EVALUATING PHYSIOLOGICAL INDICATORS FOR EARLY SEASON WATER MANAGEMENT IN WALNUT

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ABSTRACT

Midday stem water potential (SWP) was used to delay the start date of irrigation in the spring in a randomized complete block experiment (5 blocks) established on a 9 year old commercial Chandler/Paradox orchard near Red Bluff, CA. Compared to a grower control, irrigation was delayed until a treatment threshold of 1, 2, 3, or 4 bars below baseline SWP was observed, after which each treatment was irrigated on the growers schedule. The ongoing objective of these treatments is to determine if there is any long term benefit to root system health and/or tree productivity by delaying the start of irrigation in spring. For the first year of this trial, the grower started irrigation on April 28, which was the same time as the 1 bar threshold was reached. For the higher stress thresholds, the start of irrigation was delayed from 4 to 7 weeks, depending on the threshold level and the block. There were no statistically significant yield effects of the treatments based on whole plot harvests, but nut weight was reduced in direct proportion to the level of individual tree stress, with a reduction of about 10% for the most stressed (4 bar below baseline) treatment. There were no treatment effects on nut quality (color, mold, damage, etc.) other than effects directly related to nut size. The 2 and 3 bar threshold treatments also exhibited significantly lower tree stress in the fall compared to the grower control, even though these treatments received less seasonal applied water than did the control, indicating the possibility for water savings as well as beneficial carry-over effects of these mild levels of stress when used as triggers for the start of irrigation. In pilot greenhouse studies using potted walnut trees the relative sensitivity of shoot and leaf growth and tree transpiration to soil drying was evaluated. The ongoing objective of these studies is to identify the most sensitive physiological indicators of stress in walnut and to identify a corresponding technology to measure these indicators in real time for the purpose of irrigation management. Preliminary results show that tree transpiration was substantially reduced by soil drying, and that to a large extent this response was reversible, indicating a sensitive stomatal response to water stress in walnut. Growth responses to soil drying were not as apparent, contrary to expectations based on the sensitivity of growth to water stress in other crop plants. Physiological measures of water stress such as in-situ psychrometers and automated dendrometers are currently being developed for use on walnuts.

OBJECTIVES

1) Field test four threshold levels of SWP for the start of irrigation in the spring (1, 2, 3, and 4 bars below baseline) in a commercial walnut orchard.
2) Conduct greenhouse studies on the physiological responses of walnut to different levels of water stress.
3) Determine the accuracy of automated devices for measurement of plant water stress in the greenhouse, and test the most promising devices in the field.

SIGNIFICANT FINDINGS

(Objective #1)

- Grower irrigation commenced on April 28, and was equivalent to the 1 bar below baseline threshold. Lower (more stressed) thresholds allowed a substantial delay (4 to 7 weeks) in the start of irrigation.
- Longer delays caused more tree stress until irrigation commenced, but trees recovered to near control SWP levels within a few weeks of initiating a normal irrigation schedule. Even though less total irrigation water was applied for the 2 and 3 bar thresholds, significantly less tree stress was exhibited in these treatments later in the season (September-October) compared to the grower control.
- There was no statistically significant effect of irrigation threshold on yields at the plot level, but the order in plot yields corresponded to the order of threshold, with about a 10% yield loss corresponding to the lowest (most stressed) threshold. Based on individual tree samples, there was also a statistically significant (about 10%) reduction in nut weight (grams/nut) associated with the lower SWP thresholds (3-4 bars) and the longest delay. There was a significant linear reduction of nut weight with lower SWP based on individual tree samples.
- Other than nut size, there was no measureable effect of irrigation threshold on nut quality (% shrivel, color, off-grade, RLI, or relative value).
- This was the initial year for this objective, but it appears that substantial delays in the start of irrigation can be tolerated by walnuts with minimal effects on overall nut quality. Cumulative effects on yield and the potential for long term benefits to root and tree health remain as our long term objectives.

(Objectives #2 and #3)

- Potted walnut trees in the greenhouse responded to soil drying and re-irrigation by substantial reductions and increases respectively in plant transpiration, undoubtedly a result of stomatal regulation.
- The rate of daily leaf and shoot growth varied markedly over organ development, but also showed less dramatic responses to soil drying than did plant transpiration, counter to expectations based on literature data for other plants.
- Physiological measures of water stress such as in-situ psychrometers and automated dendrometers are currently being developed for use on walnuts.

PROCEDURES

(Objective 1)

<u>Orchard Features:</u>

- 9 year old Chandler/Paradox orchard about 4 miles SE of Red Bluff, CA.
- Orchard spacing 28 x 18 ft, 86.4 trees per acre.
- Irrigated with Nelson R10 minisprinklers, offset, every other tree.
- Hedging commenced in 2012 using a four center pattern. Every fourth center is hedged annually. Supplemental pruning is done in the non-hedged centers with pole loppers to minimize breakage and stimulate growth of smaller trees and promote orchard uniformity. Every other tree is considered "non-keeper" trees.

