
Effect of early post-harvest irrigation on tree health

Project No.: HORT42.Buckley

Project Leader: Tom Buckley
Plant Sciences, UC Davis, 1 Shields Ave, Davis, CA 95616
530-752-0320, tnbuckley@ucdavis.edu

Project Cooperators and Personnel: *Patrick H. Brown (UC Davis)*

Grantee(s) of the Almond Board are REQUIRED to address sections A through G. These should be **submitted in PDF**, using Arial font size 12 for the main text, and be five to seven pages in length.

A. Summary (*In laymen's terms – emphasize key findings and recommendations*)

This project aimed to assess effects of withholding water at harvest time on photosynthesis, water relations and carbohydrate stores. We installed sap flow probes in 12 trees (half Monterey and half Nonpareil) in a commercial orchard east of Turlock and modeled canopy photosynthesis using canopy conductance estimated from the sap flow data.

We encountered two major difficulties that prevented satisfactory completion of the study. Firstly, we had expected to be able to impose a drought of substantial duration on half of the trees, similar in duration and magnitude to what is common throughout the industry – on the order of 10-30 days, depending on variety. However, because the grower partner was an 'early adopter', they were already using elements of advanced harvest – including sweeping nuts to row centers and using microsprinklers to enable quick return of irrigation after shaking – so it was not commercially viable for them to impose such severe droughts on six of their trees. As a result, we were only able to impose a small treatment difference: withholding a single 6-hour irrigation set from half the trees 3 days after shaking. All trees were then heavily irrigated 4 days later. This treatment difference was too small to detect. Secondly, our probe installations in the Monterey trees caused a dramatic wound response, which led to compartmentation of flow around the probes, preventing useful measurements. This reduced our sample size by half, making it even more difficult to detect any treatment effect that may have occurred.

In January 2020, by mutual agreement with the Almond Board, we elected to terminate the study early and return the unspent funds. **Thus, this report serves as both the Annual and Final Report** for this project.

We emphasize that these results should not be taken to suggest that harvest stress has negligible impact on canopy function. To address this question properly, we suggest the following would be necessary: (1) a much larger number of sampled trees, to overcome effects of random differences in physiology and responses; (2) modification of the sap flow probe design to minimize bark removal, in order to reduce or eliminate the large wound/compartmentation response that occurred in the Monterey variety trees; and most critically (3) the ability to impose a large difference in irrigation treatments between control and stressed trees, more representative of the actual differences that would result from growers shifting from traditional on-ground harvest techniques to advanced harvest techniques.

B. Objectives (300 words max.)

1. Specify the goal(s) and specific objectives of the proposal – if a collaborative effort, identify who is the lead for each objective
2. Identify annual outputs or milestones for each of the objectives

Main Goal: Assess the effects of water stress around harvest on canopy photosynthesis and carbohydrate stores.

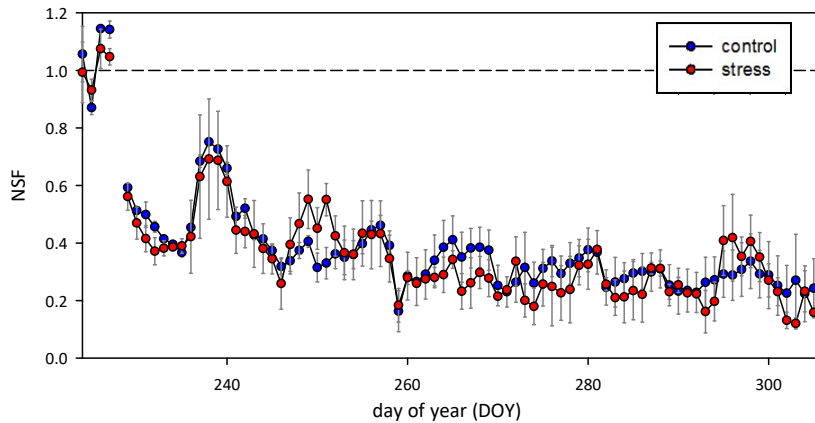
Objective(s)	Date to be accomplished	Milestones and deliverables associated to the objective
Monitor canopy conductance, photosynthesis and water status during harvest and post-harvest period of 2019 season.	31 Dec 2019	<p>Milestones:</p> <ol style="list-style-type: none"> 1. sap flow probes installed (31 Jul 2019). 2. photosynthetic parameters measured (31 Aug 19). 3. stem water potential measured (biweekly from harvest through leaf-fall).
Stem carbohydrate stores monitored during harvest and post-harvest period of 2019 season.	31 Jan 2020	<p>Milestones:</p> <ol style="list-style-type: none"> 1. stem samples collected (biweekly from harvest through leaf-fall). 2. all samples analyzed for carbohydrate content (by 31 Jan 2020).
Analyze effect of harvest stress and post-harvest irrigation on tree carbon balance.	29 Feb 2020	<p>No milestones. Deliverables:</p> <ol style="list-style-type: none"> 1. report to Almond Board describing results of analysis (31 March 2020).

