**FINAL PROJECT REPORT**

**Project Title**: How does fruit acclimation to sunburn affect sunburn management?


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**Total Project Request:** **Year 1:** 86,621 **Year 2:** 87,846

**Other funding sources**

None

**Budget:** Kalcsits, Waliullah, Waite
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**Email:** arcgrants@wsu.edu/kim.rains@wsu.edu

| **Item** | 2018 | 2019 |
|---|---|---|
| **Salaries**[1] | 49,920 | 51,917 |
| **Benefits**[2] | 18,201 | 18,929 |
| **Travel**[3] | 1,500 | 1,500 |
| **Goods and Services**[4] | 17,000 | 15,500 |
| **Total** | 86,621 | 87,846 |

**Footnotes:**
[1,2] Salaries and 36.5% benefits for Post-Doctoral Research Associate (Dr. Sumyya Waliullah, and Dr. Jessica Waite)
[3]For frequent travel to orchard site (Quincy) where trials are being conducted
[4]Goods and services include irrigation supplies, fruit respiration chamber, basic physiological and molecular lab supplies including molecular biological enzymes and chemicals for gene expression analysis and pigment analysis, liquid nitrogen tank rental and lab consumables.

## OBJECTIVES

1. **Identify how acclimation to high light and near sunburn threshold temperatures influences fruit susceptibility to sunburn**

Two postdoctoral researchers worked on this project. In 2018, Dr. Sumyya Waliullah left for a new position at the University of Georgia after completing the summer experiments. Since then, Dr. Jessica Waite has joined the Kalcsits lab and took over responsibilities for this project under the guidance of Lee Kalcsits. Jessica has since taken a position at the USDA-ARS Tree Fruit labs in Wenatchee focused on pear molecular biology and genetics.

2. **Link physiological and biochemical changes in the fruit to sunburn development.**

In 2018 and 2019, we completed a set of experiments that included controlling fruit surface temperature and examining the impact of fruit surface temperatures on susceptibility to future sunburn inducing events. These controlled experiments will form the foundation of future lines of research into understanding the genetic and physiological controls underlying susceptibility to fruit sunburn. These experiments are important for better understanding sunburn risk for current and emerging cultivars and eventually contributing to the development of future cultivars that are less susceptible to sunburn.

3. **Use information provided on temperature and light conditions that stimulate natural resistance to guide evaporative cooling and sunburn protectant applications and reduce losses to sunburn**

Here, we tested the use of automated evaporative cooling triggers when air temperature is either 85 or 90 °F. This experiment was completed in 2020 in collaboration with Cameron Burt.

## SIGNIFICANT FINDINGS

1. **Sunburn Management:** Cooling applied when temperatures exceeded 85°F had significantly less sunburn than when cooling began when air temperatures exceeded 90°F

2. **Sunburn Management:** Honeycrisp has elevated fruit surface temperatures compared to Granny Smith, WA 38, and Cripps Pink under the same light and heat conditions. This contributes to a greater sunburn risk compared to the other three cultivars tested.

3. **Understanding Risk:** Fruit are less susceptible to sunburn early in the season due to differences in stomatal conductance (transpiration) and pigmentation.

4. **Understanding Risk:** Exposure to near threshold temperatures in June increased sunburn resistance in July and August for experiments completed in 2018.

5. **Scientific Knowledge:** Anthocyanins were found to increase in response to higher heat treatments in fruit that had received no priming stimulus, and did not respond to heat in fruit that had been previously primed.

6. **Scientific Knowledge:** Candidate genes were selected based on acclimation studied in a variety of plant species. At three days after fruit were heated, no differences were detected between treatments, suggesting either changes in gene expression occur earlier, or these genes are not involved in apple acclimation to sunburn.

## RESULTS & DISCUSSION

### Honeycrisp are less able to keep fruit cool under high light and heat conditions

Fruit surface temperatures monitored throughout the 2018 growing season indicate that there are cultivar level differences in response to light and air temperature (Figures. 1-3). This was further supported by research comparing Cripps Pink and Honeycrisp in 2020 (Figure 4). Honeycrisp apples maintain greater fruit surface temperatures under similar conditions, and under the most extreme temperature and light conditions can vary by as much as 10°F more than Cripps Pink, Granny Smith, or WA 38. Approximately three-quarters of the variation in fruit surface temperature can be explained by two variables: air temperature and light intensity. Wind speed and unknown physiological factors contribute to the other 25% of variation. For experiments conducted to determine whether fruit can physiologically acclimate to elevated fruit surface temperatures, fruit that was exposed to near threshold fruit surface temperatures in June when temperatures were relatively cool showed reduced sunburn compared to fruit that was exposed to normal conditions (Figure 5). When the temperature rapidly increased from highs in the mid-70s to about 100 °F in early July, sunburn incidence and severity was greater for unexposed fruit (Figure 5). This was true for both Honeycrisp and Granny Smith; although overall incidence was lower for Granny Smith than Honeycrisp, which further supports the observations that Honeycrisp, with elevated fruit surface temperatures, is more susceptible to sunburn. Fig. 6 shows two images of sunburn development in untreated fruit but no sunburn development in adjacent fruit that was exposed to near threshold temperatures. These conditions did not exist in 2019 and temperatures gradually increased rather than suddenly increased like in 2018. Sunburn pressure was relatively low in 2019 and a small number of days in August were conducive to the development of fruit sunburn.

