FINAL PROJECT REPORT
WTFRC Project Number: AP-16-102

Project Title: Risk assessment for delayed sunburn and sunscald

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Budget: **Year 1**: $67,427 **Year 2**: $70,865 **Year 3**: $72,595

Collaborators: Christine McTavish, Omar Hernández, Brenton Poirier, Loren Honaas

Other funding sources

Agency Name: CONICYT, Chile (proposed)
Amt. awarded: $88,700 (total over 3 years)
Notes: Funds for supplies and materials, travel, and analytical services.

Budget
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
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Item	**2016**	**2017**	**2018**
Salaries	$40,757	$43,342	$44,620
Benefits	$13,450	$14,303	$14,755
Wages			
Benefits			
Equipment			
Supplies			
Travel	$2,000	$2,000	$2,000
Miscellaneous*	$11,220	$11,220	$11,220
Plot Fees			
Total	$67,427	$70,865	$72,595

Footnotes: One-third instrument service contract

Objectives:

1. Identify changes in apple peel chemistry associated with response to light prior to and during cold storage.
2. Determine if changes in peel chemistry are specifically indicative of delayed sunscald and other sun-related postharvest peel disorder risk prior to symptom development.
3. Develop protocols to establish tissue viability before and during cold storage.

SIGNIFICANT FINDINGS:

1. Peel appearance (symptoms) changes in different ways during storage depending upon cultivar.
2. Peel chemistry changes differentially depending upon pre-harvest sun exposure, indicating continued stress on the exposed side of the fruit during cold storage.
3. Sun exposure alters cutin consistency and, possibly, cutin structure and, thereby, epidermal cell protection.
4. Heating in the orchard can lead to symptoms with similar appearance and etiology to sunscald.
5. Heating in the orchard can inhibit sunscald development.
6. Sunscald severity can be altered after harvest.
7. Simple UV reflectance imaging targeting metabolites associated with light exposure can non-destructively detect relative sun exposure.
8. Identifying changes in multiple metabolic targets detected in the UV-vis-NIR range may be a reliable basis for non-destructive detection of sun stress and/or cumulative sun exposure.

Methods:

2016-2017 (primarily Objective 1)

The influence of the pre-harvest light environment alone and during the transition to cold storage on peel metabolism is different among apple cultivars. Granny Smith, Gala, September Fuji, and Honeycrisp were picked at commercial harvest (see 2017 continuing report) and stored at 33 ºF for up to 6 months. At 0, 2, 4, and 8 weeks (and then monthly until 6 months) sun damage and/or sunscald incidence was monitored and peel chemistry data were analyzed to determine differential changes between the exposed and unexposed sides of the fruit upon chilling stress, possibly linking specific changes in peel chemistry with specific delayed conditions. Additional samples consisting exclusively of sunscald tissue were analyzed to determine where peel chemistry was most linked with the disorder.

2017-2018 (Objectives 1 and 2)

At 4, 2, and 1 week(s) before commercial harvest, 180 hand-thinned Granny Smith apples in the Sunrise experimental orchard were heated on both the sun facing and shaded sides to 130 ºF for 3 minutes (60 apples per timepoint). Fifteen fruit were bagged with green sleeved paper apple bags at each time point and remained so until harvest. Another 15 fruit per timepoint were heated to 130 ºF on the sun facing and shaded sides and then bagged until harvest at each timepoint for a grand total of 270 apples (90 apples treated per timepoint, excluding controls). Four days before harvest, 45 additional fruit were treated at 130, 115, and 100 ºF (15 apples at each temperature). Unheated controls were included at every treatment date. Injury on each fruit was tracked using image analysis as well a rating system (see 2017 continuing report). Peel from the front and back was sampled at 0, 3, and 6 months and specifically from injured zones to determine the levels of compounds most associated with injury, as well as if compounds associated with sunscald in year 1 were more associated with sun stress, heat stress, or a combination. Peel from both heat damage and sunscald from unheated fruit were compared.

2018-2019 (Objectives 1-3)
Twenty-one hand thinned Granny Smith apples per timepoint were heat treated as detailed above to 125 ºF for 3 min at 2 months, 1 month and 2 weeks prior to harvest to clarify last season's results using an optimized temperature. Additionally, 100 apples were bagged at each timepoint to determine when sun damage leading sunscald may have occurred for a total of 363 apples for the experiment. Temperature optimization improved our assessment of orchard heat and the bagging trial was improved by a better plot design.