- Blended fertilizers are broad cast and followed by irrigation routinely during the growing season. Mid-summer leaf tissue was sampled in all experimental plots. No nutrient deficiencies were apparent in the plant tissue levels or visible in the crop.

Experiment Features:

- The experiment consists of 25 experimental plots, with 5 treatments and 5 replicates (=blocks).
- Experiment plots are 112 feet wide and approximately 200 foot long.
- Applied water is measured approximately twice weekly and volumetric soil water content (to 6 feet), measured weekly for all plots in 2 of the blocks.
- Midday stem water potential (SW) is measured in 2 trees of each plot approximately twice weekly. Data collection is concentrated in the center rows of each plot to guard against edge effects.
- Canopy light interception is measured mid-summer in the middle two centers of each plot using the Lampinen light bar.
- Walnut yield was measured in middle two centers of each plot with a weigh wagon.
- Walnut quality was evaluated at Diamond Walnut labs for each individual SWP tree.

Treatments and management:

The irrigation system to all experimental plots was turned off until there were 2 consecutive SWP measurements that met or exceeded the following threshold levels: 1, 2, 3, or 4 bars below baseline SWP. Baseline SWP for the time of measurement (typically midday, 13:00 – 15:00) was determined using weather data from the closest CIMIS station (Gerber #8 until 8/14/14, and Gerber south #222 after 8/14/14). Once irrigation commenced in each experimental plot, the subsequent irrigation timing and duration was determined by the grower and managed the same in all experimental plots. Applied water was measured in all plots of blocks 2 and 4 with flow meters and compared to the calculated ETc based on CIMIS ETo values and published walnut crop coefficients (Kc values).

(Objectives 2&3)

Bare root trees were established in 30 cm diameter X 30 cm deep pots of UC mix in a greenhouse at the environmental horticulture facilities at UC Davis. Pots were irrigated by hand as needed until a sufficient leaf area was established, then were micro-spray irrigated using an automatic irrigation system. Pilot experiments were performed to determine the accuracy and stability of self-made load cell scales for measuring hourly weight loss (plant transpiration, ET) and time lapse cameras for measuring leaf and shoot growth. Standard sensors and procedures were used to measure environmental variables with data-loggers. A preliminary drought experiment was performed by withholding water from one of a pair of potted plants while maintaining the other as a control, in order to determine the timing and degree of leaf and shoot growth responses as well as plant ET responses to water withholding.

RESULTS

Objective 1

Irrigation: The first grower irrigation was on April 28, which corresponded to the time that the 1 bar threshold was also reached, so both the grower and the 1 bar treatments observed the same irrigation schedule for the growing season. Water meter readings were made in 2 of the 5 blocks in all treatments, and in many instances these indicated that different amounts of water were being applied to different blocks of the same treatment (data not shown, but see below). It is not clear if these differences were reflective of a true non-uniformity of the irrigation system or to a difference in water meter calibration, and this will be determined in 2015. Since both the grower and 1 bar treatment received the same irrigation schedule, the apparent difference between these treatments in average applied water (Fig. 1) must be regarded as our current level of uncertainty in the applied water amounts. In any case, figure 1 clearly shows about a 1 month average delay for the start of irrigation in the 2 bar treatment, and approximately an additional week delay for the 3 and 4 bar treatments. Once irrigation was started however, the cumulative irrigation amount in all treatments paralleled the cumulative calculated ETc, indicating that the grower's irrigation rate during the season was similar to that predicted for walnut ETc.

<u>SWP and soil moisture:</u> Starting in early May, prior to any irrigation in the 2, 3, or 4 bar treatments, consistent differences in SWP were found between blocks (data not shown), with trees generally showing lower SWP (more stress) in blocks 3-5 than those in blocks 1-2. These block effects were associated with naturally variable soils and differences in micro topography that affected runoff from rainfall and irrigation. Hence it was decided to start irrigation in each treatment based on the average SWP for that treatment in blocks 1-2 as a group and blocks 3-5 as a group. As a result, the start date of irrigation ranged from 4 to 7 weeks (May 26 – June 16) after the first grower irrigation, depending on the treatment and block. From mid-May through the end of June, significant to very highly significant differences in SWP occurred due to the irrigation treatments, with a pattern of SWP which was consistent with that expected based on the ranking of the treatment thresholds; namely, the grower and 1 bar treatment having the highest SWP (least tree stress), the 3 and 4 bar the lowest (most tree stress), and the 2 bar intermediate tree stress (Fig. 2). SWP data was pooled for periods of time before during and after treatment imposition for statistical analysis. Prior to treatment imposition (April 15 – May 10) there were no significant differences in SWP among treatments, but during treatment imposition (May 11 – June 30), the treatments differed significantly in rank order (Table 1). Interestingly, the only other period of time during which significant treatment differences occurred was late in the season (September 1 – October 21), and during this time two of the deficit treatments (2 and 3 bar) exhibited significantly higher SWP (less tree stress) than the grower treatment (Table 1), even though the grower treatment had received substantially more seasonal applied water than these treatments. Soil moisture was only measured in 2 of the 5 blocks, but soil volumetric water content appeared to have little relation to the treatments imposed, with the 4 bar treatment showing generally higher soil moisture and the grower and 1 bar treatments showing generally lower soil moisture compared to the rest of the treatments for most of the season (Fig. 3). This may reflect the natural variability in soils and differences in water holding capacity and soil moisture release characteristics. Methods of soil moisture monitoring will be expanded to include monitoring of soil matric potential in 2015 to help understand the relationship between SWP and soil moisture status.

