FINAL PROJECT REPORT

Project Title: Testing biomarker-based tools for scald risk assessment during storage

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Cooperators: Dr. Jinwook Lee, Dr. Bruce Whitaker

Other funding sources

Agency Name: NIFA, USDA (Grant no. 2010-51181-21446**)**
Amt. awarded: $1,483,438 (federal total over 4 years)
Notes: Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle. David Rudell (Project Director) will manage and participate in the project and James Mattheis (Co-PI) and Yanmin Zhu (Co-PI) will also participate. This is a multi-state, multi-national project. The proposed project extends and compliments the activities of this SCRI project.

Agency Name: AgroFresh, Inc.
Amt. awarded: $232,253 (for Rudell and Mattheis role in SCRI project over 4 years)
Notes: Cash donation to support activities and objectives outlined in the Specialty Crops Research Initiative grant to develop biomarker based storage management tools for multiple apple postharvest physiological disorders was awarded during the last cycle (see above).

Total Project Funding: **Year 1:** $24,750 **Year 2:** $24,750 **Year 3:** $24,350

Budget History:

Item	**2011**	**2012**	**2013**
Salaries			
Benefits			
Wages	$15,038	$15,038	$5,263
Benefits	$4,962	$4,962	$1,737
Equipment			
Supplies[1]	$1,000	$1,000	$1,000
Travel			
Miscellaneous[2]	$3,750	$3,750	$3,750
Miscellaneous[3]			$12,600
Total	$24,750	$24,750	$24,350

Objectives:

1. Determine if scald risk assessment tools indicate when delayed CA imposition leads to high scald risk and high scald incidence.
2. Indicate if and when scald risk is high during CA storage based on risk assessment tools and determine if storage conditions can be changed to alter biomarker levels and scald incidence.
3. Assess effectiveness of scald-risk assessment tools in pilot scale and commercial CA storages.

SIGNIFICANT FINDINGS:

1. Changes in scald risk assessment biomarkers (SRAB) levels directly reflect how the fruit is reacting to the CA and DPA or 1-MCP chemical conditions imposed.
2. Validated 21 of 25 scald risk assessment biomarkers (SRABs) as predictors of scald risk.
3. Levels of 13 SRABs most effectively reflected scald risk altered by delaying CA imposition.
4. SRAB levels correlated with scald risk as early as 3 months after CA storage imposition. Scald was first detected at 9 months.
5. Changes in SRAB levels can detect scald risk early in fruit stored under suboptimal atmospheres and storage outcome improved (reduced scald) by subsequently adjusting CA conditions.
6. Storage monitoring using SRABs can rank different lots by risk during later phases of storage.
7. Spectrophotometric method for SRAB evaluation produced results similar to more expensive techniques.
8. How full a room is may impact SRAB levels.

RESULTS & DISCUSSION

The current report has evaluated and implemented scald risk assessment biomarker (SRAB)-based tools designed to monitor the impact the storage environment has on superficial scald risk**.** SRAB-based storage monitoring is one facet of risk assessment (Fig. 1). Discovery of other SRABs that indicate risk at harvest and just after harvest provide an earlier indication and perhaps a more accurate risk assessment. This work has been funded by our SCRI project.

Scald risk assessment following delayed CA storage and DPA treatment

Granny Smith scald was reduced or eliminated by DPA drenches. Scald symptoms developed on air-stored fruit at 6 months and on CA stored fruit at 9 months. In our small experimental chambers, delayed (up to 1 month) CA had little relationship with final scald incidence and severity after 10 months in 1% O_2 plus 7 days at 68 °F. Levels of 22 out of 25 scald risk assessment biomarkers (SRABs) increased at least 3 months prior to scald symptom development at 9 months in CA storage. Levels of 13 SRABs were highly reflective of scald final incidence and severity; very little increase in fruit that received treatments that didn't develop scald, and increasing levels as final scald incidence increased. For example, elevated scald risk was detectable by measuring SRAB N24 at 2 months in air, air+DPA, and CA fruit not treated with DPA, all treatments which developed scald by 10 months.