A technique to profile cutin composition using Granny Smith peel powder samples from year 1 was developed and these samples analyzed. Briefly, free metabolites are washed from the peel using multiple solvents. The resulting powder is freeze dried followed by incubation in an enzyme buffer to rid the sample of pectin, cellulose, and hemicellulose leaving primarily cutin. The enzyme buffer is changed 5 times over 2 weeks. The cutin is then hydrolyzed, partitioned, the fractions resuspended, and analyzed using 2 different LC-MS analyses. This analysis detected expected aliphatic and hydroxycinnamoyl components as well as some entirely novel monomers.

Samples were taken from sunscalded, shade side, and sun side undamaged peel from the 2017-2018 heat treated apples using a biopsy punch and frozen in cryo-matrix for cryo-sectioning, staining, and confocal microscopic imaging of the cuticle layer. The samples were sectioned by Dr. Loren Honaas using the Leica Cryo Jane. The cuticle was stained and imaged using a Zeiss LSM. Images provide a detailed physical 3-D physical assessment of the cuticle.

Sunburned Granny Smith apples were selected from a commercial lot. Apples were warmed overnight to room temperature and sealed in 1gallon pickle jars and the CO_2 concentration was raised using $^{13}CO_2$ to 2% for 3 min. Apples were removed from jars and peeled immediately and at 4, 8, 24, 48, and 36 h. Peel metabolites were analyzed from frozen peel powder using our polar analysis for sugars, organic acids, amino acids (Leisso et al., 2016) to detect presence of the label through pathways responsible for life-sustaining energy production in the peel. This evaluation was repeated a second time in year 3 using stainless steel chambers linked together for a 15 min $^{13}CO_2$ treatment. Apples were sampled until 72 h for this experiment.

A subset of sunburned Granny Smith were selected 2 weeks after harvest and treated with combinations different test active ingredients dissolved in carrier and surfactant to form emulsions when added to water. Controls only contained carrier and surfactant. One tray (per treatment) was treated with each combination. Additional apples were treated with half active ingredient and half untreated.

Sunscald prediction model, Vis-NIR reflectance characterization, and targeted near UV imaging
Using 90 Granny Smith fruit from 5 different lots, a model to predict delayed sunscald after 6 months 33 ºF air storage based on the degree of sun exposure at harvest has been developed for Chilean apple producers by the Torres laboratory. Ten other Granny Smith lots were picked from bins for analysis using a Vis-NIR reflectance spectrometer at harvest. Sunscald ratings were taken at 4 months of air storage. Vis-NIR spectra from 108 fruit from each lot were incorporated into existing models from Chile and were tested against actual sunscald incidence. An additional tool was developed using a camera adapted to detect specific wavelengths of light. Target metabolites associated with sunscald risk absorb light in specific wavelengths. Peel will be taken from regions of different absorbance in this bandpass (region of the spectrum) to verify if target peel chemicals are represented by absorption (darkness).

RESULTS AND DISCUSSION:

Storage disorder symptoms resulting from solar stress are cultivar specific
Evaluation of the sun damage incidence indicated differential changes of appearance depending on sun exposure of Honeycrisp and Granny Smith (Fig. 1). For example, Honeycrisp developed severe lenticel blotch on the sun side of many fruit and Granny Smith developed sunscald during the 6 months air storage. These changes and the timing of the changes in every one of these cultivars reference those from multiple previous studies. It is important to note that solar radiation also includes heat which is thought to be a principal cause of sunburn rather than only ultraviolet/visible light. Delayed sunscald, defined by the progressive darkening or browning of the exposed side, is thought to be the continuation of the effects of pre-harvest irradiation well into storage and perhaps cold stress.

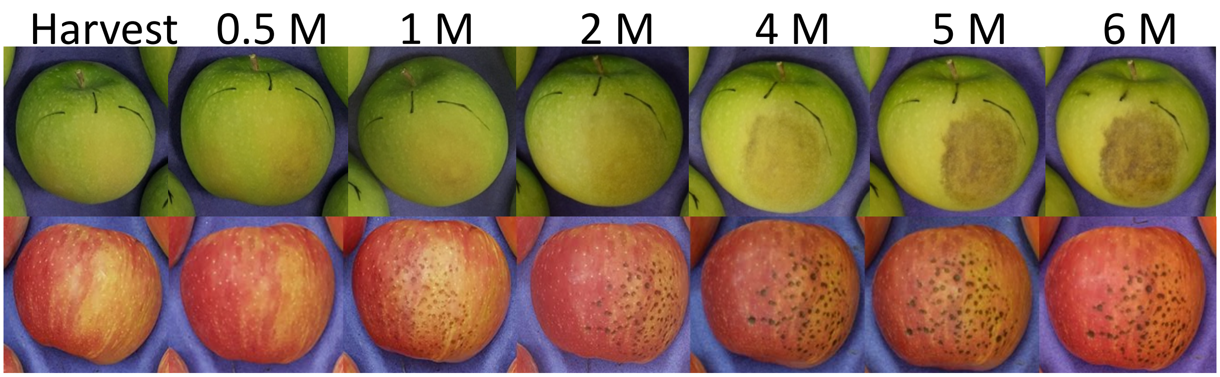

Fig. 1. Progression of peel disorders occurring on the sun side of 'Granny Smith' (sunscald) and 'Honeycrisp' (lenticel blotch) apples. In each case, the injury was not present at harvest and began to develop after 1 month in 33 ºF air storage.

