FINAL PROJECT REPORT

Project Title: Finding scald control tools using apple peel chemistry

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Cooperators: Dr. Bruce Whitaker, Dr. Maarten Hertog, Dr. Renfu Lu, Dr. Mike McCarthy

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 final year's funding of this WTFRC project provided cash support for ARS-Wenatchee's activities in the first year of this SCRI project.

Agency Name: AgroFresh, Inc.
Amt. awarded: $232,235 (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:** $53,305 **Year 2:** $55,784

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	$37,927	$39,065	
Benefits	$11,378	$11,719	
Wages			
Benefits			
Equipment			
Supplies	$4000	$5000	
Travel			
Miscellaneous			
Total	$53,305	$55,784	

Objectives:

1. Find peel apple peel chemicals that link scald to cultivar, harvest maturity, and other factors.
2. Identify additional apple peel chemicals that are important to fruit quality, ripening, and scald.
3. Identify apple peel chemicals or genes useful as early scald prediction or breeding selection tools.

Significant Findings:

Objectives 1 and 3

1. A total of 202 peel chemicals were selected as candidate biomarkers for assessing scald risk based on differences among storage duration, DPA treatment, and 1-MCP treatment.
2. Other peel chemicals associated with scald symptoms may provide a diagnostic "scald fingerprint", distinguishing scald from other similar looking disorders.
3. 53 candidates that reflected final scald incidence and severity as affected by harvest maturity were selected as *scald risk assessment biomarkers* (SRABs).
4. 27 SRABs were selected and may be useful for monitoring (monthly) and managing scald risk during CA storage after further lot by lot validation.
5. Delayed warming treatments of 1 week (68 ^{o}F) following 1-4 weeks of storage significantly reduced scald development. Peel content of related SRABs, acylated steryl glycosides (ASGs), reflected changes in scald development provoked by intermittent warming treatment.
6. ASG levels rose prior to a variety of peel disorders including scald indicating these SRABs may be more general biomarkers of risk for other peel disorders.
7. Candidate biomarkers that could potentially assess at-harvest risk were identified.

Objective 2

8. Between additional 'Honeycrisp' and 'Granny Smith' peel chemistry experiments, an additional 100+ apple peel chemicals were characterized.
9. Important SRABs including ASGs and CTs (conjugated trienols) were either identified or tentatively identified.

Results and Discussion

Categorizing candidate biomarkers. Candidate biomarkers were selected and categorized from the first experiment in collaboration with Dr. Hertog into groups that may predict or diagnose scald development. An in-depth evaluation of data from the first year of the study provided a more focused picture of the role(s) different chemicals may play in a biomarker-based superficial scald storage management system. Peel chemicals with different levels between control and DPA-treated fruit during the pre-symptomatic, predictive period were segregated from compounds reflecting scald symptom presence or absence. Candidate biomarker quality was ranked and overlap between candidates that predict and those associated with superficial scald symptoms were revealed. Candidate biomarkers associated with DPA- treated or control fruit during each period (Table 1) were evaluated in subsequent validation experiments. Results indicate candidate biomarkers can predict and may have uses beyond predicting scald for storage management. Other uses include accurate diagnosis and/or distinguishing superficial scald from other peel defects with similar visual symptoms to troubleshooting storage, packing-line, and other supply chain issues.

Relationships among peel chemistry, superficial scald incidence, and post-storage ripening after different storage durations and final selection of candidate biomarkers. Another goal was to evaluate how post-storage ripening may influence peel chemistry and possibly illuminate new candidate biomarkers or potential testing protocols. 'Granny Smith' apples were picked 1 month prior to commercial harvest and treated with 0 or 2000 ppm DPA, or 1 ppm 1-MCP then stored for 0, 1, 2, 4, or 6 months in 33 ^{o}F air. Scald incidence and peel chemistry was evaluated 0, 7, and 14 days after removal from storage. The storage stress leading to scald, as in previous years, principally transpired within the first month which was 3 months before symptoms were apparent. Peel chemical levels following 0, 7, and 14 days of ripening demonstrated that peel chemistry was different in control and DPA-treated fruit as early as 1 month after harvest confirming our results from the previous year. Results also show that levels of many candidate biomarkers change over the 2 week post-storage ripening period, either accentuating or reducing differences between control and DPA-treated fruit. Levels of a specific group of aroma volatiles associated with scald during the previous year's experiments only after scald symptoms appeared, the methanol based esters, were elevated in control peel after only 1 month storage plus 7 or 14 days. This indicates that post-storage ripening after shorter storage periods could provide earlier information related to scald status and storage stress.