Rather than relying on changing levels of just 1 or 2 SRABs, we envision monitoring multiple SRABs to provide a more complete risk assessment. Using multiple SRABs may be a useful way to monitor many different indicators of the causes and effects of scald, painting a more complete picture of the condition of the peel during storage. For instance, many of the SRABs that closely reflect final scald levels may reflect oxidative stress that ostensibly leads to scald development. By monitoring these SRABs, we are actually providing a direct assessment of damage caused by adverse storage conditions that lead to scald.

Real-time scald assessment (experimental chambers)

Experiments were conducted to determine whether detection of high scald risk can be used to indicate if storage environment should be changed and whether this change can extend the symptom free life of the product. Experimental CA chambers containing 'Granny Smith' apples were held in air, 0.5 or 5% O_2 at 33 ^oF. SRABs were monitored monthly. Changes in levels of 3 SRABs indicated scald risk in fruit held at 5% O_2 at 2 months and in 6 SRABs after 3 months. O_2 in one of the 5% O_2 chambers was lowered to 0.5% O_2 at 3 months + 2 weeks. Scald was present on air stored fruit starting at 3 months and on 5% O_2 stored fruit starting at 6 months + 7 days at 68 °F. Scald incidence and severity was substantially reduced at 6 months + 7 days and less so at 9 months + 7 days by altering the storage conditions when risk was detected (3 months + 2 weeks). Results indicate that scald risk monitoring reflects fruit response to the storage environment. Also, scald outcome can be improved, once risk is accurately assessed, by optimizing conditions farther into the CA storage period than previously considered.

Real-time scald assessment (pilot and commercial rooms)

SRAB monitoring of multiple lots of fruit under pilot (Stemilt RCA rooms) and commercial conditions was performed to indicate whether risk assessment tools work in larger rooms and can rank risk among different lots of fruit. Results from the "real-time" risk assessment/storage monitoring were confirmed by monitoring research rooms set at 2% O_2 (rather than 5%) and reducing O_2 to 0.5% if SRAB levels suggest there is a risk. Elevated risk was detected in fruit from all 4 orchards stored in air or 2% O_2 (and not in fruit stored in 0.5% O_2) at 1 month (air) and at 2 months (2% O_2) (Fig. 2). O_2 levels were reduced in one of the 2% rooms after 3 months improving the storage outcome (reducing scald) after 9 months CA + 7 days at room temp (Fig. 3). Storage outcome may have been improved more had the O_2 levels been reduced when risk was first detected at 2 months. All orchards eventually developed scald when storage conditions were conducive to scald development. Spectrophotometry, an affordable and accessible means (platform) of measuring SRABs, was investigated alongside measurements using our other instrumentation. This platform provided similar estimations of scald risk (Fig. 4). SRAB levels ranked orchards according to risk starting by 3 months indicating storage risk monitoring could provide useful information about further supply chain performance of a lot compared with other lots from the same room, although we expect other SRABs, which can be evaluated earlier in the storage period, may provide the most accurate ranking. SRAB levels in fruit from the same orchards stored in the commercial room increased more rapidly than those in the RCA rooms that were set at a higher O_2 level. This indicates that ripening and stresses associated with the amount of fruit in these rooms compared with the others and the length of room loading and O_2 pull down may impact SRAB levels. This result is the subject of further investigation in our subsequent project.

Other related findings

In collaboration with Dr. Bruce Whitaker, a group of unknown candidate SRABs and other apple peel chemicals in our data base have been identified as *p*-coumaryl acyl esters. These apple peel chemicals are part of the waxy surface layer and are likely part of the wax structure. A few of these compounds have potential as SRABs.