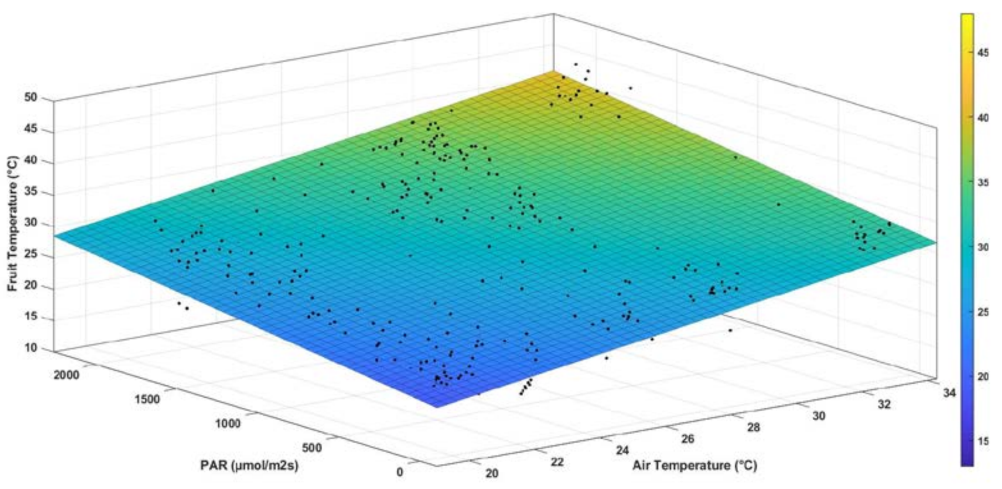


**Figure 1.** Fruit surface temperature of WA 38 fruit as a function of light intensity at the fruit surface and air temperature. Each point represents one fruit measured from June 1 through to August 24, 2018. The surface represents the best fit model using air temperature and light intensity to explain fruit surface temperature

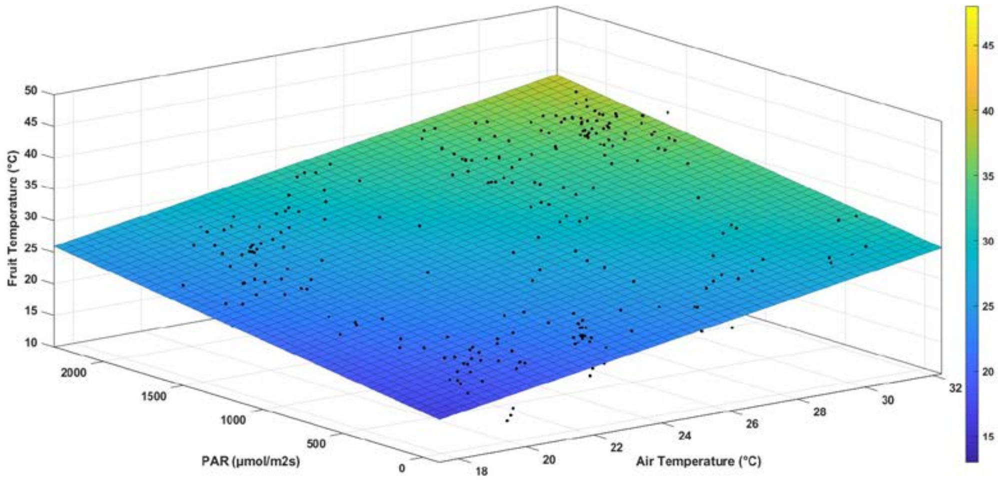


**Figure 2.** Fruit surface temperature of Granny Smith fruit as a function of light intensity at the fruit surface and air temperature. Each point represents one fruit measured from June 1 through to August 24, 2018. The surface represents the best fit model using air temperature and light intensity to explain fruit surface temperature

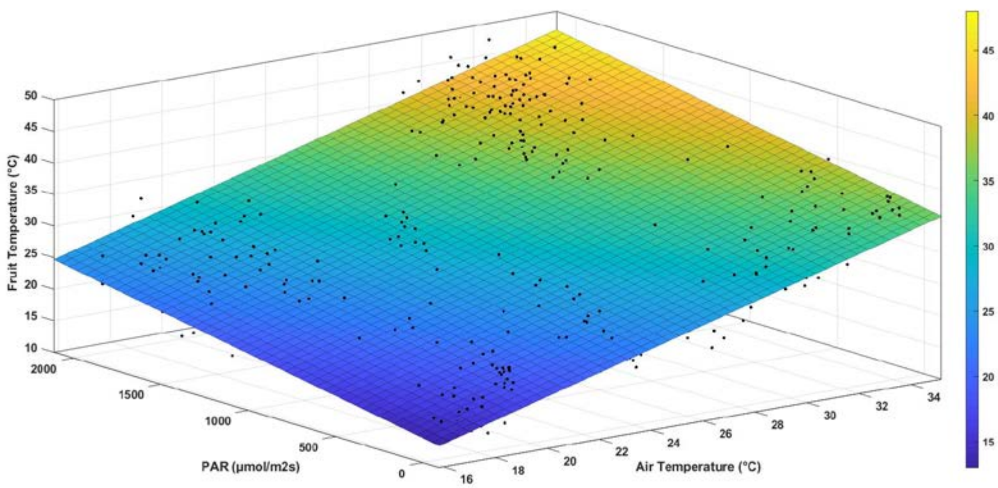


**Figure 3.** Fruit surface temperature of Honeycrisp fruit as a function of light intensity at the fruit surface and air temperature. Each point represents one fruit measured from June 1 through to August 24, 2018. The surface represents the best fit model using air temperature and light intensity to explain fruit surface temperature

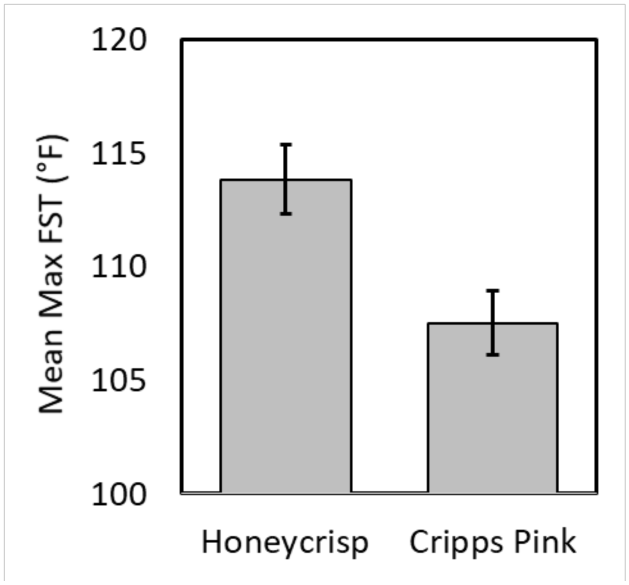


Figure 4. Mean daily maximum fruit surface temperature (FST; °F) for Honeycrisp and Cripps Pink during the period of July 3- August 10, 2020.

