study (Fig 3c, d). However, neither mean g_{sw} nor mean RWC were consistently lower in the reduced-irrigation treatment, let alone significantly so (Fig 3a, b).

Relationships between water status parameters. None of the variables were correlated significantly with one another as environmental conditions fluctuated either within given days or among days (Figure 4). Considering variation among trees and plots on specific days, correlations came close to significance on a few specific days (solid-colored lines in Figure 4; e.g., $\mathbf{\Psi}_{\text{stem}}$ vs RWC on 7-24, p = 0.070; $\mathbf{\Psi}_{\text{leaf}}$ vs RWC on 7-02, p = 0.096; g_{sw} vs RWC on 7-23, p = 0.069).

Effect of RWC sample size. Given that mean RWC was nearly identical between irrigation treatments, it is unlikely that increasing sample size (from 12 leaves total per treatment, nested within trees [n=2 per plot] and plots [n=3 per treatment]) would have enabled detection of significant differences.

Changes in leaf thickness during rehydration. Although rehydration was expected to increase leaf thickness, leaf thickness almost always decreased in response to laboratory rehydration, and as a result, relative thickness loss (100*[rehydrated thickness - initial thickness]/[rehydrated thickness]) was typically negative (Figure 5). To test whether repeated measurements with the digital micrometer would cause systematic and artefactual decreases in apparent leaf thickness, I measured the same spot on several leaves left intact in the field, weekly, but found no systematic change in thickness (Figure 6).

Leaf thickness as a proxy for RWC. RTL and RWL were not consistently related overall, either between treatments or over measurement days, although a linear mixed-effects model did detect a marginally significant interaction between RTL and irrigation (p=0.016), such that RTL and RWC were significantly correlated within the deficit irrigation treatment, with a slope of approximately 0.10 (i.e., a difference of 10% in RTL corresponds to a difference of 1% in RWL)

(Fig 5a). This indicates that within deficit-irrigated trees, changes in leaf thickness could serve as a rough indicator of leaf water loss.

Discussion

This experiment delved into whether leaf relative water content (RWC) could be a reliable proxy for water status in *Pistachio*, specifically by comparing RWC with $\mathbf{\Psi}_{\text{stem}}$, $\mathbf{\Psi}_{\text{leaf}}$, and g_{sw} over two irrigation treatments. Overall, the experiment did not find any consistent significant relationships between response variables, within or across measurement days, to provide evidence for using leaf thickness and RWC as a proxy for plant water status. Although we did see an overall significant difference in $\mathbf{\Psi}_{\text{stem}}$ and $\mathbf{\Psi}_{\text{leaf}}$ between treatments (Figure 2), this difference was minimal considering irrigation was reduced by 50% in the reduced irrigation treatment, and it was negligible during the first several weeks of the study.

Across measurement days, RWC did not consistently correlate with $\mathbf{\Psi}_{\text{stem}}$, $\mathbf{\Psi}_{\text{leaf}}$, or g_{sw}, which highlights the variability in how water content converts to physiological function in plants across field conditions (Figure 4). RWC was correlated with g_{sw} on 7-23 but not on any other day. However, g_{sw} was correlated with RWC, $\mathbf{\Psi}_{\text{stem}}$ and $\mathbf{\Psi}_{\text{leaf}}$ *within* individual days, indicating very weak control of g_{sw} by leaf water status (Figure 4). The data clearly indicates that RWC does not appear to be a useful proxy for $\mathbf{\Psi}_{\text{stem}}$, $\mathbf{\Psi}_{\text{leaf}}$ or g_{sw} in *Pistachio* when comparing different trees under different irrigation conditions.

The results of the analysis are also consistent with osmotic adjustment, with water potentials decreasing in drought as the season went on but with RWC staying within a range of around 90% (Figure 3). However, the variation in each of these measures was so large that I cannot conclude with any confidence that osmotic adjustment actually occurred.

Curiously, and contrary to my expectations, leaf thickness almost always decreased after rehydration in individual leaves; I had expected the increase in leaf water content caused by re-hydration to result in the leaf becoming thicker, not thinner. The mechanism of this phenomenon is unclear. It could be a real effect resulting from some previously unknown biological or biomechanical features of *Pistachio* leaves. Alternatively, it could be an artefact of the measurement procedure, possibly caused by the leaf having been previously compressed by the digital micrometer in the same spot where it was later re-measured. To test whether repeated measurement using the digital micrometer might somehow weaken a leaf in that spot, causing subsequent measurements to underestimate the true leaf thickness due to reduced reaction force against the micrometer's torque clutch, I measured leaf thickness in the same spot weekly, for numerous individual leaves that were left intact on the tree in the field, but found no consistent directional trend in leaf thickness over time (Fig 6). This suggests that, if the apparent decrease in leaf thickness during rehydration is an artefact, it may only occur when leaf water content and thickness are in the high ranges associated with rehydration, and perhaps only during laboratory rehydration. I did not conduct repeated in situ leaf thickness measurements at pre-dawn, which is the only time of day when water content would be expected to be in the range associated with laboratory rehydration.

I also suspected that the magnitude of leaf thickness decrease caused by rehydration (which manifests as unexpectedly negative values of relative thickness loss (RTL)) might be related in some way to conditions of leaves in the field. For example, perhaps leaves that were more water stressed would be more prone to this phenomenon. However, the relationship between relative thickness loss and relative water loss was generally weak, and was only significant in the 50% irrigated trees, where it had a positive slope (Figure 5); that is, leaves that were more well hydrated at the time of collection (higher RWC or RWL closer to zero) tended to experience a larger decrease in thickness in response to rehydration. Quantitatively, a 1% decrease in RWL (=1% increase in initial RWC) translated into a roughly 10% decrease in RWL

(10% more thickness loss during rehydration). However, given that the range of both RWL and RTL were similar between the irrigation treatments, but the two variables were only correlated in the reduced irrigation treatment, it is difficult to conclude anything from these data about the reason for leaf thickness decreasing during rehydration.