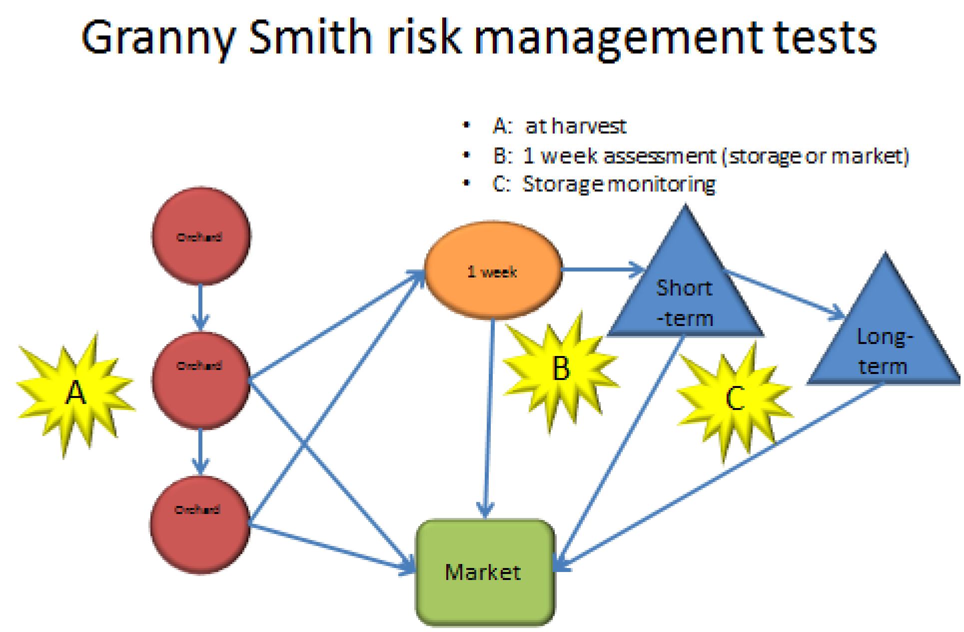

Fig 1. SRAB testing scheme for Granny Smith storage. The tools evaluated by this project primarily cover the storage monitoring phase (C) once postharvest treatments (DPA, SmartFresh) have been applied and storage conditions imposed. At-harvest and early storage (1 week) SRABs have been discovered using samples from this project and will be evaluated using samples for our continuing project (A and B). Assessing scald incidence during the latter phase of storage is another possible outcome of this project.