**<u>Evaporative cooling for Honeycrisp is best initiated at 85 °F</u>**

In 2020, solar-powered, temperature-activated solenoids were set up for a replicated experiment at the WSU Sunrise Research Orchard in Wenatchee. The orchard used was a Honeycrisp orchard that was top-worked in 2016 from Granny Smith on M9-T337. There were three replicates of each treatment; evaporative cooling activated when air temperatures reached either 85°F or 90°F compared to an uncooled control. It was set to cycle between on and off for 15 minutes on and 45 minutes off. Cooling sprinklers were standard Nelson R10's with an output rate of 45 gallons per acre per minute. Over the entire period of the middle of June to harvest, there were 402 hours when temperatures exceeded 85°F (Figure 5). That equals approximately 0.88 acre feet of water. That represents approximately 20% of the yearly irrigation needs just applied through evaporative cooling. The amount reaching the soil is limited because of cycling but the added irrigation is substantial and needs to be accounted for when making irrigation decisions. When evaporative cooling was activated when air temperatures reached 90°F, the amount of water applied was approximately 60% that of the 85°F activation temperatures. Early July had some of the highest cooling requirements but cooling was required at least one day per week for June through August. With small amounts of elevated temperatures required for sunburn damage on fruit, conservative systems are most frequently adopted. Fruit was harvested August 31, 2020. 100 pounds of fruit was picked at random from each replication. Each fruit was individually assessed for sunburn severity and incidence. Further quality metrics and bitter pit development will be assessed in January 2021. In these experiments, trees that were cooled starting at 85 had less overall sunburn and lower severity than fruit from trees where cooling started at 90 F or were uncooled overall. Cooling started at 90 F reduced the amount of severe sunburn compared to the uncovered control. While these results suggest that EC should be started at 85, other cultivars may be less susceptible compared to Honeycrisp similar to what we observed in elevated fruit surface temperatures compared to other cultivars.

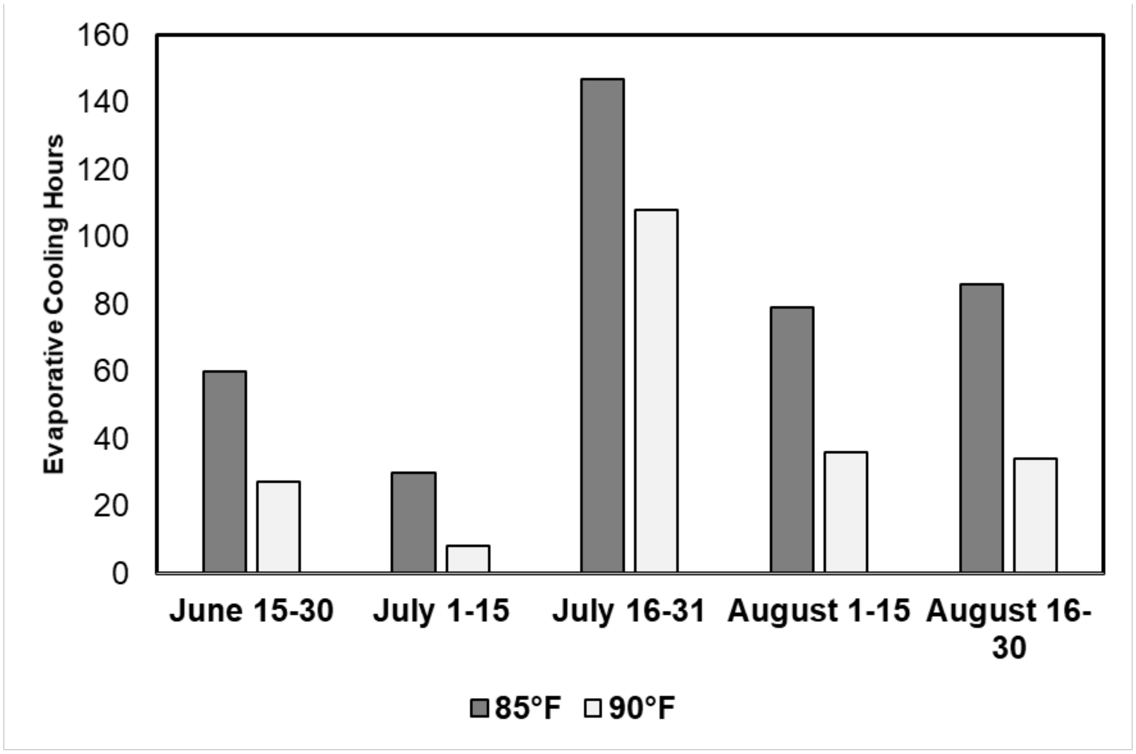


**Figure 5**. Total evaporative cooling hours for late June, July, and August of 2020 when cooling was initiated at air temperatures of either 85°F or 90°F