B. Annual Results and Discussion (*This is the core function of this report*)

1. Describe activities and outputs for each objective
2. Discuss significance of these in terms of progress toward goals, change in approach, next steps or other conclusions based on this year's results

Objective (1): Monitor canopy conductance, photosynthesis and water status during harvest and post-harvest period of 2019 season.

We installed sap flow probes in six Monterey and six Nonpareil trees in adjacent rows in August 2019. The Monterey trees rejected the probes, responding with an extreme wound response and flow compartmentation, which precluded collection of meaningful data. Results described hereafter refer to Nonpareil trees.



Daily normalized sap flow, NSF (daily sap flow expressed relative to its mean value on 12 and 13 August 2019 [day of year, DOY, 224 and 225]; DOY 225 was the last irrigation set before shaking) is shown in **Figure 1**. However,

normalized canopy conductance, NCC (sap flow divided by the evaporative demand of the air, VPD, and then normalized to pre-harvest values; **Figure 2**), is a better indicator of the

Figure 1. Normalized sap flow.

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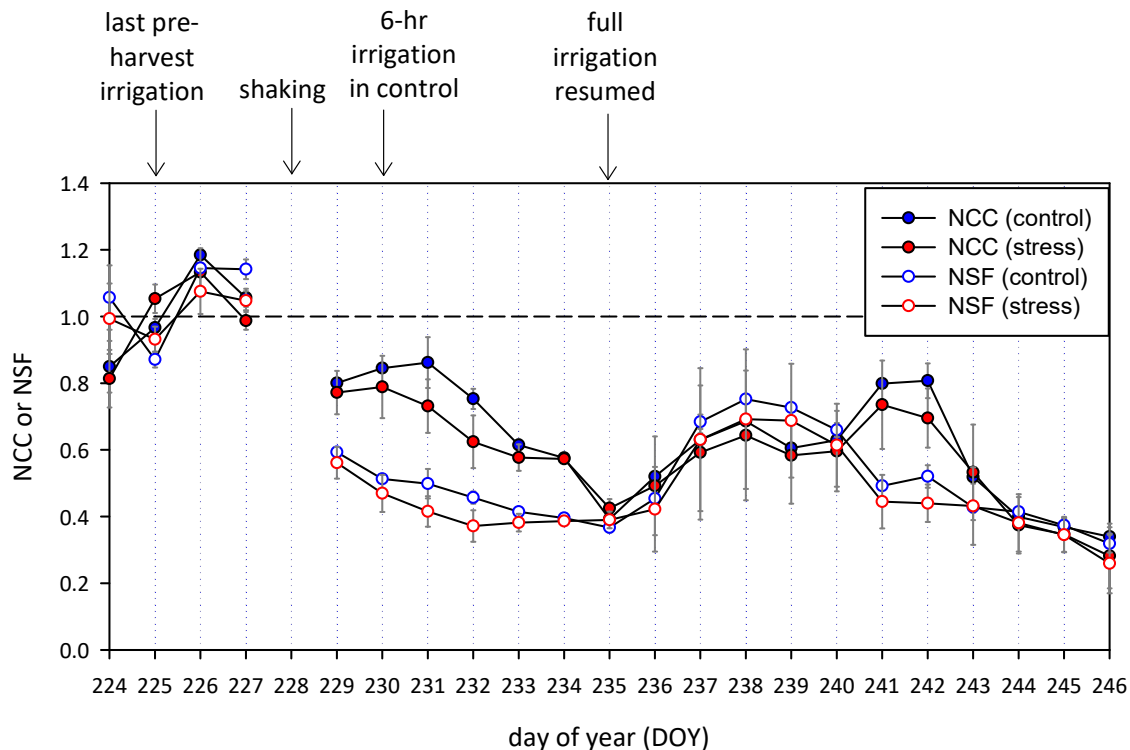