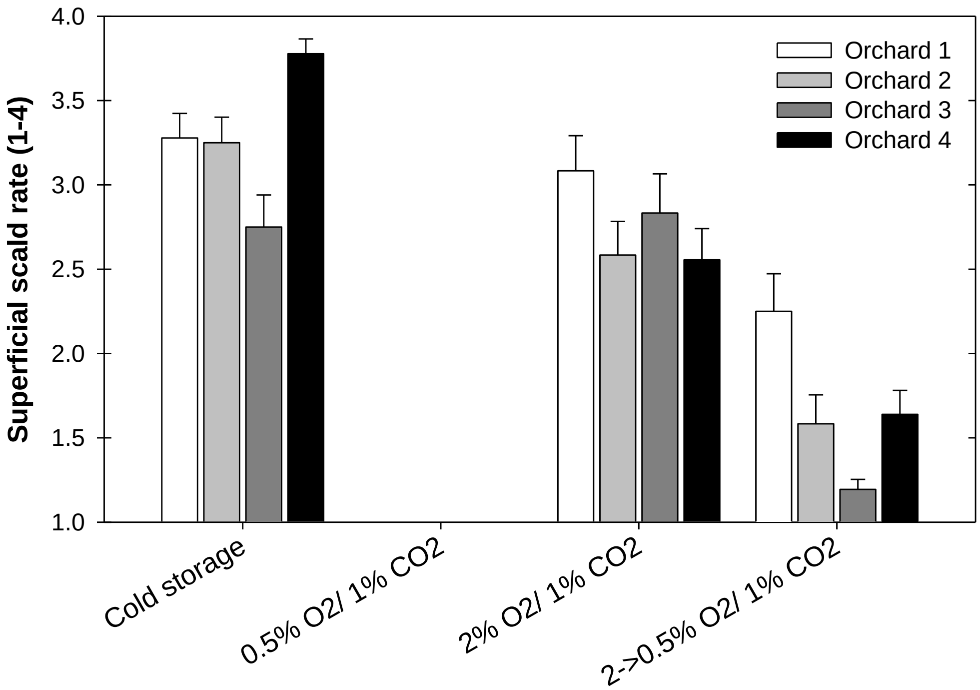

Fig 2. Superficial scald levels of 4 organic Granny Smith lots after 9 months (+ 7 days at 68 °F) at 33 °F in air or CA storage (0.5% O_2, 2% O_2, or 2% → 0.5% O_2). Superficial scald was evaluated by estimating the percent fruit peel with superficial scald symptoms: 1=0%, 2=1-25%, 3=26-50%, and 4=51-100%. SRAB levels were higher in the 2% O_2 rooms by 2 months, however the O_2 levels were not adjusted to 0.5% until after 3 months. Storage outcome may have been improved had the atmosphere been corrected in a more timely manner.