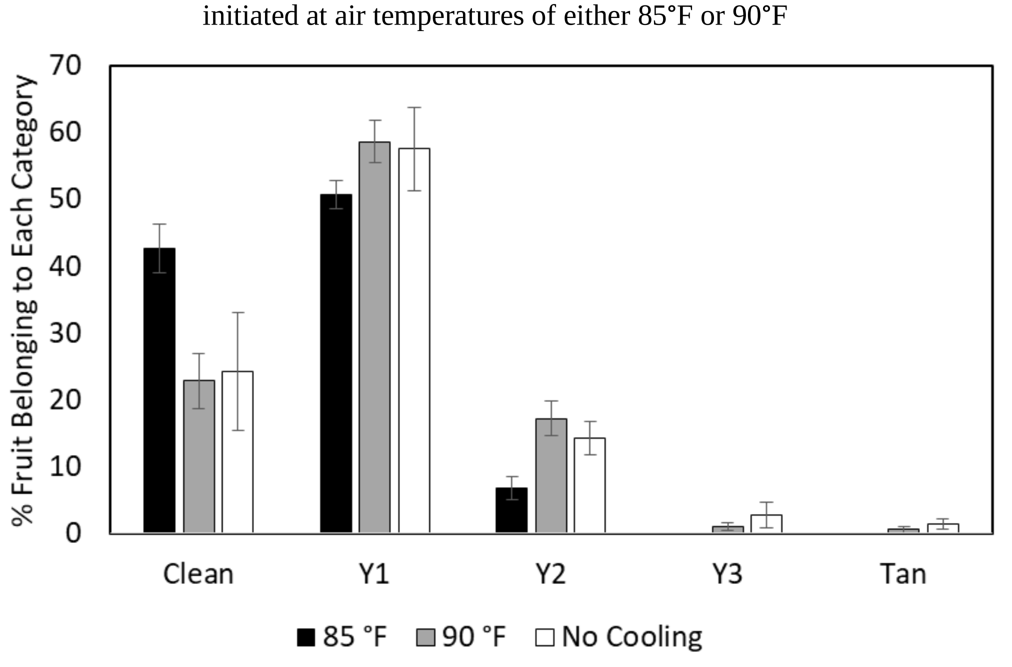

**Figure 6.** The proportion of fruit with either no sunburn (Clean) or belonging to three classes of sunburn browning (Y1-Y3) or showing leathery sunburn tanning of the fruit peel harvested from trees that were either cooled when temperatures exceeded 85 °F or 90 °F compared to an uncooled control.

**<u>Fruit can acclimate to high temperatures to resist sunburn development</u>**

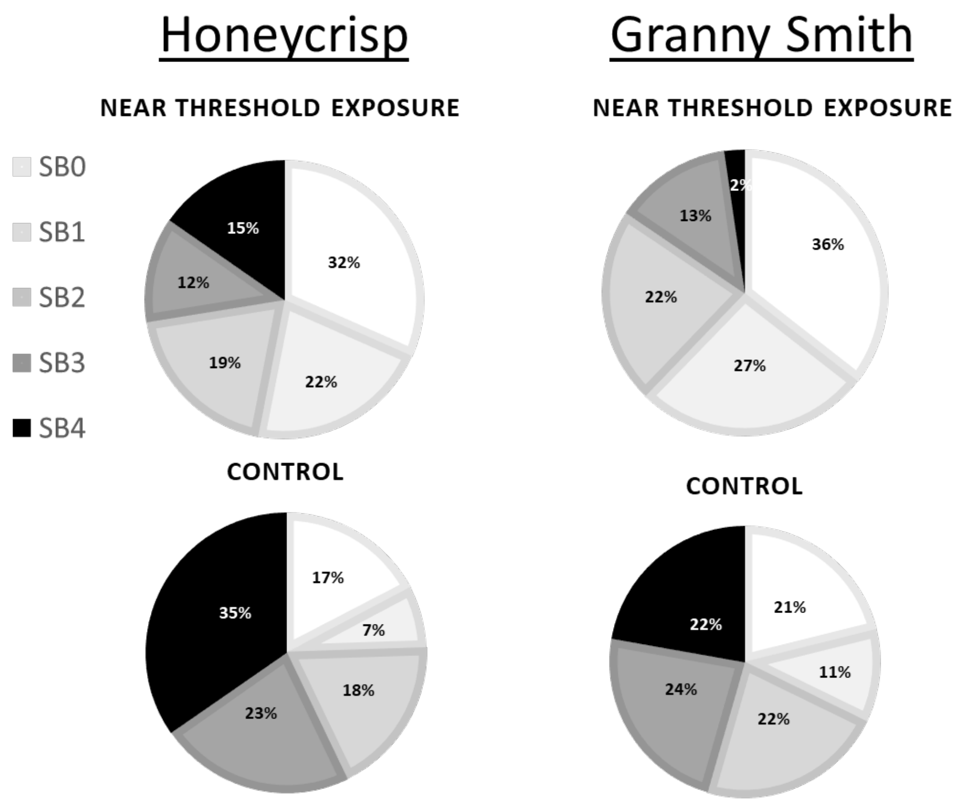


**Figure 7** Fruit sunburn severity of Honeycrisp or Granny Smith apples (N=90) after being exposed to sunburn inducing temperatures in July that were either exposed to near sunburn threshold temperatures in June or only exposed to cooler ambient conditions that were present in June, 2018. The sunburn scale used was a 5-point scale where SB0 is where there is no sunburn present and SB5 is where there is browning formed on the fruit surface.