Sunscald development both caused and inhibited by heat
During the first season of our trial, we noticed that a disorder similar in appearance to sunscald occurred following 3 months of air storage on peel that contacted a heated surface (which was?) prior to storage. Our subsequent treatments in year 2 using a heat gun (130ºF for 3 min) produced severe injury immediately following treatment at all treatment dates (1 month prior to harvest, 2 weeks, 1 week) in most, but not all, cases. Also, in most cases, the injury worsened during the 6 months of air storage and had a pronounced "halo" of less colored (both red and green) peel around it (Fig. 2). In a few cases, where injury was not as severe, it had an appearance typical of sunscald. The most interesting result of all was the sunscald-free tissue within the halo where heat injury overlapped with natural sunscald on the sun side of a few apples (Fig. 2B). It appears that peel that was damaged by the sun and subsequently treated with the heat gun was rendered resistant to developing sunscald.

Fig. 2. (A) Granny Smith apple treated with a heat gun until reaching 130 ºF for 3 min while on the tree 1 week before harvest and stored in air for 7 months. Apples were treated on both the sun facing (upper) and shade side (lower). Apples were imaged monthly. Damage severity increased with storage although sunscald on the exposed side (A and B) was inhibited in the area immediately surrounding the heat treatment damage (halo). This indicates that sunscald symptoms can be inhibited.

The experiment was refined in year 3 using an improved orchard block design as well as an optimized heat treatment temperature (125ºF). Results-to-date support those from year 2 although, the symptoms provoked by the lower temperature heat treatment appeared approximately a month into storage and, in many cases, were similar in appearance to sunscald on both sides of the fruit. Prior to symptom development, the treated region with greater chlorophyll degreening surrounded by a halo of slightly more discolored peel was apparent, appearing much like slightly sun damaged peel in the orchard. Sunscald also began to appear on the sun side of some un(heat)treated apples at the same time.

These results indicate heat likely contributes more to sunscald incidence than ultraviolet/visible light and, as is the case with sunburn, warmer air temperatures enhance the risk. However, unlike sunburn,

Fig 3. Drenching with soap and water can impact sunscald development on Granny Smith. Apples were selected for sun exposure, treated with carrier and surfactant in water (right) or left untreated (left). While we do not suggest treating apples with high rates of carrier and surfactant, this is one instance indicating sunscald outcome can be altered by postharvest factors and may be worth further examination in future research.

this injury can occur in areas where there is little evidence of sun-stress at harvest. In year 3, heat treatment largely simulated sunscald symptom appearance and disorder progression. As in the