While this study found no consistent relationship between leaf thickness and water status in *Pistachio*, other studies utilizing leaf thickness sensors and pressure probes have shown promising results in other crops (Afzal, Duiker, & John, 2017; Afzal, Duiker, & Watson, 2017; Marino et al., 2016; Seelig et al., 2012). The findings in these studies suggest that leaf thickness monitoring can be a valuable tool for irrigation scheduling in crops where leaf thickness changes are more responsive to water status. The lack of responsiveness in *Pistachio* could be attributed to its inherent physiology and morphology or experimental and measurement error.

Ecophysiological interpretation of observed patterns. *Pistachio* has evidence of exhibiting anisohydric behavior which could have altered the effectiveness of the treatments on each water status variable. A study on optimizing *Pistachio* irrigation intervals from Sedaghati & Hokmabadi (2015) showed that there was no significant difference in RWC between treatments of 30 days between irrigations and 110 days between irrigations. The same experiment from them also showed that the trees maintained high photosynthetic activity for all drought treatments. Another study from Osku et al. (2025) have results that also suggest that *Pistachio* is resilient to drought and that its tolerance may be due to something other than stomatal regulation. Our results and other experimental results suggest *Pistachio* is doing something physiologically such as osmotic adjustment to prevent RWC from fluctuating in mild to severe water stress. Another experiment done by (Goldhamer et al., 1985) where water use in a *Pistachio* orchard was measured to a soil depth of 20 ft in plots found that the trees used water throughout the whole profile as well as, significant water in the 17-20 ft range suggesting water was depleted below 20ft. Much of this evidence leads me to question whether the irrigation treatments in this experiment were drastic enough to cause any meaningful variation between them. Without a large treatment effect on soil water availability, any physiological differences could easily be swamped by the many sources of variability, including measurement error, anatomical variation, and/or the inherent biological nature of water relations and anatomy in this species. The studies mentioned above highlight the drought tolerant capabilities of *Pistachio*; the combination of anisohydric behavior, phreatophytic root systems, and xerophytic palisade mesophyll leaf morphology could have contributed to the limited meaningful differences in water status parameters between the control and reduced irrigation treatments.

Implications of diurnal patterns for experimental adequacy. It is worth noting that, despite the little effect of the irrigation treatment on $\boldsymbol{\Psi}_{\text{stem}}$ and $\boldsymbol{\Psi}_{\text{leaf}}$ and no effect on RWC and g_{sw}, these variables all changed dramatically during each day, with $\boldsymbol{\Psi}_{\text{stem}}$, $\boldsymbol{\Psi}_{\text{leaf}}$ and RWC decreasing from mid-morning to late afternoon, while g_{sw} increased (Figure 7). This indicates that the increasing transpiration rate (caused by stomatal opening in morning and high VPD in afternoon) led to large changes in leaf water status, as one would expect. This suggests that in-situ RWC sensors might be useful for monitoring *diurnal* changes in water status in a research context. Unfortunately, the background variation in the absolute values of these parameters among leaves was so large that RWC by itself is probably not useful for detecting differences in the degree of water stress among trees in relation to soil moisture, with anywhere near the sensitivity that would be needed for irrigation scheduling, at least not in *Pistachio*.

Conclusion

Together, the findings emphasize the challenge of using volumetric based metrics like RWC to infer water potentials or stomatal behavior under field conditions. Although RWC captures important leaf physiological water status/tissue hydration it may not fully reflect water status in the way $\mathbf{\Psi}_{\text{stem}}$ can provide for growers. Future research should focus on different species as well as pairing RWC with measurements of osmotic potential to better understand how the leaf is physiologically and mechanically adjusting to evaporative demand under periods of reduced irrigation.

References

Afzal, A., Duiker, S., & John, W. (2017). Leaf Thickness and Electrical Capacitance as Measures of Plant Water Status. *Transactions of the ASABE*, *60*(4), 1063–1074.

Afzal, A., Duiker, S. W., & Watson, J. E. (2017). Leaf thickness to predict plant water status. *Biosystems Engineering*, *156*, 148–156. https://doi.org/10.1016/j.biosystemseng.2017.01.011

Al-Saghir, Mohannad, G., Porter, D. M., & Nilsen, ErikT. (2006). Leaf anatomy of Pistacia species (Anacardiaceae). *Journal of Biological Sciences*, *6*(2), 242–244.

Bennett, J. M., Sinclair, T. R., Muchow, R. C., & Costello, S. R. (1987). Dependence of Stomatal Conductance on leaf Water Potential, Turgor Potential, and Relative Water Content in Field-Grown Soybean and Maize1. *Crop Science*, *27*(5), cropsci1987.0011183X002700050033x. https://doi.org/10.2135/cropsci1987.0011183X002700050033x

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment*, *40*(1), 4–10. https://doi.org/10.1111/pce.12800

Buckley, T. N. (2019). How do stomata respond to water status? *New Phytologist*, *224*(1), 21–36. https://doi.org/10.1111/nph.15899

Chalmers, D. J., Mitchell, P. D., & Heek, L. van. (1981). *Control of Peach Tree Growth and Productivity by Regulated Water Supply, Tree Density, and Summer Pruning1*. https://doi.org/10.21273/JASHS.106.3.307

Esmaeilpour, A., Van Labeke, M.-C., Samson, R., Boeckx, P., & Van Damme, P. (2016). Variation in biochemical characteristics, water status, stomata features, leaf carbon isotope composition and its relationship to water use efficiency in pistachio (*Pistacia vera* L.) cultivars under drought stress condition. *Scientia Horticulturae*, *211*, 158–166. https://doi.org/10.1016/j.scienta.2016.08.026

Ferguson, L., Polito, V., & Kallsen, C. (2005). The pistachio tree; botany and physiology and factors that affect yield. In *Pistachio production manual: Vol. 4th ed. Davis, CA, USA, University of*

California Fruit&Nut Research Information Center (pp. 31–39).