Re-examination of predictive candidate biomarkers found in the previous year indicated over 60% of the candidates distinguished fruit with and without scald during symptom development. This experiment also identified an additional 137 candidate biomarkers. Candidate selection was based on the most important factors that reduce scald: storage duration, DPA treatment, and 1-MCP treatment (Fig. 1). Candidate biomarkers were screened in subsequent validation experiments to find only the most useful biomarkers. Further harvest maturity, CA, and intermittent warming experiments sought to validate this list of candidate markers.

Selection of scald risk assessment biomarkers (SRABs) based on harvest maturity. 'Granny Smith' apples were harvested 4 (H1) or 2 weeks before (H2) commercial maturity, at commercial maturity (H3), or 2 (H4) or 4 weeks (H5) following commercial maturity. Control and DPA (2000 ppm) treated fruit were then stored in air for up to 26 weeks (6 months). Levels of candidate scald biomarkers were screened at 0, 2, 4, 8, 16, and 26 weeks. Scald was evaluated monthly from 8-26 weeks and 26 weeks + 7 days at 68 ^{o}F. Scald symptoms developed first (at 16 weeks) in H1-4 and were more severe at 26 weeks + 7 days than H5 (Fig. 2).

The experiments identified SRABs useful regardless of harvest maturity and reflect the severity of scald that developed by the end of the test. A total of 53 SRABs met these criteria (Fig. 1). In most cases SRAB levels reflected not only the magnitude of scald developed at the end of the study but also the timing (first appearing at 16 weeks as opposed to 26 weeks) (Fig. 3). These SRABs were further tested to determine which can be used as tools to assess scald risk.

Partial validation of SRABs under CA conditions. 'Granny Smith' apples were harvested 1 month prior to commercial maturity, treated with DPA (0 or 2000 ppm) and placed immediately into 33 ^{o}F air, or CA (0.5%, 1.0%, or 5% O_2; all 1% CO_2). Fruit were stored for up to 9 months and SRABs (validated by the harvest maturity experiment) were screened at 0, 1, 2, 6, and 9 months. Scald incidence and severity and incidence of other peel injuries were rated after 0 and 7 days at 68 ^{o}F.

Fruit stored in 0.5% O_2 developed a dark anomalous peel injury after 9 months storage. However, superficial scald developed only on air-stored apples not treated with DPA starting at 6 months, and at 9 months on air-stored, DPA treated fruit. As scald did not occur on the untreated, air-stored fruit until 6 months in this study, it was not surprising that none was observed on any of the CA-stored fruit by 9 months. However, it is possible that scald would have developed later than 9 months, especially in 5% O_2 which would be expected to have the least scald control potential. Using this assumption, we set up selection criteria to find which SRABs can be employed as risk assessment tools.

Line plots of levels of all 53 SRABs during storage in every CA regime were visually evaluated and categorized. SRABs were further ranked within each category by a factor representing the % change in level during storage and the % difference between peel from DPA treated and untreated fruit. Highly ranked biomarkers changed the most during storage and had the greatest difference between control and DPA treated peel. Of the 53 SRABs, 15 were selected as scald risk assessment tools for CA (Fig. 1) with another 6 that require further validation. Still another 6 SRABs may actually indicate risk for multiple types of peel injury as they were linked with scald and the anomalous browning injury occurring on fruit stored 9 months in 0.5% O_2 (Fig. 4).

Impacts of intermittent warming on scald incidence and peel chemistry. 'Granny Smith' apples harvested 1 month prior to commercial maturity were placed in 33 ^{o}F air storage immediately or following 1 week at 68 ^{o}F. Of the fruit placed in storage immediately, additional subsets were removed after 1, 2, 4, and 8 weeks for 1 week at 68 ^{o}F after which they were placed back into cold storage. Apple peel chemistry was evaluated at harvest, before and after warming treatment, and after 26 weeks (6 months) storage. Scald severity was evaluated following 13, 17, 21, and 26 weeks. No scald developed after 26 weeks on fruit held 1 week at 68 ^{o}F beginning 1 or 2 weeks after harvest, and warming beginning at 4 and 8 weeks significantly reduce scald development. Levels of one SRAB group, the acylated steryl glycosides (ASG) were permanently lowered by intermittent warming and reflected scald development. Evaluation of other SRABs is ongoing for this experiment.