<u>Yield, nut size/weight, nut quality, and PAR:</u> For this first year of treatments, based on whole plot yield data there was no statistically significant effect on yield, although the ranking of treatment yields was in the same order as the treatments, with the 4 bar treatment showing about a 10% reduction compared to the grower and 1 bar treatments, and the 2 and 3 bar treatments intermediate (Table 2). For the whole plot data there were no statistical differences or trends

related to treatments in PAR or yield/PAR (Table 2). Nut size effects were also evaluated using individual tree samples, and for these samples, there was a statistically significant reduction in nut weight, also of about 10%, for the 4 bar treatment compared to the grower and 1 bar treatment, with the 2 and 3 bar treatments being intermediate (Table 2). For individual tree samples there was also a very clear reduction in nut weight associated with a lower value of SWP, with a nut weight reduction of about 0.4 grams per bar of reduced SWP (Fig. 4). This correlation was the strongest for tree average SWP during the month of June (shown in figure 4), but the correlation was also positive for SWP in other months, indicating that even for the first year we are seeing that lower SWP (more tree stress) will reduce nut weight within the same growing season. Analysis of the nut quality data based on individual tree samples showed that the only significant effects were those related to nut weight, with significant effects as well as the identical treatment ranking in percent Large and Jumbo and percent Large Sound walnuts (Table 3), but no significant effects and no trend in ranking related to the irrigation treatment in other aspects of nut quality (e.g., shrivel, mold, off-grade, color or relative value, Table 3).

Objectives 2 and 3

Preliminary greenhouse experiments: A set of 6 custom fabricated load cell scales were obtained from H. Lieth in the plant sciences department, and tested for stability of calibration under typical greenhouse conditions. It was determined that all of the load cells showed varying degrees of error caused by environmental conditions (data not shown), but for two scales the error was small enough to allow for experimentation. Plant transpiration, measured by weight loss, exhibited the expected daily pattern of low nighttime and high daytime rates, giving a stair-step pattern over many days for both the irrigated plant (Tree #2) as well as the non-irrigated plant (Tree #1, Fig. 5). These data were analyzed by plotting the loss of weight since midnight each day, and over the first 4 days of the experiment there were progressive increases in transpiration for both plants, presumably due to either environmental conditions, plant growth, or a combination of both (Fig. 6, August 6-9). On August 10, transpiration was similarly reduced for both plants, presumably due to a reduced greenhouse evaporative demand, but by August 11, the non-irrigated plant was exhibiting substantially less transpiration than the irrigated plant (Fig. 6). The difference between the plants in daily transpiration increased until plant # 1 was irrigated in the morning of August 15 (Fig. 6). The transpiration of plant #1 approximately doubled on the day of irrigation compared to the prior day, whereas the control plant (#2) exhibited a similar level of transpiration on these days, indicating a rapid and substantial recovery in stomatal conductance upon irrigation in plant #1 (Fig. 6). This was also followed however, by a gradual increase in transpiration in plant #1, indicating that while substantial, this stomatal recovery was not complete in the first day. By about 4 days following the resumption of irrigation in plant #1 and withholding of irrigation in plant #2, the rate of transpiration of plant #2 had dropped substantially compared to plant #1, indicating that both plants had similar stomatal responses to soil drying, and that this approach should provide reasonably reproducible results.

Periodic growth measurements were made on multiple internodes, petioles, and terminal leaflets on the non-irrigated plant #1 (Figs. 5 and 6) from July 21 (prior to irrigation withholding), to after re-irrigation (through August 20). Each organ exhibited more-or-less linear growth to a final size (Fig. 7), but for internodes and possibly terminal leaflets and petioles, there also appeared to be a progressive reduction in organ final size over plant development, even before irrigation was withheld. For some organs which began development close to the time of irrigation withholding, there was some indication of reduced growth rates during the period when transpiration was substantially reduced (August 11 – 15) and resumption of growth following irrigation, but the growth responses were not as dramatic in the short term as the responses seen in plant transpiration (Fig. 6). In order to evaluate the sensitivity of walnut shoot and leaf growth to water deprivation however, it will be necessary to conduct sufficient control experiments to determine whether organ growth changes systematically over plant development.