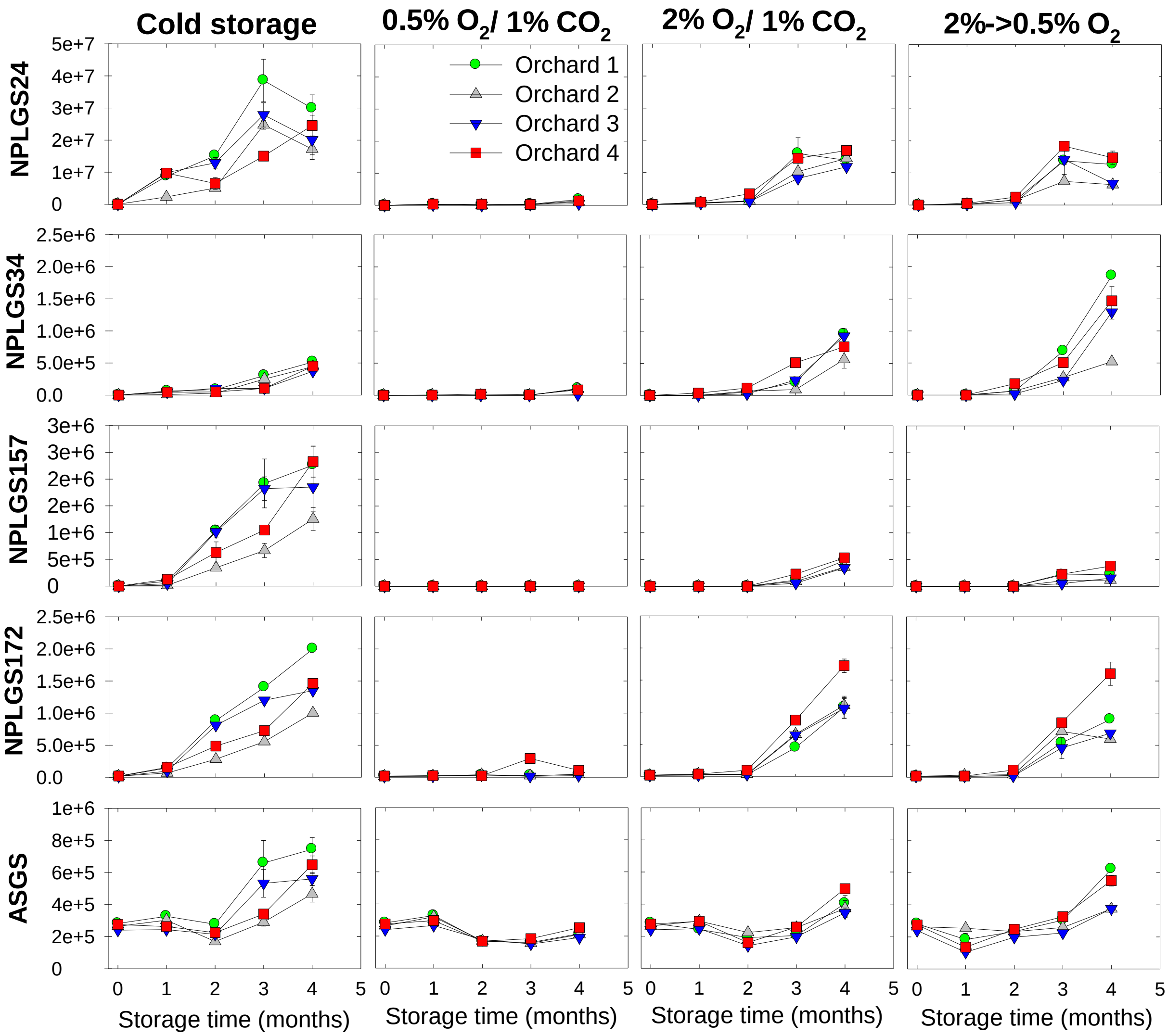

Fig 3. SRAB levels in fruit stored in Stemilt RCA rooms in air, 0.5% O_2, or 2% O_2. SRAB levels began to increase in 2% O_2 rooms beginning between 1 and 2 months storage. One 2% room was adjusted to 0.5% after 3 months. NPLGS34 levels most represented final scald outcome beginning at 3 months and ASGs at 4 months.