Expanded apple peel chemical library and identification of key scald-associated chemicals. The peel chemical analysis was expanded to include over 100 additional chemicals in 'Granny Smith' and 'Honeycrisp' experiments. In collaboration with Dr. Bruce Whitaker, ARS-Beltsville, we identified a key "family" of peel chemicals called ASGs and their chemical building blocks, the steryl glycosides (SGs) in 'Granny Smith' peel. ASGs modify important plant cell components in response to low temperatures. While there is no change in concentrations of the ASG "building blocks" (SG and phytosterols), ASG levels are elevated in control peel both prior to and during scald symptom development. ASG levels decrease in peel from both control and DPA-treated fruit after removal from storage. The same relationship between scald-risk and elevated ASG levels was confirmed and validated in subsequent experiments including differences associated with harvest maturity and CA conditions. Results indicate increasing ASG levels also precede soft-scald in 'Honeycrisp' and anomalous peel browning in 'Granny Smith'. Elevated ASG levels may indicate chilling stress leading to many of these disorders has occurred, and ASG metabolism may also serve as a potential genetic target to modify susceptibility to chilling related peel injuries. Expression of genes that possibly control this pathway was evaluated during storage with and without DPA with inconclusive results. It is possible that control of this little-known pathway is complex and requires a much more comprehensive gene expression analysis to discover the correct candidate biomarkers associated with this pathway.

Other compounds, collectively called conjugated trienols (CT), were identified. These natural peel chemicals have been associated with oxidative stress and scald in this and many other studies over the last 40 years. Results indicate that these may be useful SRABs. In addition to identified CTs, this study revealed other peel chemicals with some CT-like features. These unidentified SRABs were among the highest ranked for identifying scald risk.

Finding candidate biomarkers for at-harvest scald risk assessment. Five harvests of 'Granny Smith' [see above "Selection of scald risk assessment biomarkers (SRAB) based on harvest maturity"] were stored in air for 26 weeks (6 months) and scald evaluated at 26 weeks + 7 days at 68 ^{o}F. Apple peel chemistry (+700 natural peel chemicals) was evaluated at harvest. Statistical comparison of the apple peel chemistry at harvest with scald incidence was used to select candidate biomarkers for at-harvest scald risk assessment. Most highly-ranked candidates were associated with later harvests suggesting higher levels may indicate less likelihood of scald development (Fig. 5). A few compounds were

associated with fruit that did develop severe scald. It is important to note that this very preliminary evidence may also indicate these candidate biomarkers are associated with only harvest maturity which is, in turn, associated with scald incidence. Considerably more experimentation is necessary to establish which, if any, of these candidates would be useful at-harvest tools.

Adapting peel chemical measurement platforms for biomarker based scald-management tools. Some preliminary work has been conducted towards development of non-laboratory based platforms to estimate changes in apple peel chemistry. Dr. Renfu Lu (ARS, East Lansing) and Dr. Mike McCarthy (UC-Davis) have collaborated by performing preliminary evaluations of two different on-line devices for superficial scald prediction. Dr. Lu used near infra-red spectroscopy and fluorescent scattered light imaging to attempt to differentiate apples at 0, 1, 2, and 4 months. It was not possible to segregate untreated or DPA treated apples using either technique under these experimental conditions.