DISCUSSION

This first year of testing a range of SWP thresholds for the start of irrigation in the spring has indicated that substantial delays may be possible using only modest levels of thresholds (2-3 bars below baseline SWP). In addition to water and labor savings, a delay in the start of irrigation may generally promote improved root health by avoiding excessively wet soil conditions, but documenting these effects will require longer term data collection. It is interesting to note that even though the SWP thresholds themselves were set at equal increments (1, 2, 3, and 4 bars), the resulting delay in irrigation was not, with about 4 weeks between the 1 and 2 bar thresholds, about 1 week between the 2 and 3 bar thresholds, and only a few days between the 3 and 4 bar thresholds. This pattern suggests the difficulty of developing a reliable calendar-based criteria for the start of irrigation in the spring, as the net effect of soil and environmental conditions on tree water status are presumably changing rapidly at this time of year. The lack of any clear relation between the treatments and measured soil water (Fig. 3) may also illustrate the difficulty of using soil-based approaches to determine the start of irrigation in the spring. Soil variability may account for the unclear relation between the treatments and soil water, and this will be evaluated in the future. The overall similarity of ranking in the plot yields and tree sample nut weights, as well as the similar range in values as a percent of the grower treatment (Table 2) strongly suggests that nut weight was the primary driver in the observed pattern of yield effects, so any benefit from delaying the start of irrigation will need to be balanced against the risk of reducing nut size. The effect of the treatments appeared to be limited to nut weight however, as this was the only nut quality or orchard parameter that was affected by the treatments (Tables 2 and 3).

Midday SWP has proven to be a reliable method for trouble shooting water-related orchard problems as well as a method for routine irrigation management, but despite many advances in sensor technology, we are still in the early stages of developing methods capable of monitoring SWP, or something closely related to SWP, on a continuous basis. Based primarily on evidence from herbaceous annual crops, it is generally assumed that a reduced rate of growth is the most sensitive indicator of water stress in plants, and in part this has led to the commercial development of automated dendrometers for irrigation management. Stomata are also known to close in response to water stress, and this has given rise to commercial development of both direct (sapflow), and indirect (leaf or canopy temperature) automated measurement methods for irrigation management. Our preliminary experiments on potted plants in the greenhouse have indicated that transpiration may be a more sensitive indicator of water stress than growth in walnuts, but these results must be confirmed with further study.

ACKNOWLEDGEMENTS

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Tables and Figures

Table 1. Average SWP values for periods of time representing pre-treatment (April/May), during and shortly after treatment (May/June), and two post-treatment periods (July/August and September/October). Treatments are ranked within each period based on the value of SWP, but values followed by the same or no letter are not significantly different at the 5% level of Tukey's test. Note that these values are actual recorded SWP values, rather than the difference between the recorded values and the baseline. The treatment ranking and statistical separation are not affected in either case.

April 15 - May 10		May 11 - June 30		July 1 – August 31		September 1 – October 21	
Treatment	SWP	Treatment	SWP	Treatment	SWP	Treatment	SWP
2 bars below	-4.4	1 bars below	-5.3a	2 bars below	-6.1	3 bars below	-4.5a
3 bars below	-4.5	Grower	-5.3a	1 bars below	-6.9	2 bars below	-4.7a
1 bars below	-4.5	2 bars below	-6.2ab	3 bars below	-7.0	1 bars below	-4.9ab
Grower	-4.5	3 bars below	-7.2b	Grower	-7.0	4 bars below	-5.4b
4 bars below	-4.7	4 bars below	-7.4b	4 bars below	-7.3	Grower	-5.5b

Table 2. Plot-based yield, PAR and Yield/PAR, and tree-based average nut weight at harvest, expressed as average values as well as a percent of the grower value, for the five irrigation treatments in the study. Average values followed by the same or no letter are not significantly different at the 5% level of Tukey's test.

	Plot Yield (lbs/ac)		Tree sample nut weight (g)		Plot PAR		Plot Yield/PAR	
Treatment	Lbs/ac	(% Grower)	Weight	(% Grower)	PAR	(% Grower)	Y/P	(% Grower)
1 bars below	3700	100	10.3a	98	86	102	43.0	98
Grower	3690	100	10.4a	100	84	100	43.8	100
2 bars below	3440	93	10.1ab	97	88	104	39.3	90
3 bars below	3420	93	9.4bc	91	85	101	40.0	91
4 bars below	3360	91	9.1c	87	88	104	38.4	88

Table 3. Average nut weight (repeated from Table 2), two relative measures of nut size (percent large/jumbo, percent large sound), and other measures of nut quality (shrivel, mold, off grade, extra light, RLI and relative value) at harvest for tree-based samples. Average values followed by the same or no letter are not significantly different at the 5% level of Tukey's test.