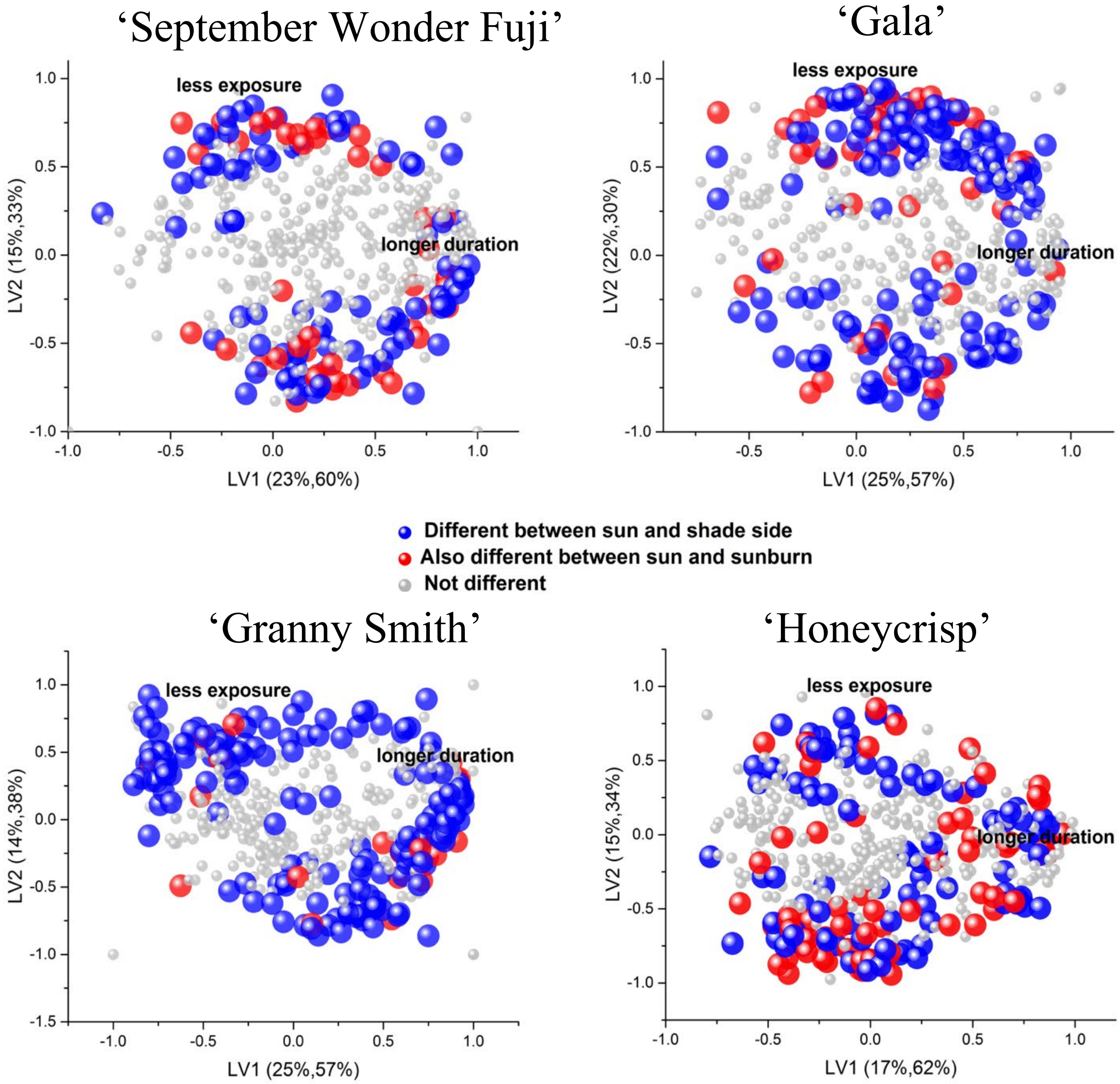

Fig. 4. Peel chemistry during 6 months air storage at 33 F is different depending upon prior sun exposure. This analysis (Partial Least Squares-Discriminate Analysis) of sun-facing and shaded peel of 'September Wonder Fuji', 'Gala', 'Granny Smith', and 'Honeycrisp' indicates how different sun facing, shaded, and sunburned tissue reacts to cold storage. Small gray bubbles indicate metabolites that had the same level in sun-facing and shaded peel. Larger bubbles indicate metabolites with levels different in sun-facing and shaded peel (blue and red) as well as metabolites different in sun-facing peel with and without sunburn (red only). The position of the bubbles on this graph indicate which sun exposure chemicals are associated with and whether levels increase or decrease during storage. We can use this to find chemistries associated with structure and physiological processes. We can also determine targets by which we can sort fruit according to cumulative sun exposure.

previous season, heating provoked an either physical and/or chemical response that renders a portion of the peel of some apples to be resistant to developing sunscald. This indicates that heat can actually correct an earlier sunscald-provoking event, although there is still no evidence of what conditions make one event damaging and the other curative. Ongoing activities are attempting to answer this and what physical and chemical changes occur upon heating, as well as how closely related the heat gun and sun provoked symptoms are to each other.

Sunscald development was reduced by drenching with a solution containing carrier and surfactant.
In a separate experiment aimed at altering sunscald development by attempting to alter cuticle composition after harvest using natural chemicals, we found that the carrier and surfactant we used for formulation reduced sunscald formation (Fig. 3, right) compared with the untreated control (Fig. 3, left). These preliminary results indicate that sunscald is more likely related to a chemical changes that alter the physical properties of the surface of the epidermis. More importantly, it emphasized that sunscald development can be altered after harvest. Far more evidence is required to validate the utility of this finding as many more factors are likely impacting this result.