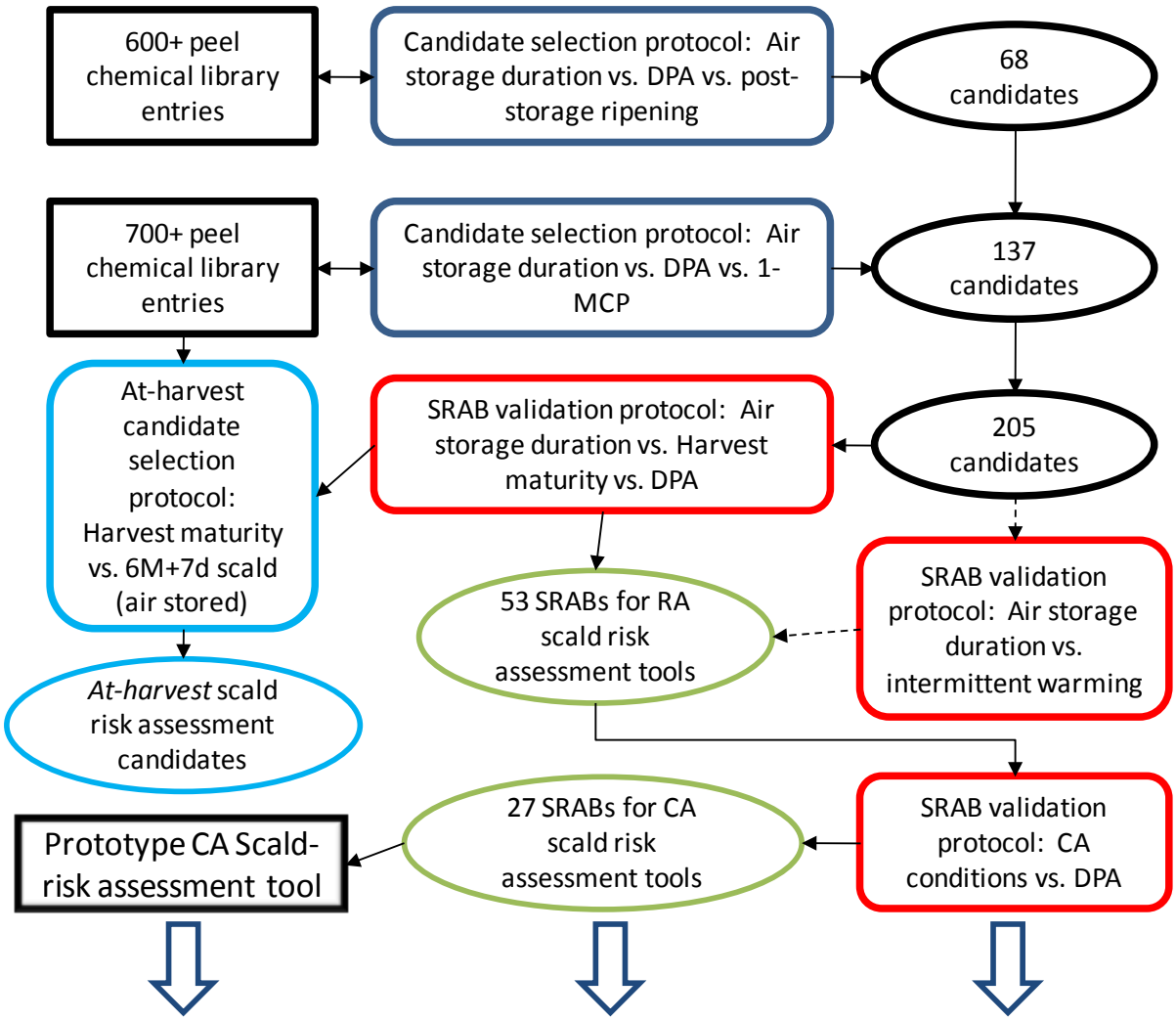
In an ongoing study, Dr. McCarthy is evaluating the possibility of using a small magnetic resonance imaging platform to differentiate untreated and DPA treated apples at 0, 1, 2, and 4 months. Both collaborators generously performed this work using our apples without direct funding.

Assessment of natural apple peel chemicals as scald-reducing crop protectants
Evaluation of natural apple peel chemicals selected in this study as pre-harvest treatments is ongoing. Preliminary results indicate a possible relationship between scald protection and one peel compound. An additional evaluation of this natural peel chemical and related metabolites is in progress.

Conclusions and future directions
Results indicate that measuring various scald-risk assessment biomarkers (SRABs) may be a useful apple storage management tool by indicating when and which fruit are at higher risk to develop scald. By selecting SRABs using storage conditions typically required for scald to develop (storage stress and ripeness) and then validation under storage conditions similar to commercial practice, risk assessment tools are expected to widely applicable. Furthermore, ASGs may have an added value of indicating risk of other peel browning disorders in addition to superficial scald. Candidate biomarkers potentially indicating scald risk *at-harvest* were also selected and require validation.