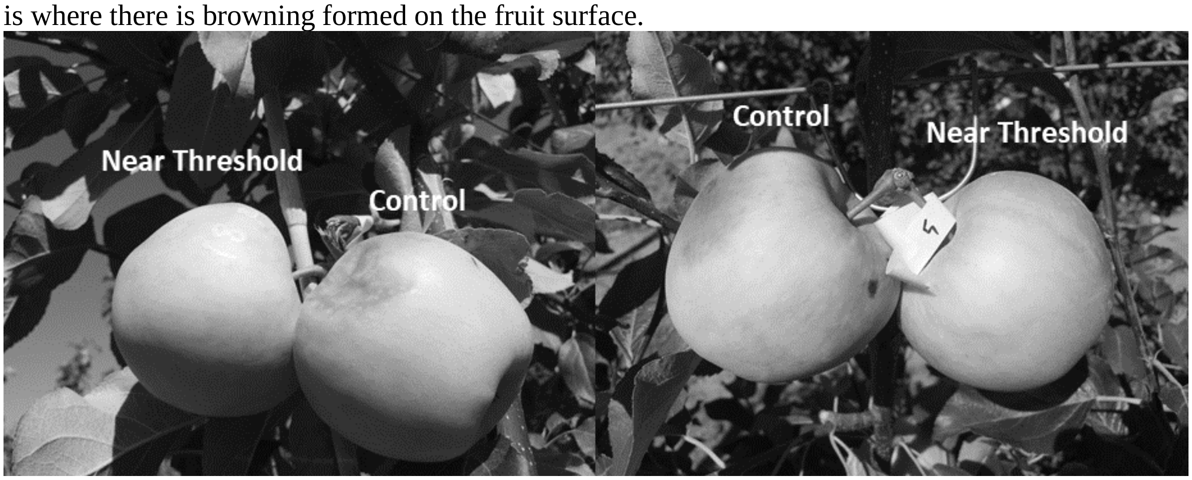

**Figure 8.** Fruit sunburn development after sudden increased in temperature during early July 2018. Adjacent fruit was either left untreated or exposed to elevated, near sunburn threshold fruit surface temperatures for one hour in June 2018. Fruit that was part of the untreated control suffered more severe sunburn than fruit that was exposed to near-threshold temperatures.

From measurements of stomatal function during fruit development, we observed that transpiration rapidly decreased in fruit during the month of June and slowly began to decrease as fruit continued to grow and mature. While changes in pigmentation during sunburn have been well documented, the changes in pigmentation that provide further protection for fruit have been less documented. Figure 7 shows changes in reflectance during fruit development where reflectance increases as fruit matures meaning that it absorbs less energy. Specifically, more green and red light is reflected. There were also differences between interior or exterior fruit where exposed fruit reflects more red light and non-exposed fruit reflects more green light. However, this will be confirmed based on a larger dataset that is being processed in December.

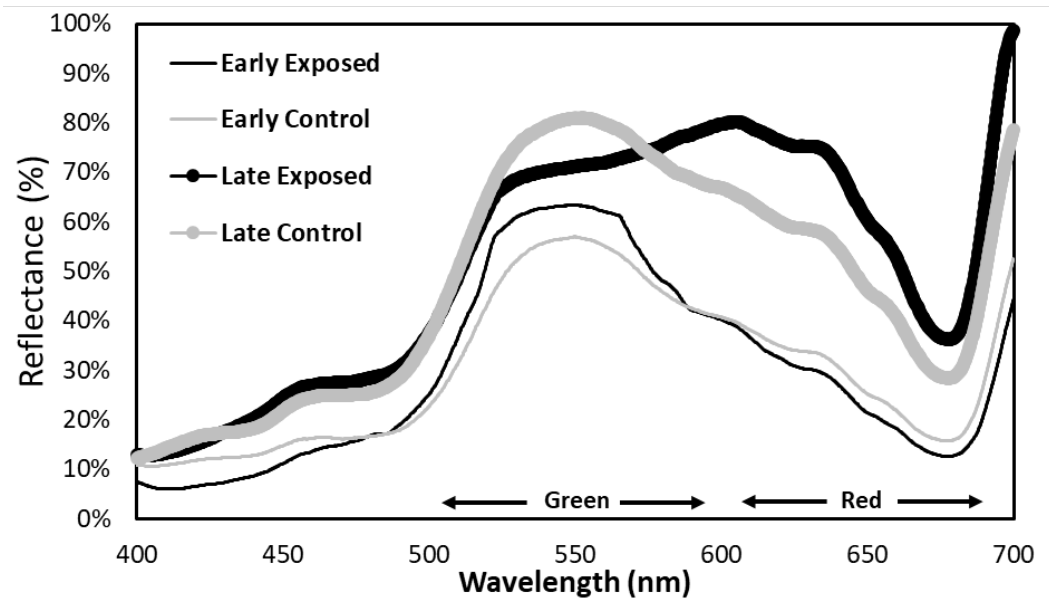


**Figure 9.** Spectral reflectance (% of incoming energy) for Honeycrisp before and after a rapid heating event for fruit that was either exposed to high radiation pressure (exposed) versus low radiation pressure (control).

For experiments conducted to determine whether fruit can physiologically acclimate to elevated fruit surface temperatures, fruit was exposed to near threshold fruit surface temperatures in June of 2018 when temperatures were relatively cool. These experiments were repeated in 2019. In addition to showing reduced sunburn compared to fruit that was exposed to normal conditions, peel tissue from these fruit also showed no increase in anthocyanin production, while fruit under normal conditions showed increased production with increased heat (Figure 13). To understand the genes involved in sunburn acclimation, we selected five candidate genes from the thermotolerance literature shown to be involved in heat stress and acclimation (Table 1). Tissue from heat-primed fruit and untreated fruit showed no significant difference in gene expression of these candidate genes three days after heat treatments (Figure 12). This could suggest that these genes are either not involved in apple heat stress acclimation, or that we did not have enough temporal resolution to see changes in expression. These experiments were repeated in 2019, with the addition of collecting tissue 24, 48, and 72 hours posttreatment to capture the dynamics of pigment accumulation and gene expression, as changes in expression may occur sooner than 3 days. During the 2019 season however, with the exception of a few days in late July and early August, temperatures throughout much of the season were not sufficient to produce much sunburn pressure on fruit, thus acclimation could not take place and control fruit were not highly stressed as in the previous season. However, due to our experimental design (Fig 10), we obtained samples from fruit that had been treated with near-threshold and above threshold temperatures, both at the beginning and late in the season, which can be used to perform an RNA-sequencing experiment in the winter of 2020 to address questions about the molecular players and pathways underlying sunburn development in apple (Fig 11), which is a largely unanswered question from our field experiments.