Figure 2. Normalized canopy conductance (NCC) and normalized sap flow (NSF) in period around harvest.

physiological status of the plant than sap flow itself. This is because fluctuations in evaporative demand can cause swings of sap flow that may create the illusion of suppression or enhancement of plant function, when in fact they are merely passive consequences of changes in VPD. NCC is a measure of stomatal opening.

A good example of the relevance of considering NCC rather than water use itself is the steep decline in NSF that began on DOY 227, the day before shaking (**Figure 2**). (Data are unavailable for the day of shaking because we had to temporarily remove the probes on that day to allow shaking.) The decline continued through DOY 235, when full irrigation was returned to the block. This decline in NSF appears to confirm the expectation that stomata should close due to withholding of irrigation after DOY 225. However, the first several days of the decline in NSF were in fact driven primarily by a coincidental steep decline in VPD (**Figure 3**). NCC – the measure of stomatal opening – was actually stable during that period, and did not decline below $79 \pm 3\%$ (mean \pm SE) of its initial value until DOY 232 – a full week after the last full irrigation set. NCC declined to a low of $41 \pm 2\%$ before full irrigation was returned, and then rose gradually over a week to a maximum of $104 \pm 17\%$.

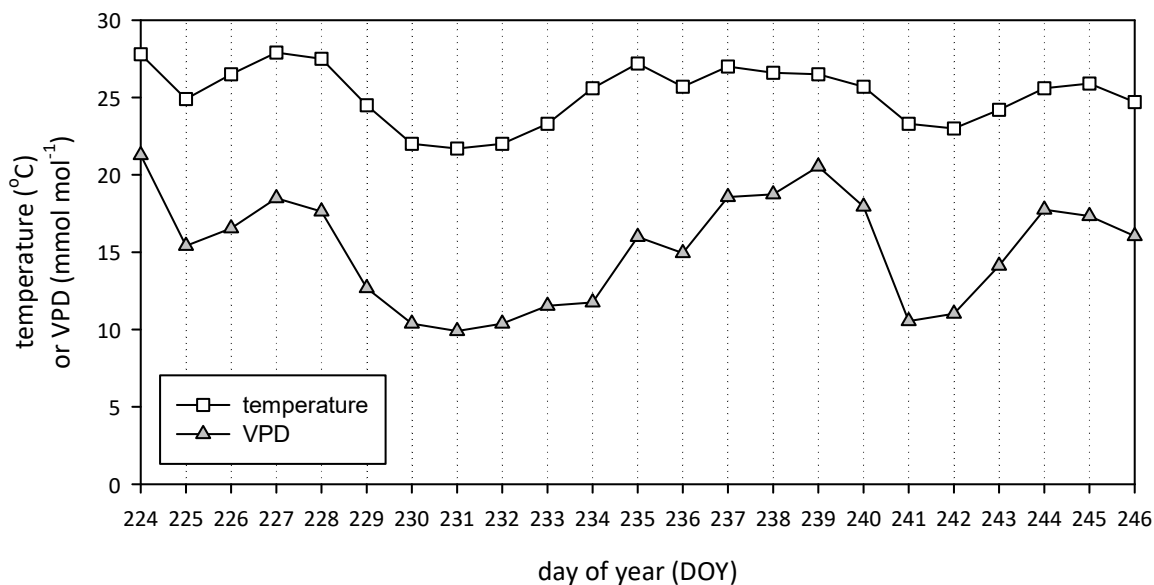


Figure 3. Air temperature and VPD in the period around harvest.