SRABs selected for scald risk assessment tools need to be validated using multiple lots in commercial settings. Measurement protocols should also be refined under these settings. Platforms used to measure SRABs must be adapted or developed for both service laboratory and field use. A similar protocol should be employed to reveal useful at-harvest scald risk assessment biomarkers. Improved protocols to find gene expression SRABs should be employed.

Figure 1. Overview of project activity coordination and accomplishments.



Project Outcomes

- SRABs for storage management tools.
- Prototype SRAB-based tool for monitoring scald risk during CA storage.
- List of candidate biomarkers to assess at-harvest risk.
- Continued federal funding to search for additional gene expression and chemical biomarkers for superficial scald and other significant postharvest disorders.
- Apple fruit mass spectral phytochemical library containing descriptions of 700+ individual phytochemicals.
- Identification of an important class of SRABs, the ASGs, which may be broadly applicable for monitoring general storage stress that leads to multiple disorders.

	High 50	VIP>0.8
Pre-scald (7d-2m model)		
total candidates	33	68
Greater in control	23	32
Greater in DPA	10	36
Scalding (2m-6m model)		
total candidates	38	232
Greater in control	14	82
Greater in DPA	24	150

Table 1. Ranked candidate biomarkers from initial selection categorized by superficial scald development period and treatment association. This demonstrates peel chemicals associated with unstressed, "healthy" peel can also be useful biomarkers indicative of storage stress levels.

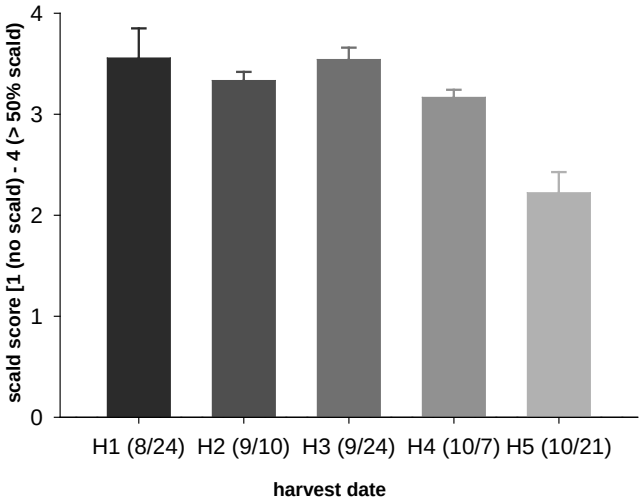


Figure 2. Scald severity after 26 weeks (6 months) 31 °F air storage (+7 days at 68 °F) of 'Granny Smith' apples harvested 4 or 2 weeks prior to commercial harvest, commercial harvest, and 2 or 4 weeks following commercial harvest. Scald developed less on H5 apples. This data was used to compare effects among harvest maturity, scald severity and candidate biomarker. This comparison provided validation to select Scald Risk Assessment Biomarkers (SRAB).