Differences in natural peel chemistry during storage are linked with preharvest sun exposure
To investigate any difference in chemical composition during the first 6 months of storage caused by the combination of cold stress and preharvest sun exposure, peel from either side of September Fuji, Gala, Granny Smith, and Honeycrisp was sampled during storage as outlined above. Sun exposure impacted levels of a number of chemicals produced by multiple metabolic pathways and residing in different layers of apple peel. Aside from many of the differences in levels of both water soluble and oily pigments, levels of compounds residing in the wax layer, cellular membranes, chemicals that act as signals to muster defensive responses, and aroma differed depending upon sun exposure (Fig. 4). Using a statistical analysis that finds the main influence of experimental factors (treatments or differences in appearance we expect or employ in our tests), we determined that peel differed depending upon sun exposure. We distinguished peel according to sun exposure for all cultivars on this basis. Peel chemistry continued to diverge between sides of the fruit during storage. Honeycrisp and Fuji were more mature when picked and the contrast between sides was not as dramatic. Some of the major differences were the elevated levels of pigments and related compounds including quercetin glycosides known to be associated with light exposure and sun damage. Other compounds included volatile metabolites that are linked with oxidative stress caused by high light in leaves, suggesting oxidative events responsible for the genesis of these compounds continue after the fruit have been removed from the orchard. Possibly some of the most striking differences were levels of compounds that potentially modify the structure of the wax and cutin layer on the outside of the fruit. Some of these metabolites, the quercetin glycosides, may make good targets for non-destructive sorting.

Cutin chemistry is altered by sun exposure and changes continue during storage

In order to answer questions about actual changes to the cutin, we developed a method to extract and hydrolyze this otherwise insoluble natural fruit coating from the frozen peel powder samples to evaluate the freely soluble chemicals. Like the freely extractable peel chemicals, the chemicals

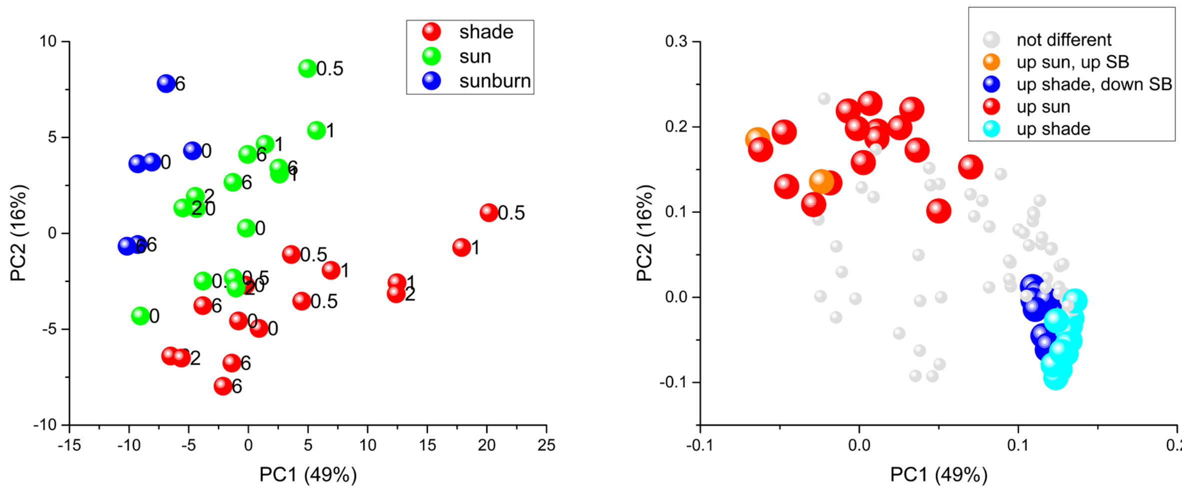

Fig. 5. The chemical composition cutin, the natural plastic plant coating, is different depending upon sun exposure and sunburn. In this analysis, Granny Smith peel was sampled from sun facing and shaded sides and stored in 33ºF air for 6 months. Sunburned peel was also analyzed at 0 and 6 months only. Cutin isolated over 2 weeks then prepared by hydrolyzing it so that our instruments could analyze is chemical structure. The figure on the left shows that sun facing, shaded, and sunburned peel were different over the entire storage period (the storage duration is included beside each bubble). The right-hand figure indicates individual chemicals that make up cutin associated with cutin coating sun-facing peel (red and orange) or cutin coating shaded peel (blue and turquoise). These differences could impact the physical properties of cutin such as elasticity or porosity that could impact liquid and gas exchange.

comprising 'Granny Smith' cutin were very different depending upon sun exposure (Fig. 5). The main components of apple cutin are lipophilic fatty acids linked together producing a polyester polymer similar to some plastics. Also, comprising a small part of this polymer are hydroxycinnamates of which coniferol, an alcohol derived from ferulic acid, was higher in the cutin of the sun side when compared to the shade side. Ferulic acid is purportedly higher with sun exposure of waxy leaves but this is the first report of that in apple. Ferulic acid may provide some sun protection in the UV-B range. Some new cutin components were also discovered that are enhanced in the sun side peel. Like the acylated hydroxycinnamates, these appear to be built from acylated monomers that are knitted into the cutin polymer. Even though it has been hypothesized that the hydroxycinnamates confer some sun protection in other species and organs, the impact that these chemical constituency changes have on cutin structure and function is not necessarily clear. Other evidence indicates changes in relative concentration of these could alter the "breathability" or consistency of these layers at different temperatures.