A small (6-hour) irrigation set was applied to half of the trees (the "control" trees) on DOY 230. This may have temporarily slowed the decline in NCC in these trees. However, NCC was already stable or even slightly increasing in both treatments prior to this irrigation set, and at any rate, NCC eventually declined to similar values in both treatments ($39 \pm 2\%$ in the control and $43 \pm 3\%$ in the stressed trees) before the return to full irrigation. Mean NCC over the ensuing 10 weeks did not differ between treatments ($62 \pm 2\%$ for control vs. $59 \pm 2\%$ for stressed). Furthermore, our measurements of stem water potential (described below) suggest that the trees chosen for the water stress treatment may have been systematically under-watered (relative to the control trees) prior to, and during, the experiment, perhaps because of microtopographical differences or variances in microsprinkler emitter efficiency. **Thus, in summary, we concluded that the sample size, experimental constraints, natural bias**

and available data precluded detection of meaningful differences between the two treatments.

Mid-day stem water potential (SWP) averaged -1.54 ± 0.02 MPa (control) and -1.92 ± 0.02 MPa (stress) on the day of shaking (DOY 228) before any controlled difference in irrigation had been imposed on the two treatments. This strongly indicated that the stressed trees may have been systematically underirrigated before and throughout the experiment. Two weeks later (DOY 242), a week after return to full irrigation, SWP was significantly lower in both treatments, and in fact the control trees had declined in SWP by a greater degree than the stressed trees (-1.91 ± 0.08 MPa (control) and -2.19 ± 0.06 MPa (stress)). We speculate that the failure of water potential to fully recover at this date may have been partly due to the fact that DOY 242 was an unseasonably cool day, 4°C cooler than most of the preceding week: low temperatures suppress water transport by increasing the viscosity for water flow in the xylem. Indeed, stem hydraulic conductance (expressed as NSF divided by the difference between mid-day leaf and stem water potentials) was 11-17% lower on DOY 242 than on the day of shaking.

Although water potentials had recovered to baseline levels by DOY 262 (-0.67 ± 0.02 MPa (control) and -0.74 ± 0.02 MPa (stress)), this largely reflected a seasonal decline in gas exchange (on DOY 262, NSF was 29% (control) and 28% (stress) of pre-harvest levels). This decline was partially due to a gradual decline in VPD through the fall (from 16.1 kPa in the week of harvest to 7.7 kPa in the week around DOY 262). However, a progressive decline in stomatal opening also contributed (NCC = 68% (control) and 65% (stress)). The reasons for the decline in NCC are difficult to determine without additional data. There is some evidence that stomatal function gradually declines as leaves age, and that stomata respond directly to lower temperatures by partially reducing their apertures. It is also well established, though, that stomata are influenced by water transport, and we found that stem hydraulic conductance had declined by roughly 78% in both treatments by DOY 262, as compared to before harvest. Whether this reflects cumulative effects of periodic minor water stress, effects of temperature or another gradual and metabolism-linked decline is unknown.

Objective (2): Stem carbohydrate stores monitored during harvest and post-harvest period of 2019 season.

This objective was abandoned due to early termination of the study.

Objective (3): Analyze effect of harvest stress and post-harvest irrigation on tree carbon balance.

We measured photosynthetic parameters (carboxylation capacity, potential electron transport rate and day respiration rate) in one leaf on each of the 12 study trees, sampled on 19 September 2019. Results are shown in **Table 1** for Nonpareil.