Flower, D. J., Rani, A. U., & Peacock, J. M. (1990). Influence of Osmotic Adjustment on the Growth, Stomatal Conductance and Light Interception of Contrasting Sorghum Lines in a Harsh Environment. *Functional Plant Biology*, *17*(1), 91–105. https://doi.org/10.1071/pp9900091

Goldhamer, D. A., Kjelgren, R. K., Beede, R. H., Williams, L. E., Moore, J. M., Lane, J., Weinberger, G., & Menezes, J. (1985). *Water use requirements of pistachio trees and response to water stress* (Annual Report of the California Pistachio Commission, pp. 85–92) [Annual Report of the California Pistachio Commission]. [object Object]. https://calisphere.org/item/5abc47a2-fb4b-4c7b-b5d6-76ef35dee750

Goldhammer, D. A. (2005). Tree water requirements & regulated deficit irrigation. In *Pistachio production manual* (Vol. 4, pp. 103–116).

Holbrook, N. M., Shashidhar, V. R., James, R. A., & Munns, R. (2002). Stomatal control in tomato with ABA-deficient roots: Response of grafted plants to soil drying. *Journal of Experimental Botany*, *53*(373), 1503–1514. https://doi.org/10.1093/jexbot/53.373.1503

Kallsen, C. E. (2022). Pistachio Climate & Cultivars | Fruit & Nut Research & Information Center. In *Pistachio Production Manual* (5th ed.). https://fruitsandnuts.ucdavis.edu/climate-cultivars

Marino, G., Pernice, F., Marra, F. P., & Caruso, T. (2016). Validation of an online system for the continuous monitoring of tree water status for sustainable irrigation managements in olive (*Olea europaea* L.). *Agricultural Water Management*, *177*, 298–307. https://doi.org/10.1016/j.agwat.2016.08.010

Marino, G., Scalisi, A., Guzmán-Delgado, P., Caruso, T., Marra, F. P., & Lo Bianco, R. (2021). Detecting Mild Water Stress in Olive with Multiple Plant-Based Continuous Sensors. *Plants*, *10*(1), Article 1. https://doi.org/10.3390/plants10010131

McAdam, S. A. M., & Brodribb, T. J. (2016). Linking Turgor with ABA Biosynthesis: Implications for Stomatal Responses to Vapor Pressure Deficit across Land Plants. *Plant Physiology*, *171*(3), 2008–2016. https://doi.org/10.1104/pp.16.00380

McCutchan, H., & Shackel, K. A. (1992). Stem-water Potential as a Sensitive Indicator of Water Stress in Prune Trees (Prunus domestica L. cv. French). *Journal of the American Society for Horticultural Science*, *117*(4), 607–611. https://doi.org/10.21273/JASHS.117.4.607

Osku, M., Roozban, M. R., Sarikhani, S., Arab, M. M., Akbari, M., & Vahdati, K. (2025). *Revealing Drought Tolerance Strategies in Pistachio Clonal Hybrids: Role of Osmotic Adjustment*. Research Square. https://doi.org/10.21203/rs.3.rs-5905176/v1

Parfitt, D. E., Kallsen, C., Maranto, J., & Holtz, B. (2007). 'Golden Hills' Pistachio. *HortScience*, *42*(3), 694–696. https://doi.org/10.21273/HORTSCI.42.3.694

Pistachio | Description, Uses, & Nutrition | Britannica. (2025, January 18). https://www.britannica.com/plant/pistachio-plant

Rodriguez-Dominguez, C. M., Buckley, T. N., Egea, G., de Cires, A., Hernandez-Santana, V., Martorell, S., & Diaz-Espejo, A. (2016). Most stomatal closure in woody species under moderate drought can be explained by stomatal responses to leaf turgor. *Plant, Cell & Environment*, *39*(9), 2014–2026. https://doi.org/10.1111/pce.12774

Romero, P., & Botía, P. (2006). Daily and seasonal patterns of leaf water relations and gas exchange of regulated deficit-irrigated almond trees under semiarid conditions. *Environmental and Experimental Botany*, *56*(2), 158–173. https://doi.org/10.1016/j.envexpbot.2005.01.012

Sack, L., John, G. P., & Buckley, T. N. (2018). ABA Accumulation in Dehydrating Leaves Is Associated with Decline in Cell Volume, Not Turgor Pressure. *Plant Physiology*, *176*(1), 489–495. https://doi.org/10.1104/pp.17.01097

Sanders, G. J., & Arndt, S. K. (2012). Osmotic Adjustment Under Drought Conditions. In R. Aroca (Ed.), *Plant Responses to Drought Stress: From Morphological to Molecular Features* (pp. 199–229). Springer. https://doi.org/10.1007/978-3-642-32653-0_8

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., & Hammel, H. T. (1965). Sap Pressure in Vascular Plants. *Science*, *148*(3668), 339–346. https://doi.org/10.1126/science.148.3668.339

Sedaghati, N., & Hokmabadi, H. (2015). Optimizing Pistachio Irrigation Management Using the

Relationship between Echo-physiological Characteristics and Water Stress. *Journal of Agricultural Science and Technology*, *17*. https://dorl.net/dor/20.1001.1.16807073.2015.17.1.8.9