Micrographic analysis of sunscalded cuticle reveals extra cuticle deposition and pitted cutin

Preliminary results from our micrographic analysis of Granny Smith cuticle with sunscald reveals at least one physical difference compared with healthy peel on either side of the apple. While results

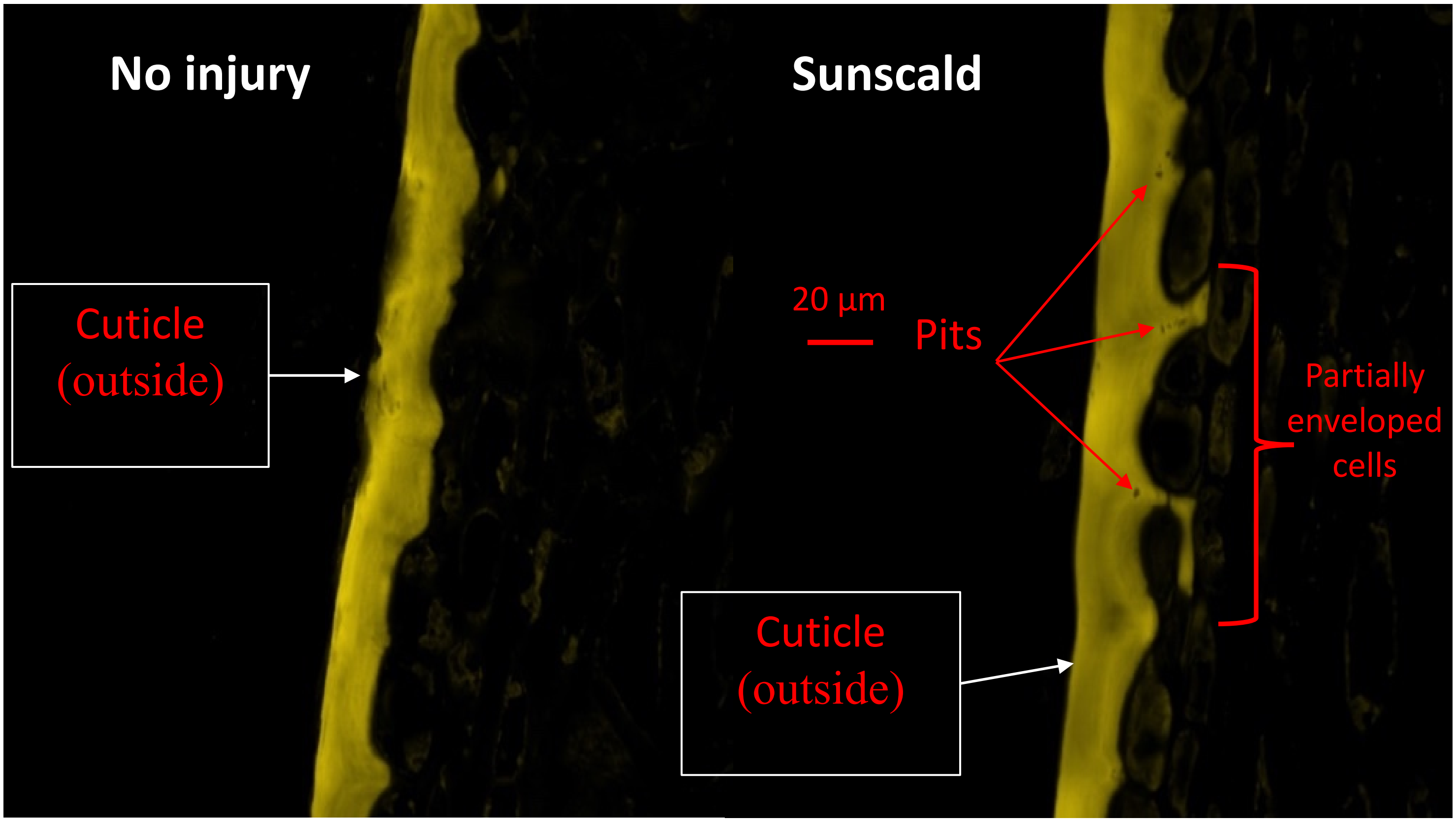

Fig. 6. (A and insets) Examples of natural peel chemicals with higher levels in the sun facing side of Granny Smith peel. These include quercetin glycosides which are a potential target for non-destructive detection as well as hydroxycinnamoyl esters that are likely building blocks of cutin, the external "plastic" coating around plant organs. These and other chemicals with a similar modification appear to be related to sun stress. We have also found that these compounds are actually integrated into the cutin itself. Their impact on the structure and function is unknown. (B) Preliminary micrographic evidence (Auromine O stain, 40X, 2 µm optical section) indicates cutin is deposited deeper in the peel and there are more defects (pits) in the layer when tissue is sunscalded.

were inconclusive regarding cuticle thickness, areas where the outer layer of epidermal cells are transected or even enveloped by cutin are more prevalent in sunscalded peel after 6 months of storage in air (Fig. 6). Also, cutin deposited in many of sub-epidermal regions contained pits and channels appearing weakened or degraded. Results point to a change in cutin deposition as an outcome of surface damage and cutin quality, possibly as an outcome of cutin composition.

Non-destructive detection of natural chemicals at harvest linked with sun stress

Sunscald prediction models using Vis/NIR reflection of both non-sunburned and sunburned apples at harvest (Grandón et al, in press) were developed by Dr. Torres's group. These models focus on relatively depleted levels of chlorophyll (green color) and anthocyanin (red color) as well as enhanced levels of carotenoid (yellow and orange color) associated with peel at risk for developing sunscald. These models were tested on lots from two local packing sheds. Both models tested revealed significant differences in accumulated reflectance between fruit that did and did not develop sunscald after storage (not shown) indicating they may be useful, with further development, alone or as part of a non-destructive prediction test for Granny Smith in Washington state.

Better non-destructive sun-related postharvest disorder assessment tools will likely focus on additional target metabolites that are even more tightly linked with sun stress compromised peel. Quercetin glycosides have a unique absorption spectrum (less interference), therefore, we chose to