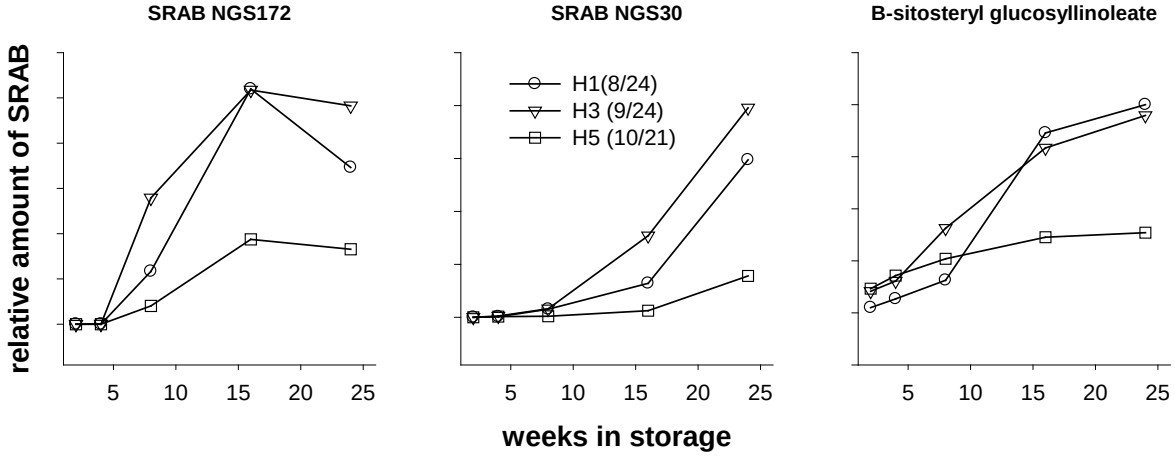


Figure 3. Levels of select Scald Risk Assessment Biomarkers (SRAB) in 'Granny Smith' peel of apples harvested 4 weeks prior to commercial harvest, commercial harvest, and 4 weeks following commercial harvest. Results demonstrate that SRAB levels reflect the earlier onset and greater severity of scald after 26 weeks (6 months) 31 °F air storage (+7 days at 68 °F) in the earlier harvests.

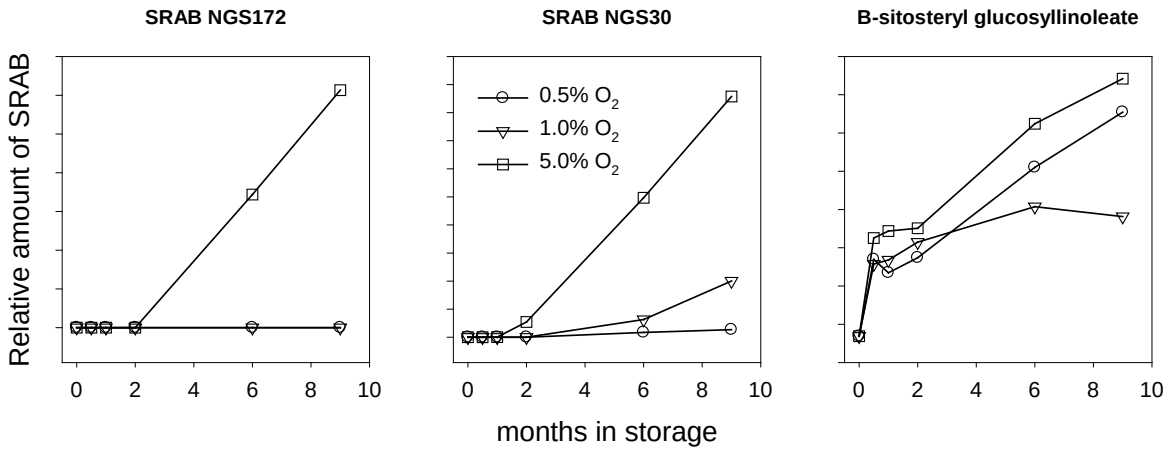


Figure 4. Levels of selected Scald Risk Assessment Biomarkers (SRAB) in 'Granny Smith' peel of apples harvested 1 month prior to commercial harvest and stored for up to 9 months in 0.5, 1.0, or 5.0% O_2 at 31 ^{o}F. Although no scald developed on any of the CA stored fruit within 9 months (it took 6 months to appear on air stored controls), SRAB levels were elevated in the 5.0% treatment, which would be most likely to develop scald. ASG levels (far right, for example) also increased prior to the development of an anomalous peel browning disorder that began to appear in 0.5% O_2. With further validation, it is expected that monthly monitoring of these and other CA-specific SRABs may be a useful storage management tool for scald.