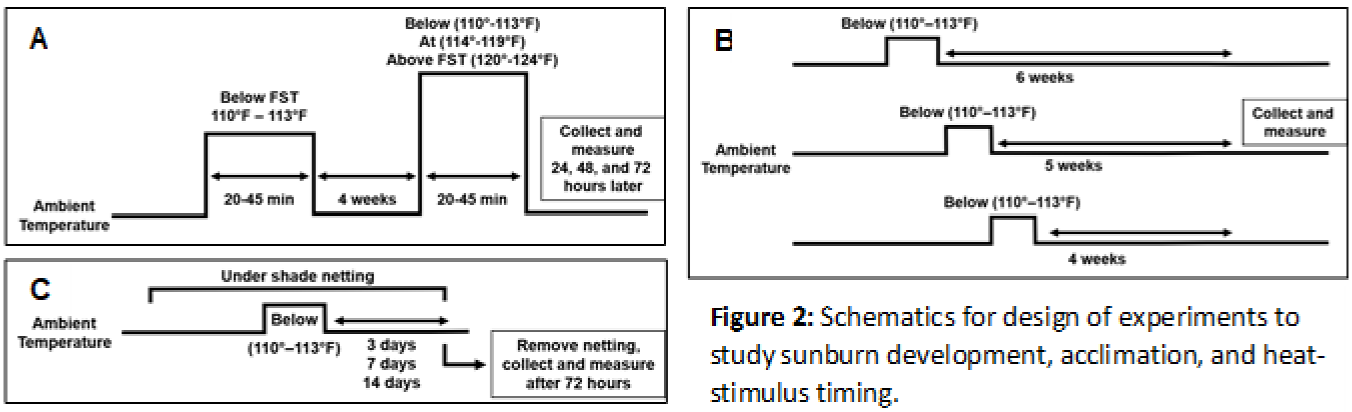


**Figure 2:** Schematics for design of experiments to study sunburn development, acclimation, and heat-stimulus timing.

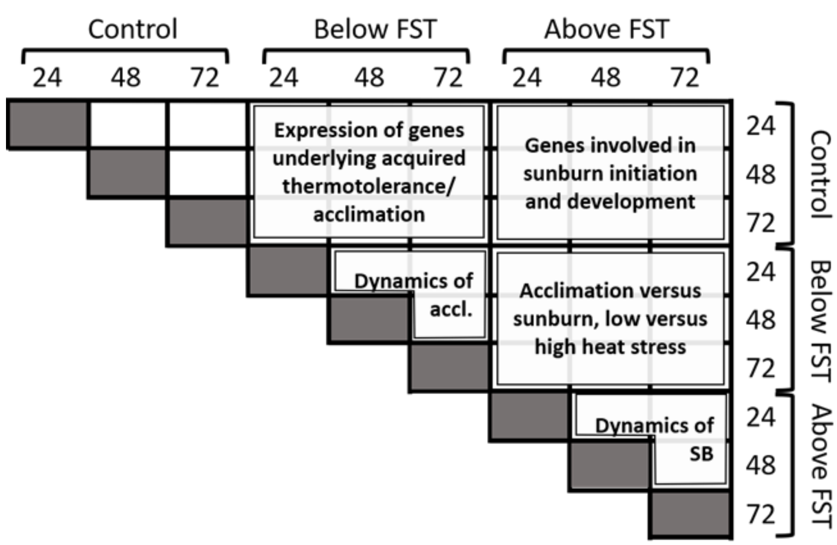


**Figure 11:** Potential comparisons to be made using RNA-sequencing. The outlined comparisons would highlight differentially expressed genes involved in acclimation, sunburn initiation and development, and useful information of the dynamics of these genes after heat treatments.

Additional experiments were designed in 2019 to understand the importance of the timing of the priming heat stimulus (Fig 10B and C). From the literature on acquired thermotolerance across plant species, the amount of time that a heat stimulus confers priming to heat stress can vary. In addition, mechanisms for short-term acquired thermotolerance (SAT), long-term acquired thermotolerance (LAT), and thermotolerance to moderately high temperatures (TMHT) involve distinct molecular pathways, and our initial experiments were not designed to tease apart which are involved in apple sunburn acclimation. These samples are currently being measured and will shed light on heat acclimation pathways that will have the potential to better inform risk in current cultivars and then, as new cultivars are released heat tolerance should also be well understood for those cultivars.