Seelig, H.-D., Stoner, R. J., & Linden, J. C. (2012). Irrigation control of cowpea plants using the measurement of leaf thickness under greenhouse conditions. *Irrigation Science*, *30*(4), 247–257. https://doi.org/10.1007/s00271-011-0268-2

Turner, N. C., Begg, J. E., & Tonnet, M. L. (1978). Osmotic Adjustment of Sorghum and Sunflower Crops in Response to Water Deficits and Its Influence on the Water Potential at Which Stomata Close. *Functional Plant Biology*, *5*(5), 597–608. https://doi.org/10.1071/pp9780597

Tyree, M. T., & Hammel, H. T. (1972). The Measurement of the Turgor Pressure and the Water Relations of Plants by the Pressure-bomb Technique. *Journal of Experimental Botany*, *23*(1), 267–282. https://doi.org/10.1093/jxb/23.1.267

Zhang, J., & Davies, W. J. (1991). Antitranspirant Activity in Xylem Sap of Maize Plants. *Journal of Experimental Botany*, *42*(3), 317–321. https://doi.org/10.1093/jxb/42.3.317

Figures

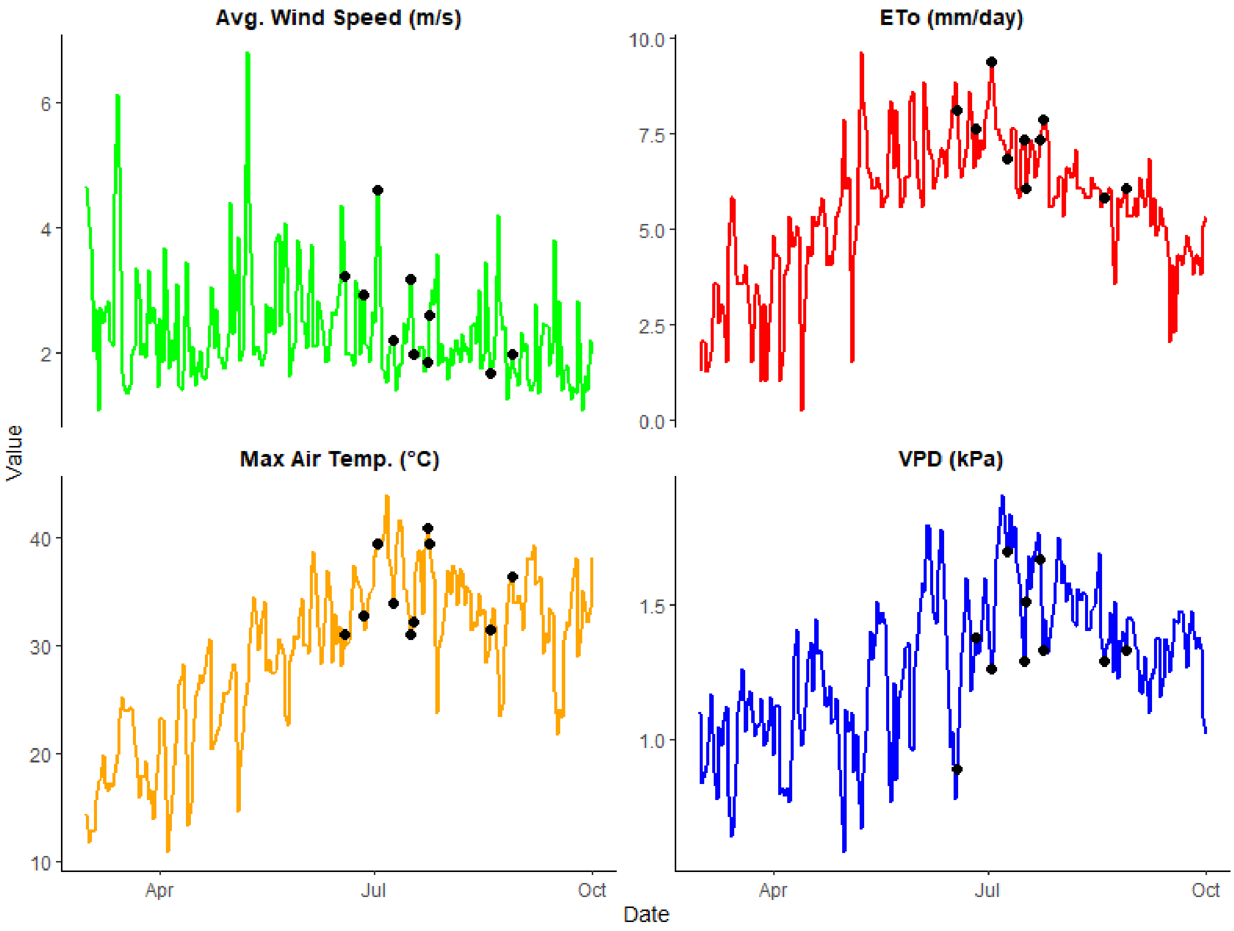

Figure 1. Environmental conditions measured at a CIMIS station 11 km from the research site. Black dots represent measurement days.