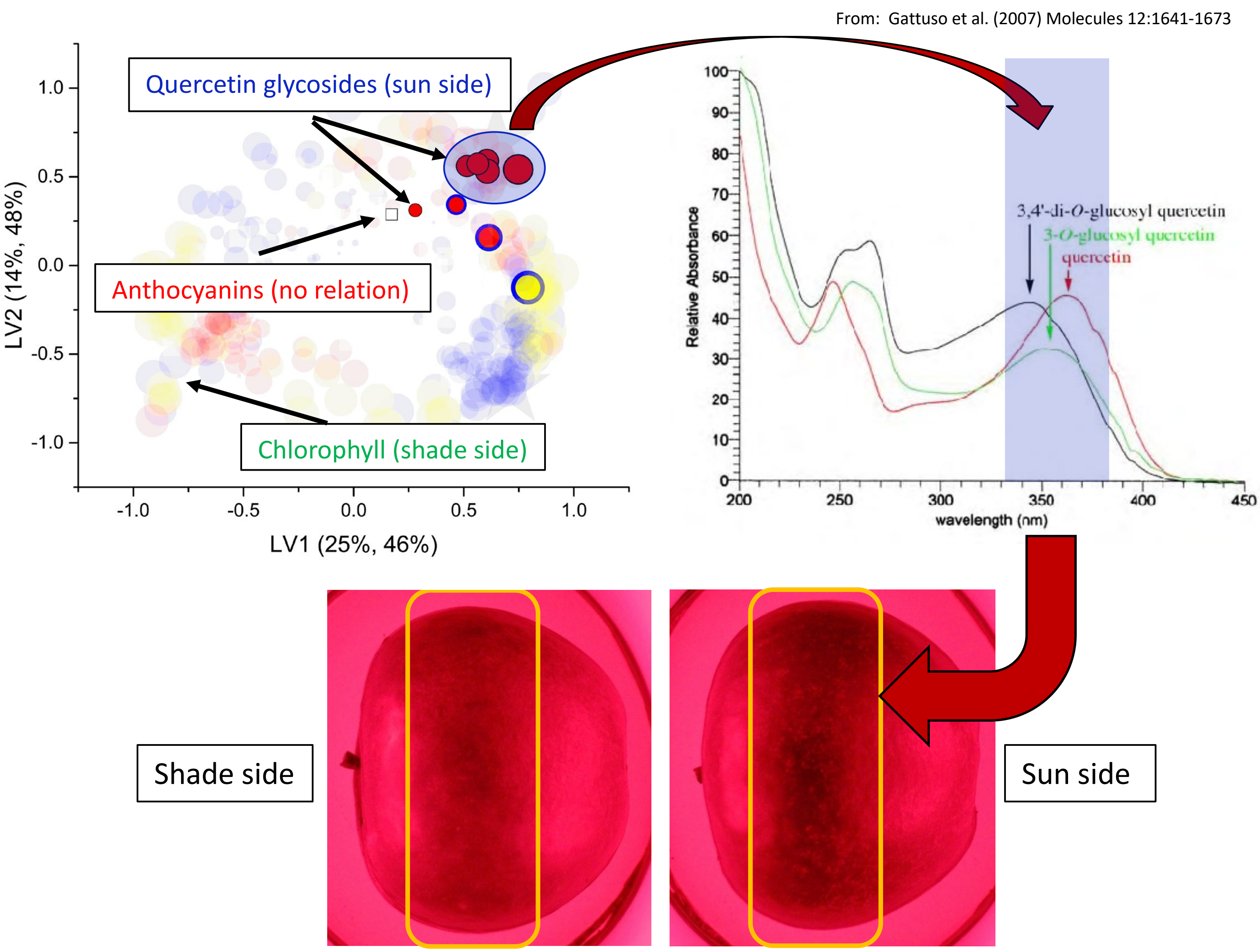

Fig. 7. Levels of apple peel chemicals associated with cumulative sun exposure can be assessed non-destructively. Quercetin glycosides accumulate in peel exposed to sun (upper left-hand figure). These natural peel chemicals absorb light at wavelengths to low to see by the human eye. The wavelengths that absorb the most energy are unique in that few other compounds in apple peel absorb light in the same wavelengths so any light absorbed in this region would be highly related to quercetin glycoside content (upper right-hand figure). A modified camera with a filter that images mostly light from these wavelengths can preferencially image these chemicals in Granny Smith peel (lower figure). Because these chemicals absorb more light at these wavelengths, the image appears darker in the region where higher concentrations are present as on the sun-facing side (lower right) as opposed to the shaded side (lower left) which is lighter.

target this area of the near ultraviolet spectrum to image peel according to light exposure. We modified a digital camera to acquire images within this absorption range. Our preliminary results indicate that peel on the exposed side is darker or absorbs more light within this spectral band ostensibly due to the elevated presence of quercetin glycosides, and fruit from internal portions of the tree, while still maintaining the contrast between sides, are overall lighter than external fruit (Fig. 7). Vis-NIR along with UV-Vis spectroscopy or imaging is expected to provide a solid non-destructive basis for assessing sun-related disorder risk at harvest.

Project Title: Risk assessment for delayed sunburn and sunscald (AP-16-102)

Executive Summary

Keywords: cold chain, sun stress, fruit finish