Treatment	Nut weight (g)	LgJm (%)	LgSd (%)	Shrivel (%)	Mold (%)	OffGr. (%)	Ex. Lt. (%)	RLI	Relative Value
Grower	10.4a	78.7a	78.9a	2.8	1.6	2.3	60	53.5	0.88
1 bars below	10.3a	76.9ab	77.8a	2.6	2.1	2.8	63	54.2	0.89
2 bars below	10.1ab	75.1ab	76.0a	1.7	2.2	2.5	59	54.1	0.90
3 bars below	9.4bc	61.1bc	62.9ab	2	1.3	1.9	56	53.1	0.88
4 bars below	9.1c	49.2c	52.0b	2.6	1.6	2.2	61	53.8	0.89

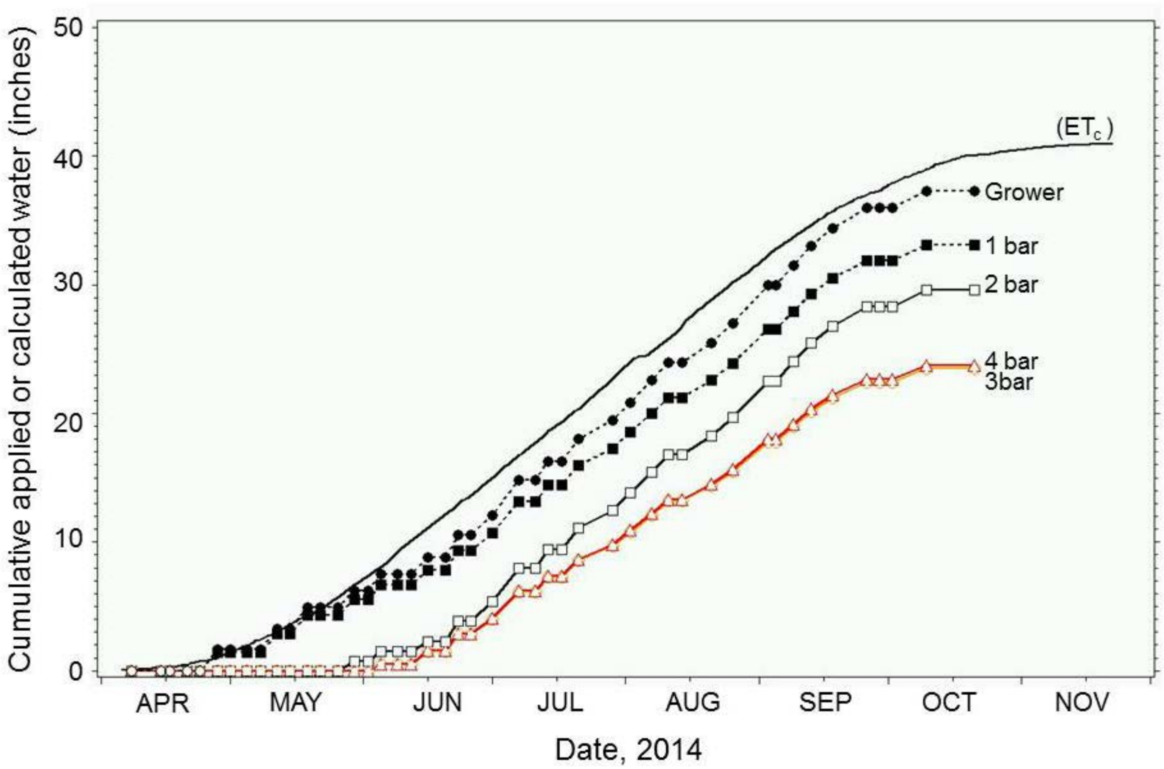

Figure 1. Treatment average cumulative seasonal water applied, as well as the calculated seasonal water demand (ET_c) for walnuts based on real-time CIMIS reference ET. Because irrigation was managed differently in different blocks, the first non-zero value of cumulative applied water for any treatment indicates the initiation of irrigation in the first irrigated blocks of that treatment. Each point is the average of two water meter readings (water meters were installed in blocks 2 and 4) for each treatment.