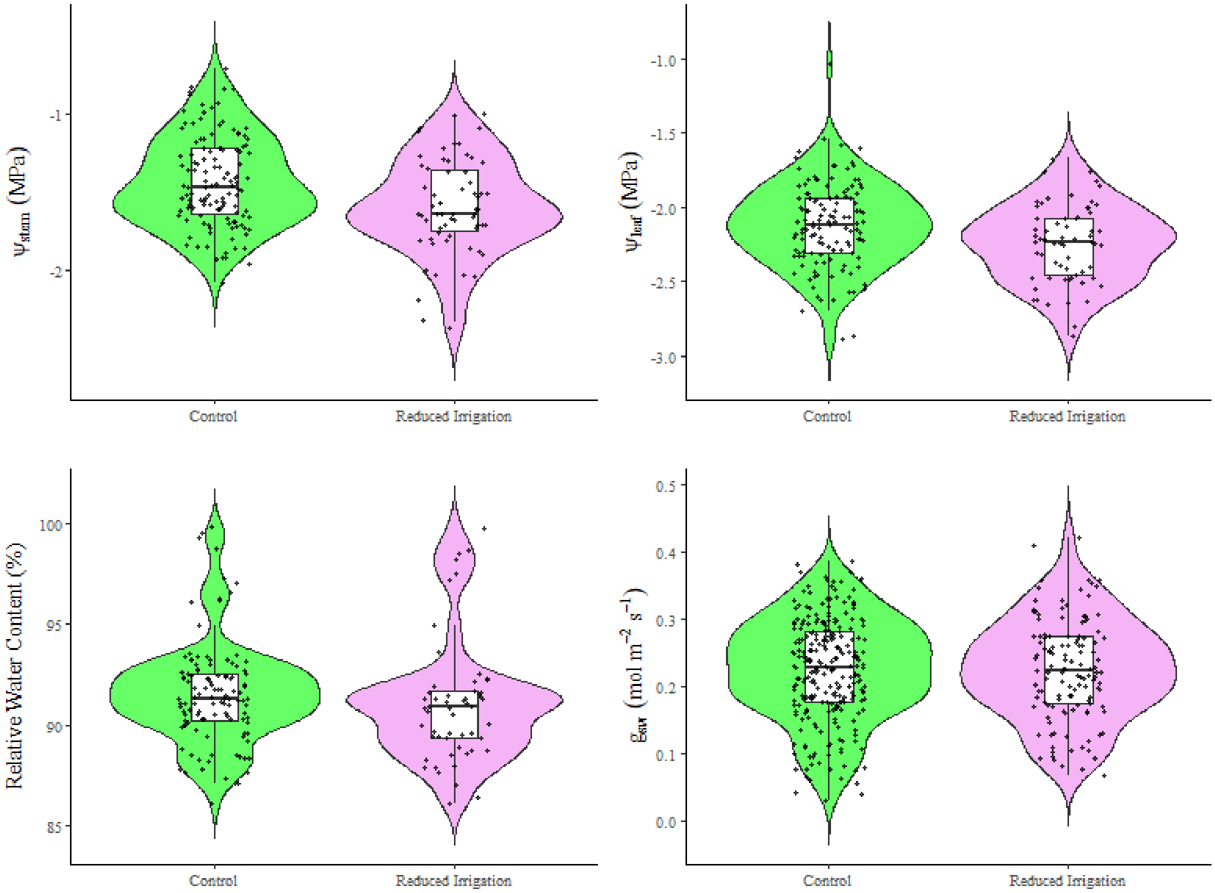

Figure 2. Distributions of stem and leaf water potential, leaf relative water content, and stomatal conductance in the two irrigation treatments.