Abstract: Apple peel appearance and chemical changes in response to orchard sun exposure were evaluated to develop solutions that reduce sun-related cold-chain losses. Our results reveal residual impact of sun exposure during the cold chain and potential strategies for reducing losses based on altering fruit surface, and disorder avoidance using non-destructive sorting.

Project outcomes:

1. Identification of continued impacts sun exposure has on fruit quality, appearance, and stress during the cold chain.
2. Identified factors related to acclimation and peel surface chemistry that could be useful to develop treatments that reduce sunscald at-harvest.
3. Chemical targets linked with cumulative sun exposure that could be used as targets for non-destructive detection of and, potentially sorting for, sunscald and other sun-related defect risk.

Significant Findings:

1. Peel appearance (symptoms) changes in different ways during storage depending upon cultivar.
2. Peel chemistry changes differentially depending upon pre-harvest sun exposure, indicating continued stress on the exposed side of the fruit during cold storage.
3. Sun exposure alters cutin consistency and, possibly, cutin structure and, thereby, epidermal cell protection.
4. Heating in the orchard can lead to symptoms with similar appearance and etiology to sunscald.
5. Heating in the orchard can inhibit sunscald development.
6. Sunscald severity can be altered after harvest.
7. Simple UV reflectance imaging targeting metabolites associated with light exposure can non-destructively detect relative sun exposure.
8. Identifying changes in multiple metabolic targets detected in the UV-vis-NIR range may be a reliable basis for non-destructive detection of sun stress and/or cumulative sun exposure.

Future Directions:

1. Evaluation of at-harvest drenches and coatings to reduce sunscald.
2. Develop non-destructive techniques to detect sun using chemical targets identified in this study.
3. Determine if at-harvest sorting according to cumulative sun exposure could be used to improve appearance and quality.