FINAL PROJECT REPORT

Project Title: Commercial testing of early scald risk assessment tools

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Cooperators: Drs. Jinwook Lee, Bruce Whitaker, and Christopher Watkins

Total Project Request: **Year 1:** $54,881 **Year 2:** $56,275 **Year 3:** $57,675

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. 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).

Agency Name: AgroFresh, Inc.
Amt. awarded: $90,000
Notes: Continued development of systems for implementation of biomarker-based tools developed from the above SCRI project as well as finding additional biomarkers.

Budget
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	**2013**	**2014**	**2015**
Salaries	$38,417	$39,302	$40,372
Benefits	$16,464	$16,972	$17,302
Wages			
Benefits			
Equipment			
Supplies[1]			
Travel			
Total	$54,881	$56,275	$57,675

Objectives:

1. Determine if risk assessment tools accurately represent scald risk in multiple commercial lots of Granny Smith apples.
2. Test scald risk assessment tools using Delicious apples.
3. Validate additional biomarkers for CA storage.
4. Extend search for biomarkers for at-harvest superficial scald risk assessment tools.

SIGNIFICANT FINDINGS:

1. SRAB levels indicate which CA room will have the highest scald incidence for Delicious and Granny Smith as early as 1 month into storage.
2. Low and unchanging SRAB levels during CA indicate that apples will not scald while in those CA conditions.
3. When scald risk is high, room conditions can be checked and changed or fruit marketed according to assessed risk of each room.
4. A protocol to monitor SRAB (281 nm) could be used in the industry as part of a quality control regime.
5. SRAB levels increase with higher O_2 levels in CA storage.
6. Delaying CA imposition results in enhanced ethylene and SRAB levels.
7. CA conditions and room environment are the most important factors in scald control without crop protectants.
8. SRAB monitoring can be used to monitor how multiple factors associated with room loading, impacts of other fruit in the same room, and room atmosphere/integrity affect scald risk.
9. Metabolic pathways potentially associated with scald risk or tolerance at harvest were identified.
10. Natural apple wax components that are also accurate SRABs were identified.
11. Identification of additional chemistries linked with scald risk at-harvest.

RESULTS & DISCUSSION

Scald risk assessment for Delicious apples

In year 1, scald risk assessment biomarker (SRAB) levels monitored in Scarlet Spur Delicious apples stored in air began to increase between 1 and 2 months (Fig. 1). SRAB levels in CA fruit increased between 2 and 3 months, then the O_2 was decreased to 0.5% in one of the 2% O_2 chambers. Increased SRAB levels preceded scald development on fruit stored in air or 2% O_2. Apples stored in 0.5% O_2 did not develop scald by 9 months and SRAB levels did not increase. Reducing O_2 after scald risk was detected in fruit stored at 2% O_2 reduced, but did not prevent, scald. These results are, in part, consistent with previous results for Granny Smith, where scald incidence can be reduced if CA conditions (if not optimal) are remedied once increased SRAB levels are detected.