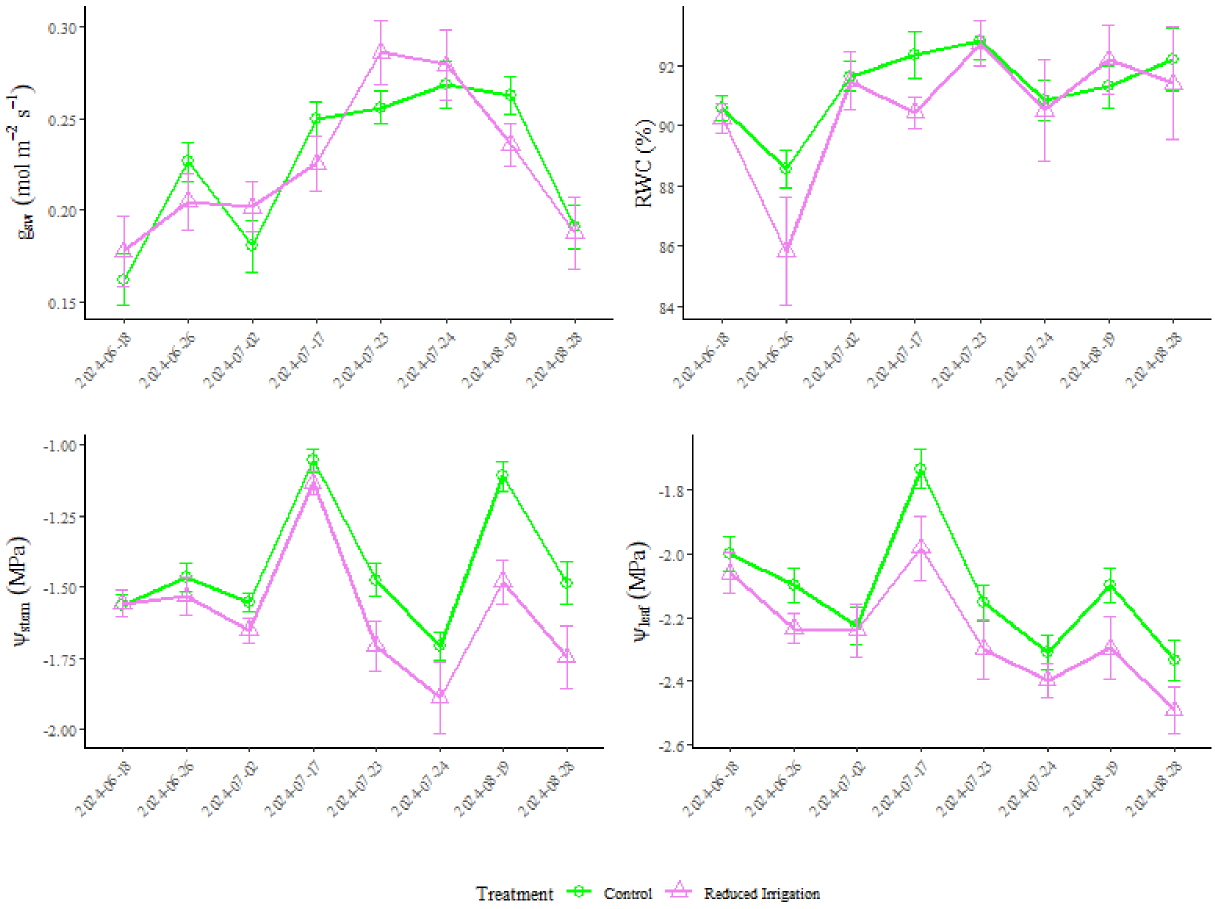

Figure 3. Variation across measurement days and between treatments (green-control; purple=deficit irrigated) in stomatal conductance, leaf relative water content, and leaf and stem water potentials.

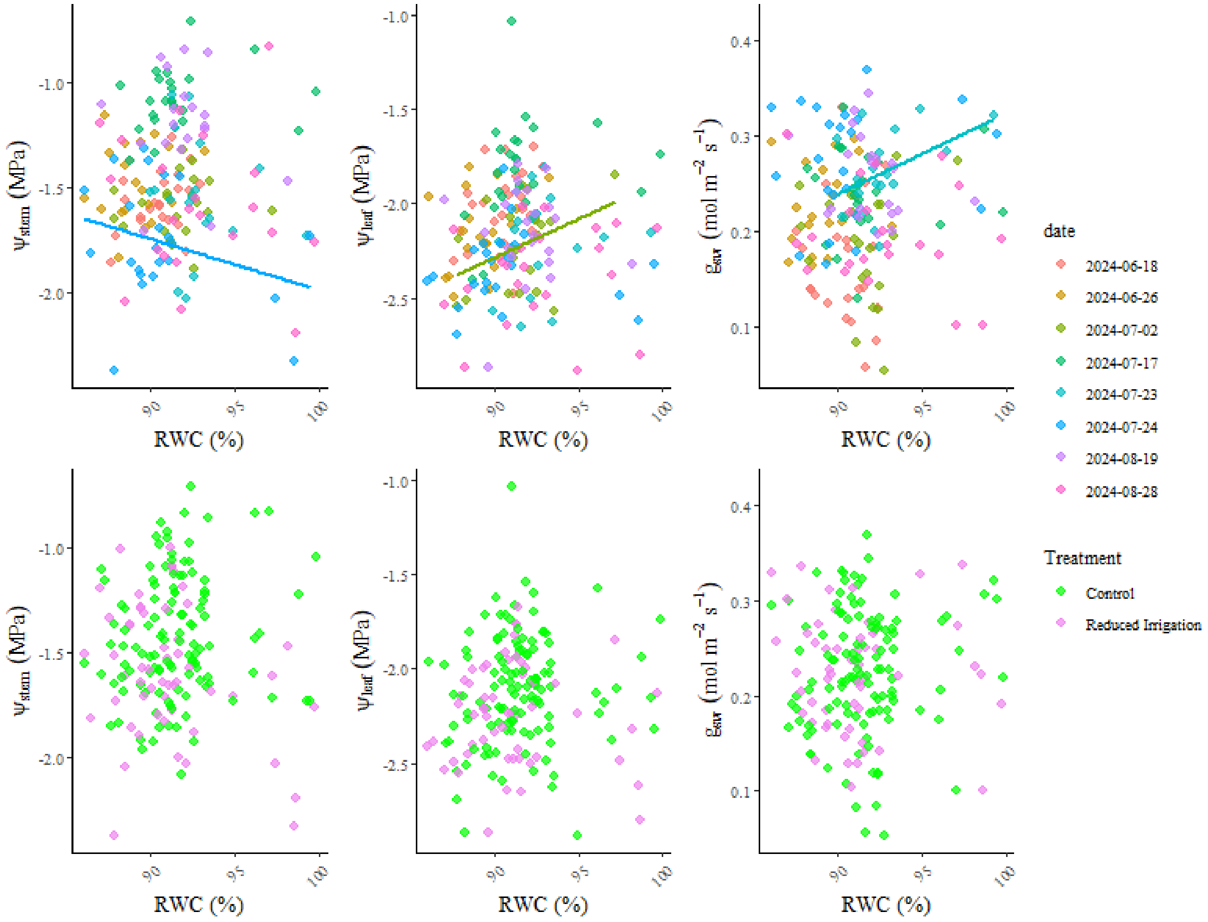

Figure 4. Relationships between leaf relative water content (RWC, x axes in each panel) and stem water potential, leaf water potential, and stomatal conductance, across trees on given days (colors in top panels) and between treatments (colors in bottom panels). Solid lines in the top panels indicate significant linear regressions on three specific days; all other regressions were insignificant and are therefore not shown here.