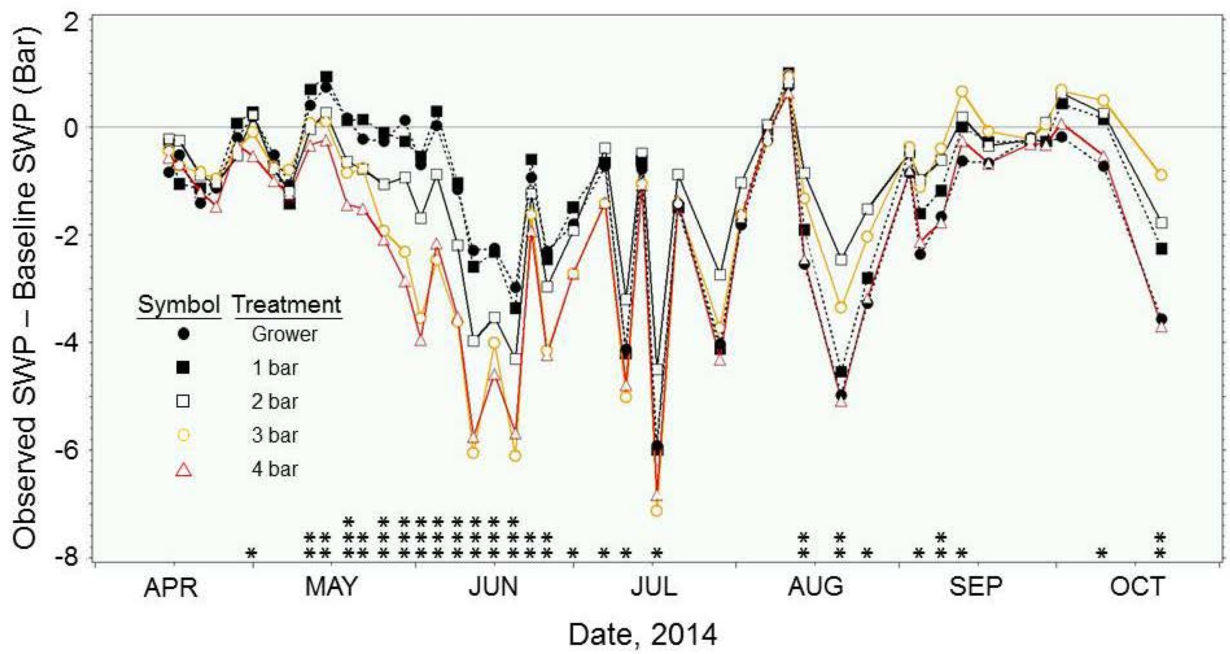

Figure 2. Seasonal pattern of SWP, expressed as the difference between observed and baseline SWP (in order to remove the effect of day-to-day changes in weather conditions), for the five irrigation treatments imposed. One, two or three stars (*) on the date axis indicate a significant (p=.05), highly significant (p=.01) or very highly significant (p=.001) treatment effect respectively, on SWP based on a Tukey's test. Each point is the average of 10 trees.

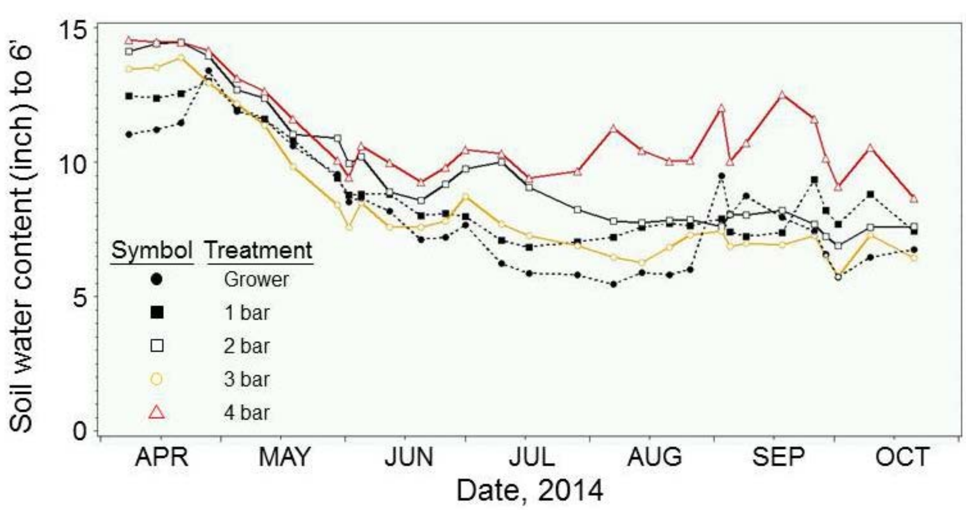

Figure 3. Seasonal pattern in soil water (total inches of water content to a 6' depth) for the irrigation treatments imposed. Each point is the average of 12 values (6 depths at 2 locations, i.e, blocks 2 and 4).