Table 1. Photosynthetic parameters measured in Nonpareil (means \pm SE; n=3 per treatment).

parameter	control	stress
carboxylation capacity $/(\mu\text{mol m}^{-2} \text{s}^{-1})$	30.3 ± 1.1	34.2 ± 1.4
electron transport rate $/(\mu\text{mol m}^{-2} \text{s}^{-1})$	62.4 ± 2.3	62.3 ± 4.2
day respiration rate $/(\mu\text{mol m}^{-2} \text{s}^{-1})$	2.6 ± 0.2	2.0 ± 0.3

With the exception of carboxylation capacity, which was marginally larger in the stressed trees, the parameters did not differ between the two treatments. We applied these values to canopy conductance and meteorological data to estimate daily canopy photosynthesis in both treatments. Photosynthesis declined by similar amounts during harvest stress, but these absolute declines were amplified by the influence of a cooling trend during the same period (low temperatures suppress photosynthetic activity). We therefore used the model to "back out" the effect of temperature variations, as well as seasonal variations in irradiance, by replacing the observed temperatures and irradiances with a constant temperature of 25°C and

a constant irradiance equal to the pre-harvest average in the model. We then divided photosynthesis by its pre-harvest average to produce **temperature-adjusted normalized canopy photosynthesis (Figure 4)**.