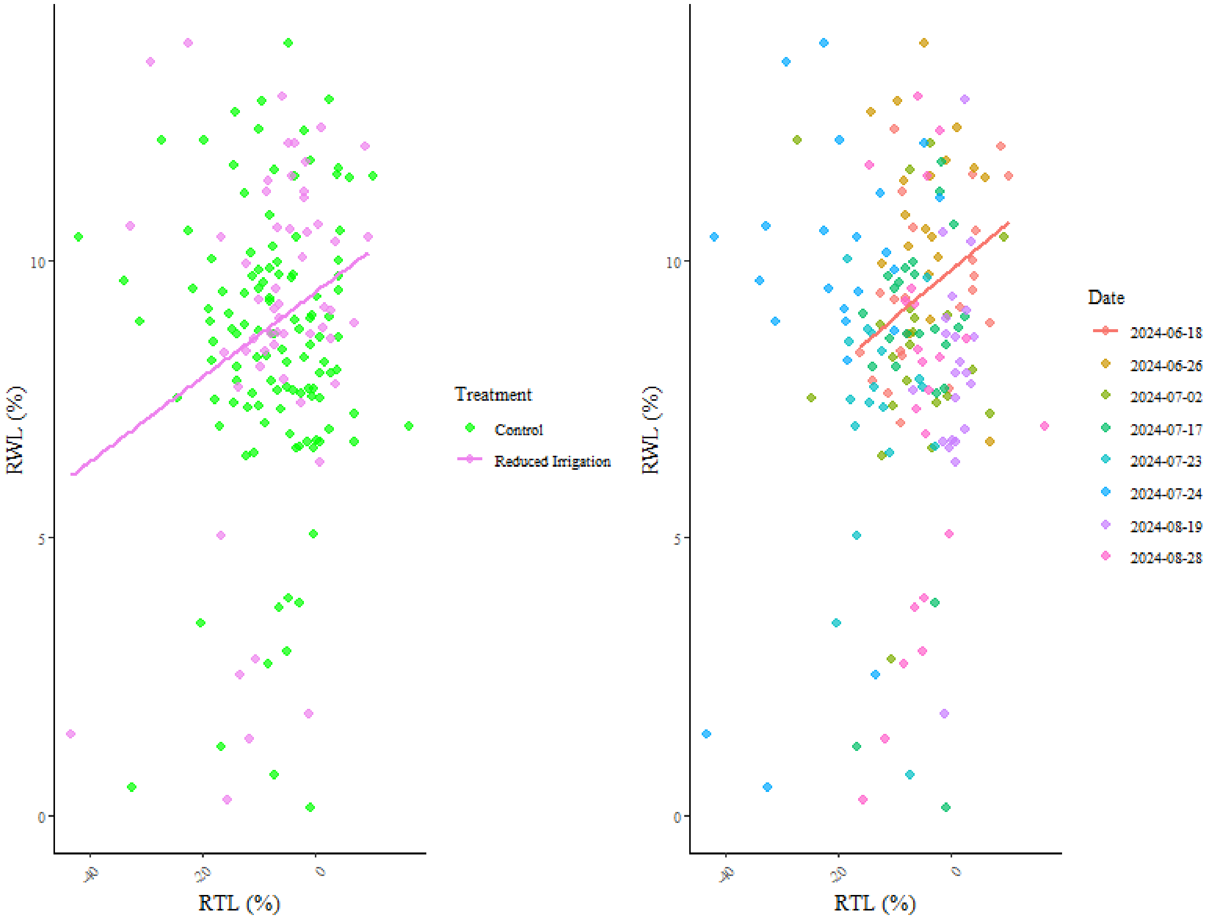

Figure 5. Relationships between relative leaf thickness loss (RTL = 100*[rehydrated thickness - initial thickness]/rehydrated thickness) and relative leaf water loss (RWL = 100 - RWC) across trees, for individual days (right panel) and irrigation treatments (left panel). Lines indicate significant regressions; insignificant regressions are not shown. RTL was nearly always negative, indicating that leaf thickness decreased in response to rehydration, rather than increasing as expected.