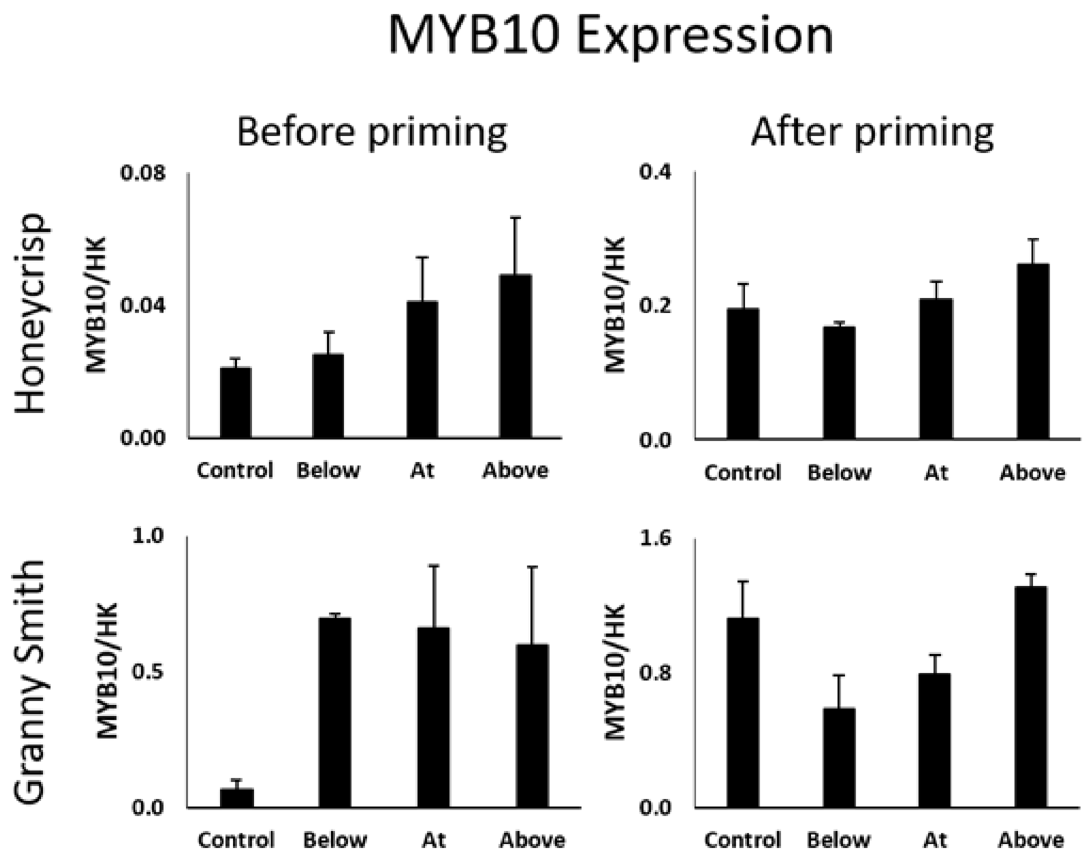


**Figure 12:** MYB10 expression in peel tissue from heat-primed fruit and fruit grown under normal conditions prior to being challenged with below-, at- and above-FST thresholds for sunburn. Similar to other genes observed, MYB10 showed no significant differences between treatment categories. This gene is involved in resistance to abiotic stress and anthocyanin production (red color)

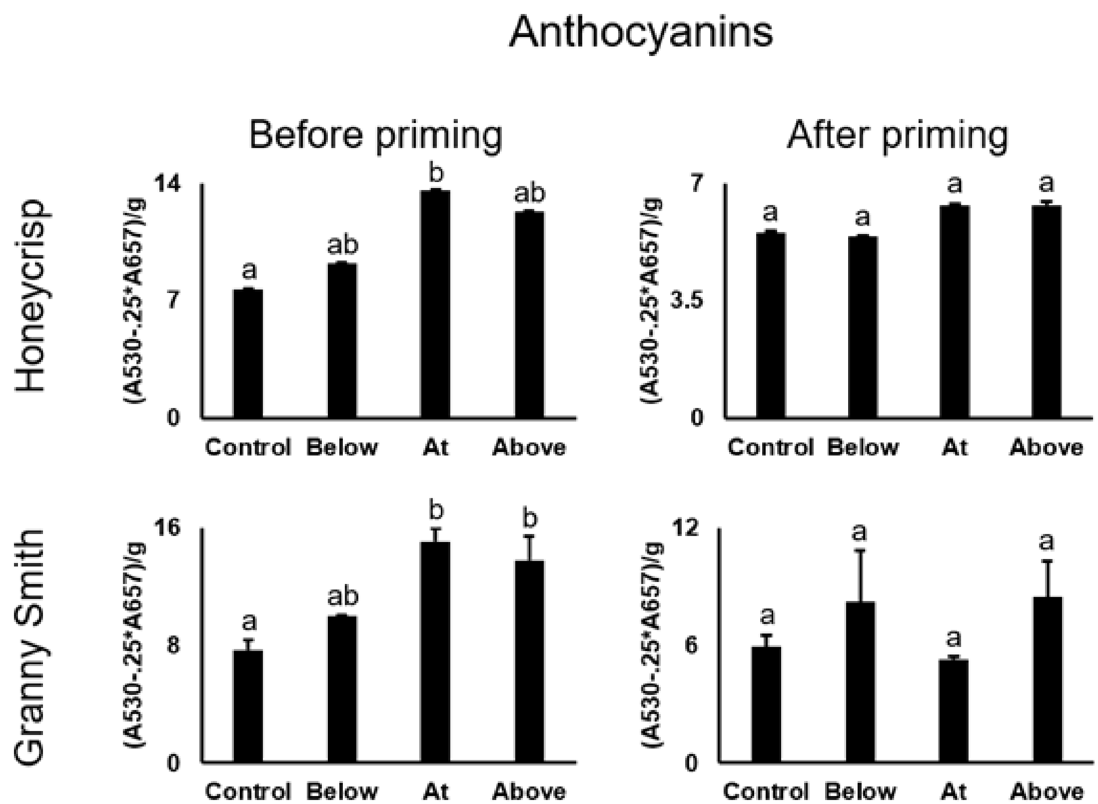


**Figure 13:** Anthocyanin levels in peel tissue from heat-primed fruit and fruit grown under normal conditions prior to being challenged with below-, at- and above-FST thresholds for sunburn. Non-primed fruit showed an increase in anthocyanins with increased heat treatments, while fruit that were primed did not.

**Table 1**. Candidate genes involved in heat stress and acquired thermotolerance

| Gene name | Candidate Gene ID | Processes involved | References |
|---|---|---|---|
| **LDOX** | MD06G1071600 | Phenylpropanoid/flavonoid pathway. Showed higher expression in sunned fruit peels. | Feng et al. Plant Phys Biochem 2013 |
| **MYB10** | MD09G1278600 | Anthocyanin biosynthesis. Peels of shaded fruit showed lower MYB10 expression and anthocyanin levels compared to sunned fruit. | Feng et al. Plant Phys Biochem 2013 |
| **APX2** | MD12G1125600 | Ascorbate peroxidase. Dependent on HSFA2, a gene required for heat shock memory after an acclimatizing stimulus. | Friedrich et al. Plant Cell Env 2018, Lamke et al. EMBO 2015, Charng et al. Plant Phys 2007. |
| **DFR1** | MD15G1024100 | Phenylpropanoid/flavonoid pathway. Showed higher expression in sunned fruit peels. | Feng et al. Plant Phys Biochem 2013 |
| **HSP17.6/ HSFA2-Like** | MD15G1209400 | Heat shock protein, similar to HSFA2, a gene required for heat shock memory after an acclimatizing stimulus. | Friedrich et al. Plant Cell Env 2018, Lamke et al. EMBO 2015, Charng et al. Plant Phys 2007. |
| **HK (reference)** | MDP0000274900 | Housekeeping gene, used as a reference for expression. | Perini et al. Mol Breed 2014. |