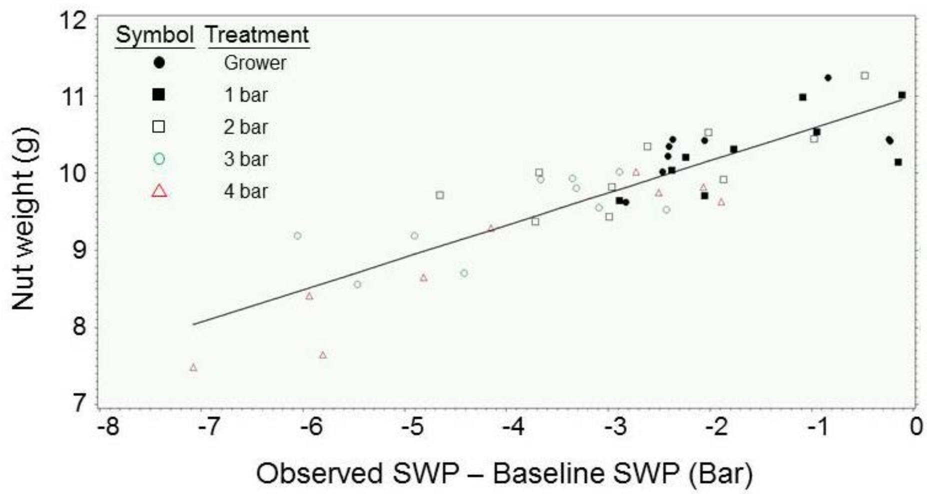

Figure 4. Relation of tree-sampled nut weight to the average observed SWP – Baseline SWP for the month of June for the five irrigation treatments. Each point is the average of 8 June SWP measurements and an approximately 100 nut harvest sample. A linear regression (Weight = 0.415*SWP+12.87, r-square = 0.77***) is also shown.

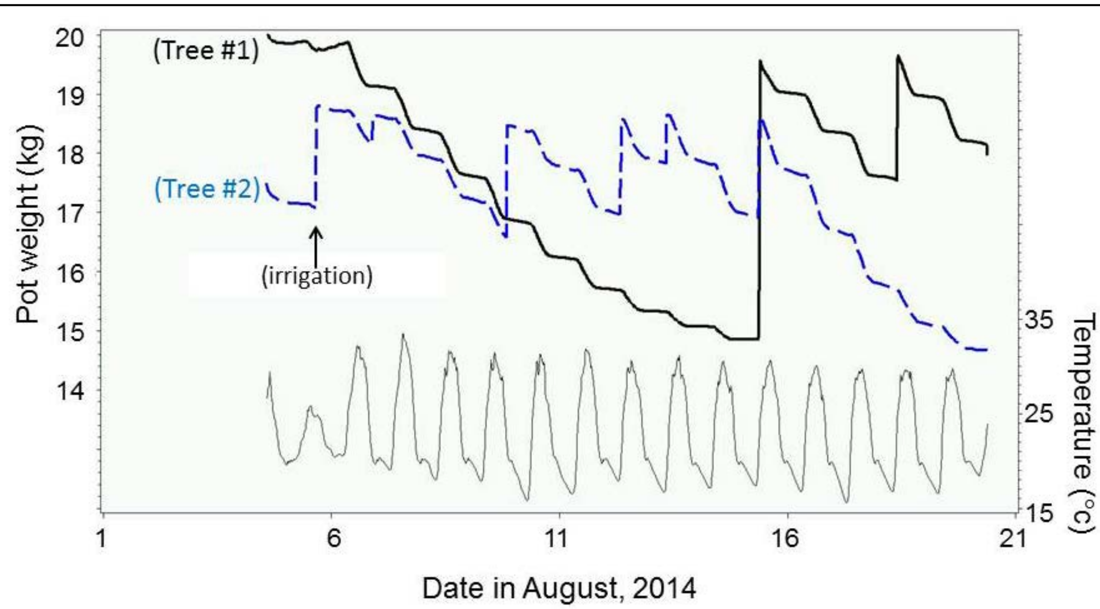

Figure 5. Example pot+plant weight (left axis, heavy lines) and greenhouse temperature (right axis, light line) data from a walnut dry-down experiment. During this experiment water was withheld from tree #1until August 15, when it was irrigated, as indicated by the sudden increase in weight. Tree #2 was irrigated periodically until August 15.