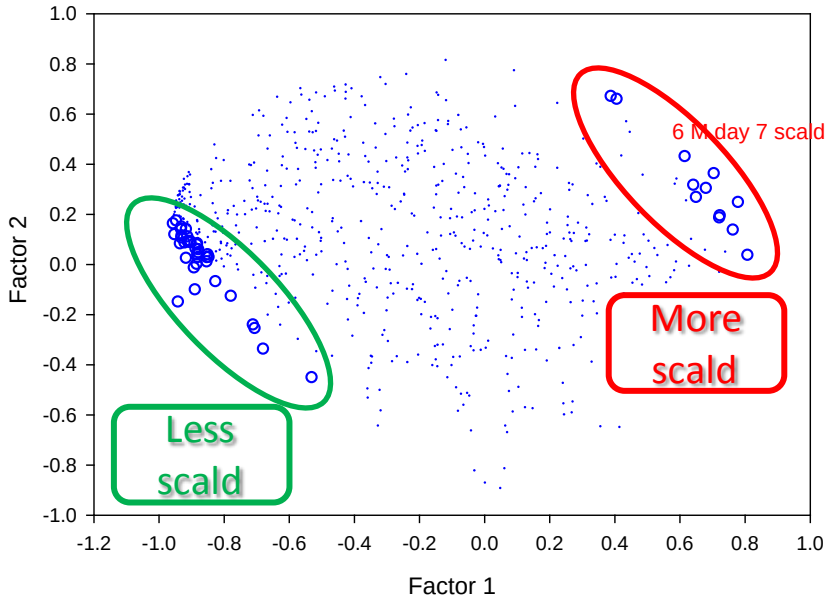


Figure 5. Selection of candidate *at-harvest* scald risk assessment biomarkers in 'Granny Smith' peel of apples harvested 4 or 2 weeks prior to commercial harvest, commercial harvest, and 2 or 4 weeks following commercial harvest. Scald was evaluated after 26 weeks (6 months) 31 ^{o}F air storage (+7 days at 68 ^{o}F). Total at-harvest apple peel chemistry was compared to final scald severity to highlight candidates associated with harvests that developed more or less scald. As this is only an evaluation of one lot, it is suggested that this experiment be repeated with multiple lots and multiple seasons to establish a final list of candidates.

Executive Summary

Project outcomes:

1. *Scald risk assessment biomarkers* (SRABs) for storage management tools.
2. Prototype SRAB-based tool for monitoring scald risk during CA storage.
3. List of candidate biomarkers to assess at-harvest risk.
4. Continued federal funding to search for additional gene expression and chemical biomarkers for superficial scald and other significant postharvest disorders.
5. An apple fruit mass spectral phytochemical library containing descriptions of 700+ individual phytochemicals.
6. Identification of an important class of SRABs, the ASGs, which may be broadly applicable for monitoring general storage stress that leads to multiple disorders.
7. Improved understanding of the peel chemistry behind superficial scald, storability, and quality to direct and expedite future research.

Significant Findings:

1. A total of 202 peel chemicals were selected as candidate biomarkers for assessing scald risk based on differences among storage duration, DPA treatment, and 1-MCP treatment.
2. Other peel chemicals associated with scald symptoms may provide a diagnostic "scald fingerprint", distinguishing scald from other similar looking disorders.
3. 53 candidates that reflected final scald incidence and severity as affected by harvest maturity were selected as *scald risk assessment biomarkers* (SRABs).
4. 27 SRABs were selected and may be useful for monitoring (monthly) and managing scald risk during CA storage after further lot by lot validation.
5. Delayed warming treatments of 1 week (68 ^{o}F) following 1-4 weeks of storage significantly reduced scald development. Peel content of related SRABs, acylated steryl glycosides (ASGs), reflected changes in scald development provoked by intermittent warming treatment.
6. ASG levels rose prior to a variety of peel disorders including scald indicating these SRABs may be more general biomarkers of risk for other peel disorders.
7. Candidate biomarkers that could potentially assess at-harvest risk were identified.
8. Between additional 'Honeycrisp' and 'Granny Smith' peel chemistry experiments, an additional 100+ apple peel chemicals were characterized.
9. Important SRABs including ASGs and CTs (conjugated trienols) were either identified or tentatively identified.

Future directions:

1. Validate CA prototype tools under commercial conditions in multiple lots.
2. Explore and develop SRAB measurement platforms that can be easily adopted in multiple areas of the apple production and service industry.
3. Continue to find gene expression biomarkers for potentially more sensitive and accurate disorder risk assessment and management.
4. Continue to identify natural apple fruit chemicals that are important to fruit maturation, ripening, superficial scald, and other postharvest disorders.
5. Screen and validate candidate biomarkers that assess *at-harvest* scald risk in multiple commercial lots.
6. Use apple peel chemistry to increase the understanding of scald and related disorders.