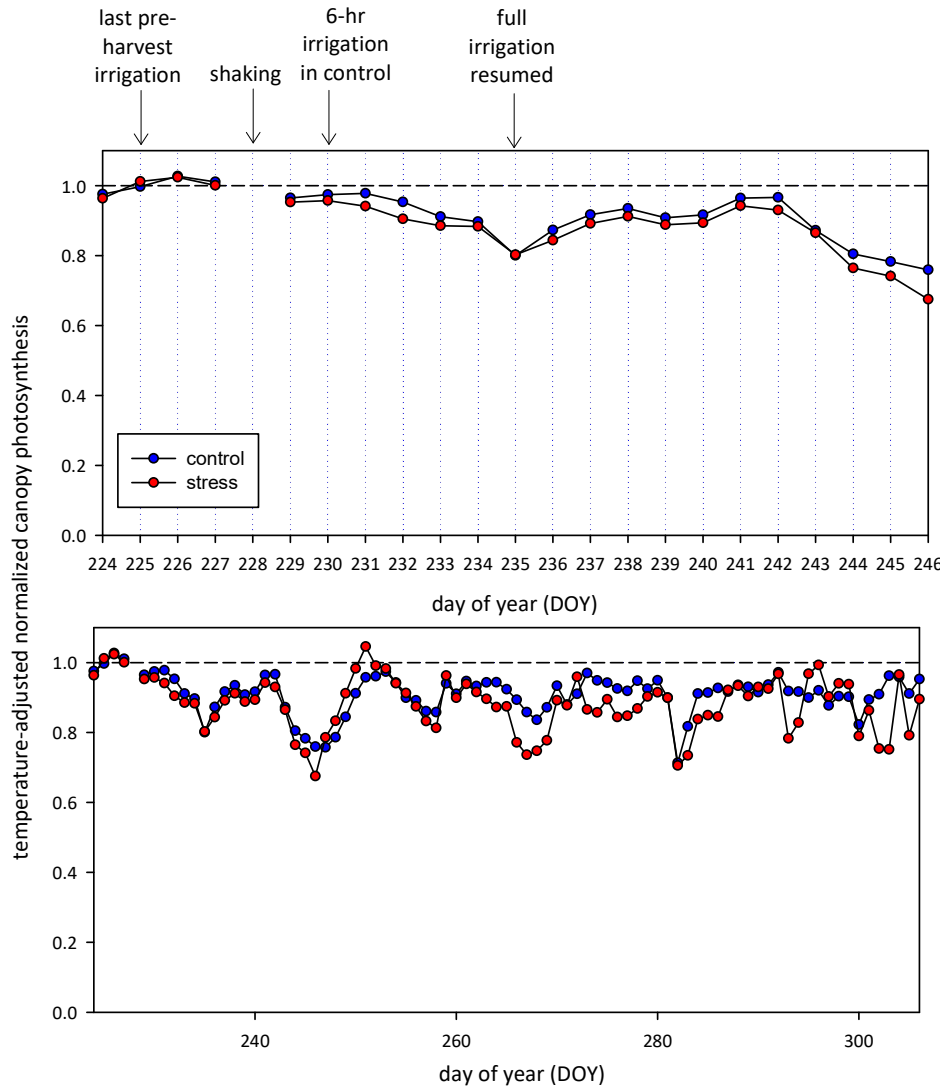


Figure 4. Simulated temperature- and irradiance-adjusted normalized canopy photosynthesis. (top) during harvest period; (bottom) through October.

This revealed a gradual decline of 20% over the 10 days that water was withheld during the harvest period, followed by a gradual recovery to 96% (control) or 94% (stressed) of pre-harvest levels. There was no systematic difference in temperature-adjusted normalized photosynthesis between the two treatments through the remainder of the season. Each treatment occasionally exhibited higher normalized photosynthesis than the other, but both mostly remained between 80% and 100% of pre-harvest values. This result is consistent with the null results from observations of sap flow and canopy conductance.

C. Outreach Activities

1. Please describe outreach activities including the event description (date, location, topic of the presentation, approx number of participants and type of audience)

Results were presented in a poster at the Almond Conference in Sacramento on 11 December 2019.

D. Materials and Methods (500 word max.):

1. Outline materials used and methods to conduct experiment(s)
2. Note any challenges or unforeseen developments that were encountered resulting in change of methodology, timeline, or scope of project

Setting and treatments. We studied 12 individual trees at Sperry Farms, 30 minutes east of Turlock, CA, of which 6 were Nonpareil and 6 Monterey. Trees were irrigated three days prior to shaking (DOY 225) and 7 days after shaking (DOY 235), and half of the trees of each variety were also given a brief, 6-hour irrigation set two days after shaking (DOY 230).

Normalized sap flow. We measured sap flux (sapwood water flow per unit of cross-sectional sapwood area) in each tree using the double-ratio method (DRM). Four needles (one heated needle and three temperature sensors, located 7.5 mm below, 7.5 mm above and 22.5 mm above the heater, respectively; each needle was 1.27 mm in diameter and 30 mm in length) were installed in each tree trunk approximately 60 cm above the ground and underneath the bark, and then insulated by wrapping the tree with mylar-coated bubble wrap (Reflectix). The DRM method was described in previous project reports for the ABC, and has been validated by lysimetry and tube flow experiments. Sap flow was normalized by dividing its value by the average on DOY 224-226.

Normalized canopy conductance. We calculated canopy conductance by dividing sap flow by estimated leaf to air water vapor mole fraction gradient (computed from air VPD). Canopy conductance was normalized by dividing its value by the average on DOY 224-226.

Canopy photosynthesis. We measured photosynthetic parameters (carboxylation capacity, potential electron transport rate and day respiration rate) in one leaf on each of the 12 study trees, sampled on 19 September 2019, by fitting the photosynthesis model of Farquhar et al. (1980) to response curves of net leaf photosynthesis to intercellular CO₂ concentration, obtained using two Li-Cor Li-6800 portable photosynthesis systems. We used previously published temperature responses for these parameters in almond. We combined these parameters with measurements of light intensity and temperature from the nearest CIMIS station and canopy conductance estimated as described above, and applied these data to the model of Farquhar et al (1980) to estimate canopy photosynthesis. We then corrected canopy photosynthesis for daily and seasonal fluctuations in temperature and irradiance by repeating simulations assuming constant values for both temperature and irradiance, to better isolate the role of physiological responses to harvest stress.

E. Publications that emerged from this work

1. List peer review publications in preparation, accepted or published
2. Other publications (e.g. outreach materials)
3. Please provide copies of publications

Nothing to report.