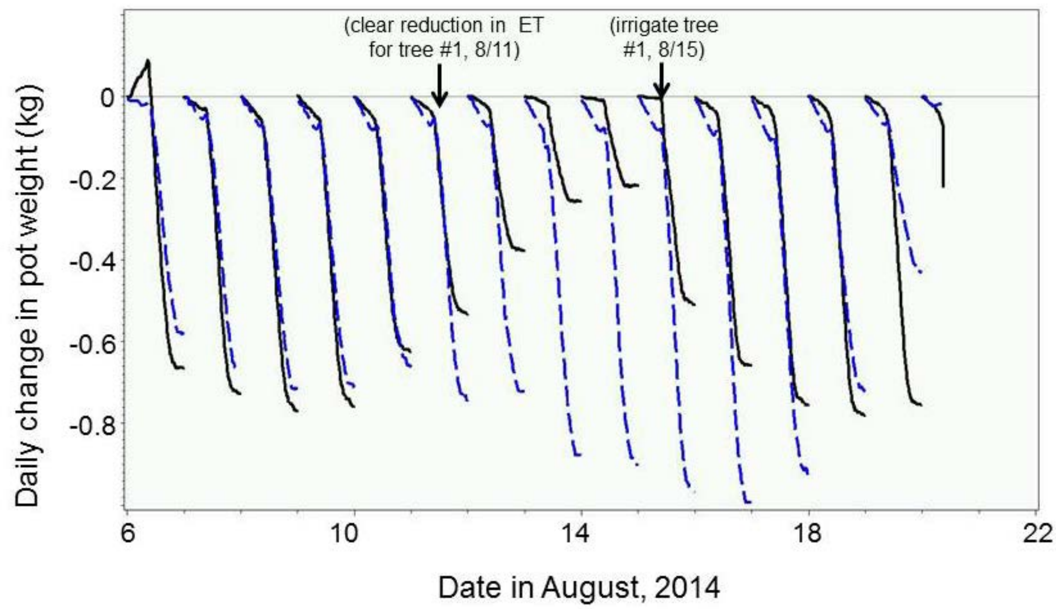

Figure 6. Pot+plant weight data from figure 5 plotted as a daily change in weight starting at midnight, in order to compare the daily water use of the non-irrigated tree (#1, solid line) with the irrigated tree (#2, dashed line). The first day when there was a clear reduction in ET for plant #1 compared to plant #2 is indicated, as well as the rewatering date for plant #1.

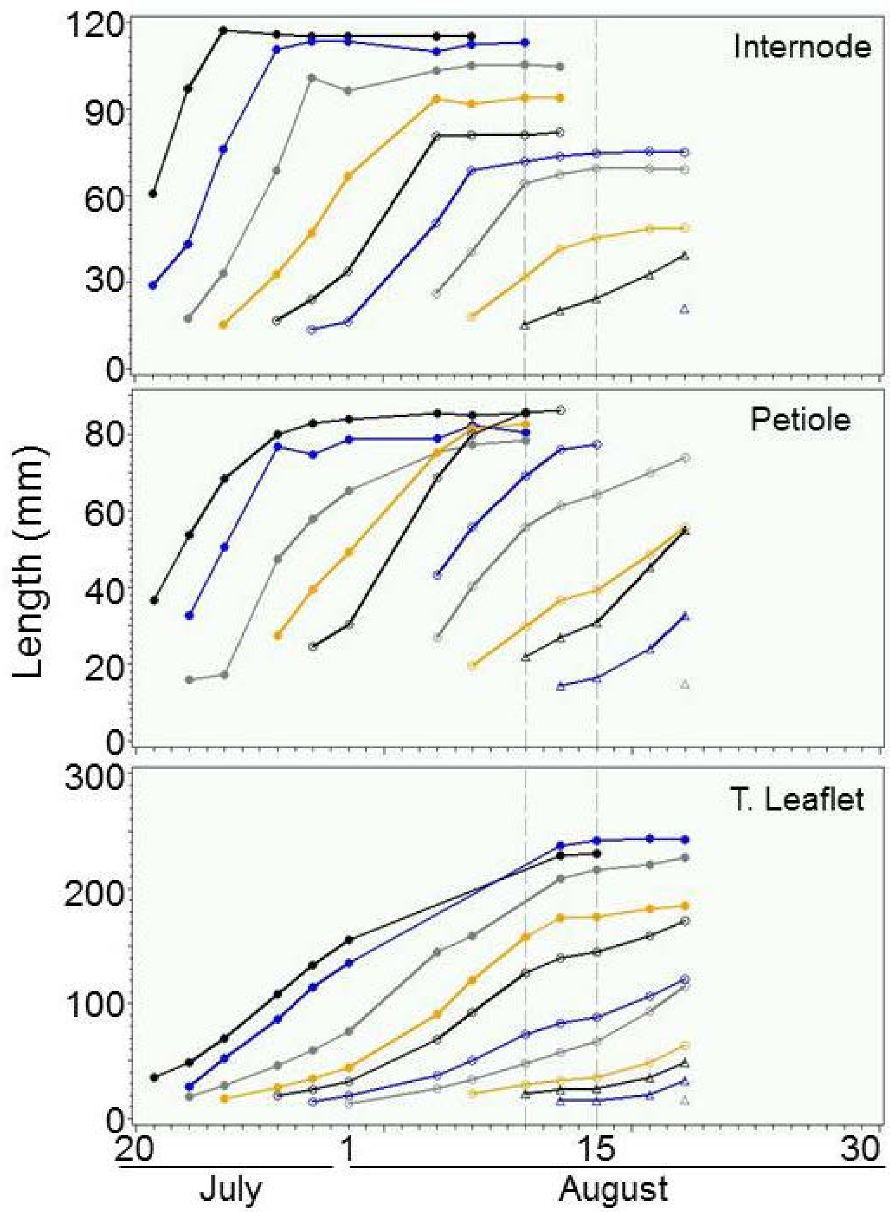

Figure 7. Growth of sequential internodes, petioles and terminal leaflets for Tree #1 in figures 5 and 6. Each line represents a single internode, petiole, or leaf measured repeatedly over time, with lines starting later in time representing more apical organs. Vertical dashed lines indicate the time period prior to irrigation during which plant #1 exhibited substantially reduced ET compared to the control.