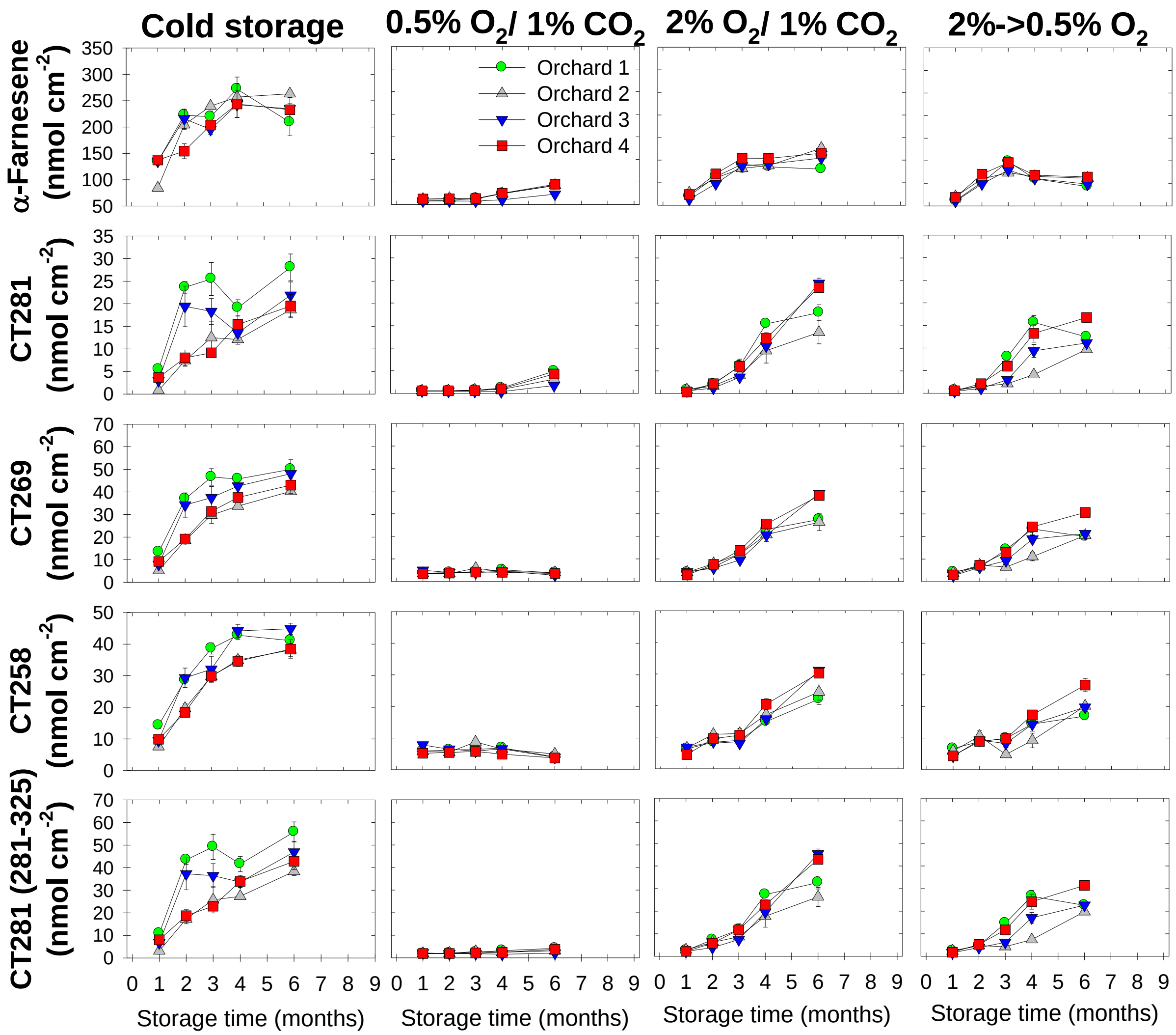

Fig 4. SRAB levels measured using spectrophotometry in RCA rooms under 3 different atmospheric conditions; air, 0.5% O_2, 2% O_2. SRAB levels were estimated using less expensive, more user friendly protocols compared with biomarker discovery instruments used in our laboratory, where CT269 estimates N24 and CT281 estimates N34.

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Executive Summary

Background: Our previous work screening hundreds of natural chemicals in apple peel during scald development has revealed many with potential for use as biomarker-based scald risk assessment tools. Further validation was needed to reveal whether tools will aid in commercial storage and supply chain management decisions by monitoring whether CA storage conditions or crop protectant usage is sufficient to prevent superficial scald. We initially developed and validated this technology by measuring peel chemistry changes related to storage stress during the scald development period of fruit stored in both air and CA. We continued this work by testing additional CA conditions in a laboratory setting as well as in pilot or commercial settings. Our objectives complemented our Specialty Crops Research Initiative (SCRI) project to continue to develop biomarker-based storage management tools for superficial scald and other key postharvest disorders.

Project outcomes:
1. Scald risk assessment tools to monitor risk during storage.
2. Less expensive platform to monitor scald risk assessment biomarkers (SRABs).
3. Potentially new recommendations for room loading.

Significant Findings:
1. Changes in SRAB levels directly reflect how the fruit is reacting to the CA and DPA or MCP chemical conditions imposed.
2. Validated 21 of 25 scald risk assessment biomarkers (SRABs) as predictors of scald risk.
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7. Spectrophotometric method for SRAB evaluation produced results similar to more expensive techniques.
8. How full a room is may impact SRAB levels.

Future Directions:
1. Continued validation of storage monitoring SRAB-based tools and defining their utility.
2. Testing and employment of tools for other superficial scald susceptible cultivars.
3. SRABs that provide scald risk assessment at harvest and for all points in the supply chain.
4. Similar risk assessment systems for other disorders such as Honeycrisp soft scald.
5. Biomarker-based tools for other fruit production uses.
6. New, better storing cultivars, with reduced postharvest disorder risk.