**Research and Extension Outputs**

Kalcsits L. **2019.** Developing Resilient Orchards. BC Agricultural Climate Adaptation Research Workshop. December 2, 2019. Kelowna, BC. **Keynote Presentation.**

Kalcsits L, Mupambi G, Waite J, Waliullah S, Reid M, Rajopalan K, Noorazar H, Jones V, and Jones M. **2019.** Impact and Mitigation of Shifting Seasons and Elevated Summer Temperatures for Apple Production in the United States. Workshop: Effects of Climate Change on Fruit Production. American Society for Horticultural Sciences Annual Meeting. Las Vegas, NV. July 25, 2019. **Invited Presentation**

Kalcsits L, Waliullah S, Mupambi G. **2018.** Taking Advantage of Climate Extremes to Grow High Quality Tree Fruit. Washington State Tree Fruit Association Horticultural Show. Yakima, WA. December 3-5, 2018. **Invited Presentation.**

Kalcsits L. **2019**. Climate Change Brings New Challenges for the Pacific Northwest Tree Fruit. October 2, 2019. Washington State STEM Education Innovation Alliance. Wenatchee, WA.

Kalcsits L. **2019**. Bitter pit and sunburn mitigation in apple. August 7, 2019. WSU Sunrise Research Orchard Field Day. Wenatchee, WA

Waite J, Waliullah S, Kalcsits L. **2019.** Physiological changes associated with heat stress acclimation for developing apple fruit. American Society for Plant Biologists Annual Meeting. The Environmental and Ecological Plant Physiology Section Meeting. San Jose, CA. August 3-7, 2019.

Waite J, Waliullah S, Kalcsits L. **2019.** Physiological changes associated with heat stress acclimation for developing apple fruit. American Society for Plant Biologists Annual Meeting. San Jose, CA. August 3-7, 2019.

In 2021, Extension programming will be developed to guide sunburn mitigation practices and the use of evaporative cooling in orchards. One component will be communicating when sunburn risk is the highest. Another section will include mitigation practices and key cultivars that are important to carefully control fruit surface temperature to limit sunburn losses.

**Leveraged Funding and future grant applications**

FUNDED - 2020-2022 'Modeling Orchard Effects on Meteorological Measurements' (Co-PI; PI – Dr. Dave Brown) WTFRC Technology Review. ($206,100)
FUNDED - 2018-2021 'Risk modelling for a future climate for growing tree fruit in WA State.' (Co-PI; PI- Dr. Kirti Rajagopalan). Washington State Department of Agriculture Specialty Crop Block Grant. ($249,502).

PENDING - 2021-2022 'Sunburn Risk Management Strategies for the Pacific Northwest Apple Producers' Western SARE ($50,000)
PENDING - 2021-2022 'Is netting removal prior to harvest to improve color a risk for sunburn' WTFRC Apple Review ($75,882)
PENDING - 2021-2026 'Enhancing resilience of U.S. pome fruit production to extreme temperatures in a changing climate' USDA-NIFA SCRI. Project Director. ($4,700,000)

EXECUTIVE SUMMARY

Project title: How does fruit acclimation to sunburn affect sunburn management?

Key words: WA 38, heat, evaporative cooling, Honeycrisp, Granny Smith

Abstract: Sunburn is the leading cause of losses across all apple cultivars grown in Washington State. As fruit develops during the growing season, its susceptibility to sunburn browning changes depending on internal physiological factors as well as the environment. This project sought to determine how apple cultivars differ in their susceptibility to sunburn and what changes occur in response to environmental conditions that lead to varying degrees of sunburn severity across different growing seasons. This information will help contribute to enhanced sunburn risk modelling, more efficient mitigation practices as well as information that will eventually lead to the identification of cultivars that are less susceptible to sunburn. During fruit developments, transpiration is elevated during the 6 weeks following petal fall. Then, transpiration dramatically decreases as fruit expands and develops thicker cuticle layers that limit cooling from transpiration. Here, we report that Honeycrisp has elevated sunburn risk compared to other cultivars. This is because the fruit surface temperatures of Honeycrisp fruit are higher than fruit from WA 38, Granny Smith, and Cripps Pink under the same environmental conditions. Through experiments conducted in 2019 and 2020, we identified the anthocyanin pathway as a potential contributor to acclimation to high temperatures. This pathway is important in plants for heat dissipation under high energy inputs which is what may help keep fruit surfaces from developing sunburn symptoms under high light or temperature conditions. We will be continuing this research in other projects and look forward to developing physiological knowledge in this area in the future. Furthermore, as fruit ripens and light harvesting chlorophyll content is reduced in the apple peel, fruit becomes more susceptible to sunburn even as red color continues to develop. The applied side of this project proposed to identify whether sunburn incidence is affected in Honeycrisp apple when evaporative cooling is initiated at either 85 °F or 90 °F. We found that cooling initiated at 85 °F was more effective at reducing sunburn although both cooling treatments reduced sever sunburn compared to the uncooled control. Water use for R10s cycled every 15 minutes and off for 45 minutes applied a total of 0.8 acre feet of water in June, July, and August. This accounts for approximately 20% of the water demand for an orchard in an average year and needs to be accounted for in irrigation decisions, especially for Honeycrisp apple. Switching to low water volume fogging or evaporative cooling systems could substantially reduce the amount of water applied to an orchard and keep most of it in the tree canopy where it has the greatest impact on fruit surface temperatures. Overall, we identified key differences in susceptibility in commercially important cultivars grown in Washington State. We determined that susceptibility changes as fruit develops and in response to the environment. Lastly, we confirmed that the conservative practice of turning on evaporative cooling at 85 °F is important for reducing sunburn losses in Honeycrisp apple and that activation at higher temperatures lead to higher losses from sunburn. Ongoing work will focus on better understanding fruit acclimation to heat and the pathways responsible. These efforts will enhance sunburn risk models and forecasting to better protect fruit from sunburn in Washington State.