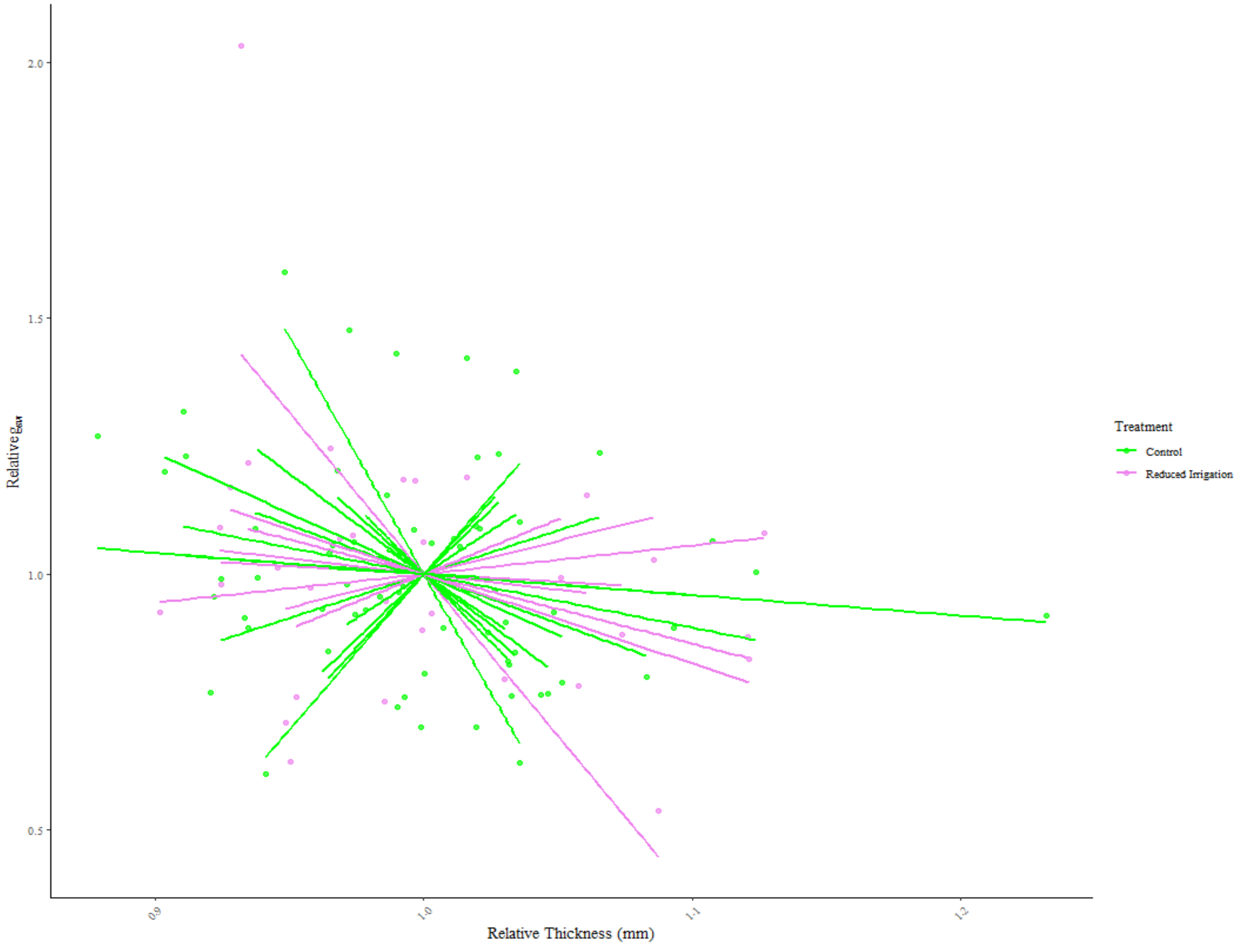

Figure 6. Relationships between stomatal conductance and leaf thickness for the same spots on the same leaves, measured repeatedly every week (values are expressed relative to means within each leaf), showing that repeated measurement does not lead to any systematic change in leaf thickness.

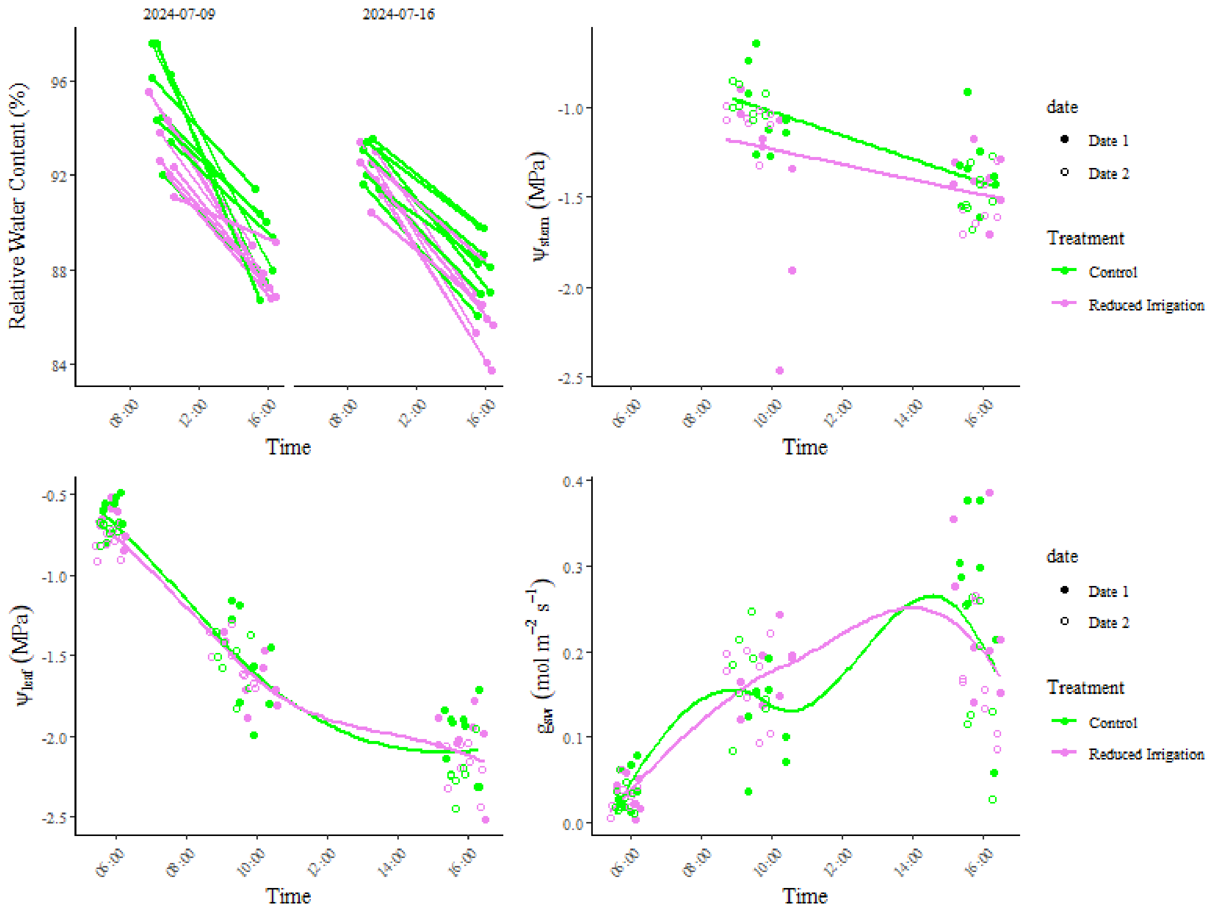

Figure 7. Changes in water status parameters during individual days, illustrating that the measurement procedures were sensitive enough to capture real physiological changes where they occurred.