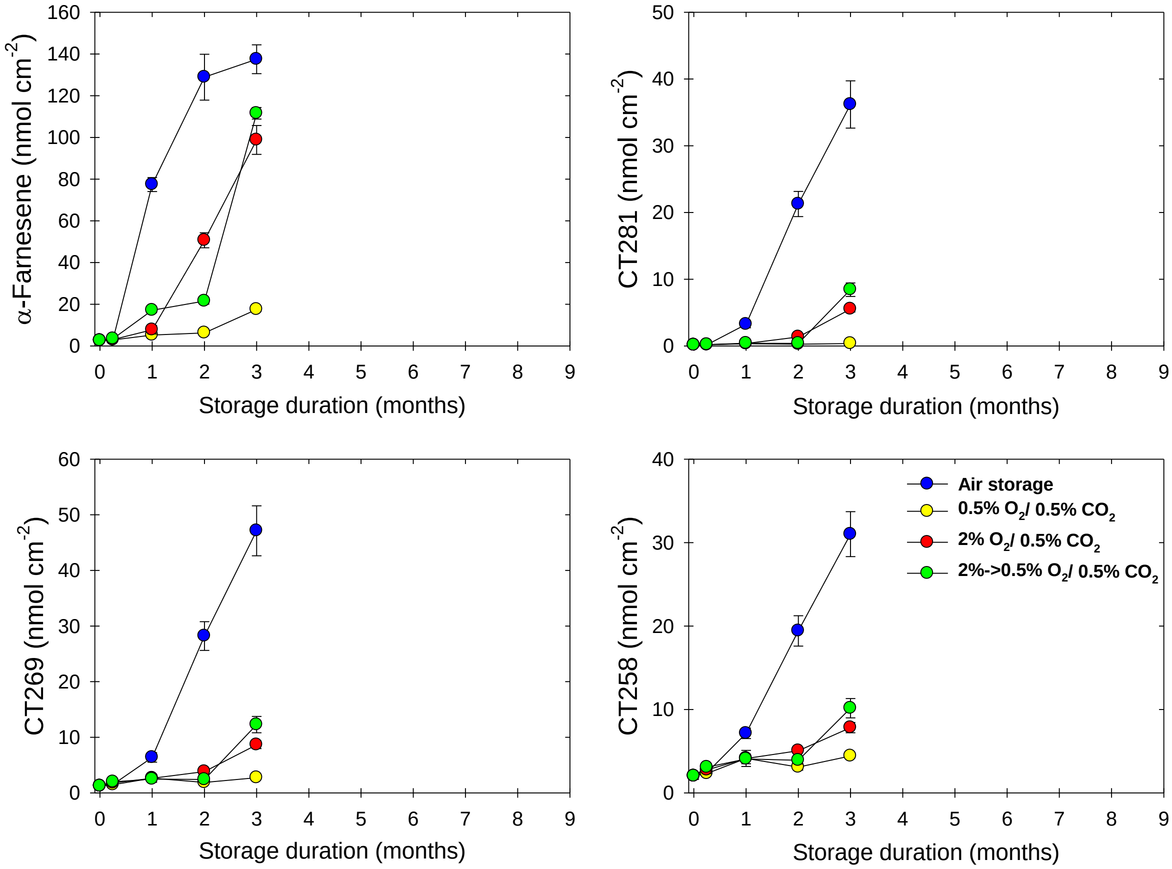


Fig 1. α-Farnesene (the "building block" of SRABs monitored in this study) and SRAB levels over the first 3 months in Delicious stored in test chambers at 33 ºF in air or 0.5% or 2% O_2. The oxygen was reduced to from 2% to 0.5% O_2 when SRAB levels were found to be increasing. Scald began to develop at 6 months in air and chambers held at 2% O_2 during the first part of storage. Scald incidence was less where the O_2 were reduced.

In year 2, three organic CA rooms containing samples from 3 orchards each were used to assess if the trial was still effective if scaled up to commercial sized. Average SRAB levels were relatively higher in rooms 1 and 3 and continued to increase in room 1 until 3 months (Fig. 2, top). At 3 months, all test samples were removed and half placed into a 36 F air room to simulate transit and retail supply chain or 33 ºF RCA room (0.6% O_2, 0.5% CO_2), monitoring scald monthly. Fruit stored in the RCA room was removed into the same air storage at 6 months to simulate a supply chain starting at 6 months. SRAB levels at 3 months accurately predicted relative scald incidence among rooms that was first detected at 4 months in air (Fig. 2, bottom left). Likewise, SRAB levels at 3 and 6 months predicted relative scald incidence among rooms after 6 months CA + 3 months air (Fig. 2, bottom right). Results indicate the test is scalable and SRAB levels were similar to those related to risk in our test chambers. SRAB levels suggested greater risk in 2 of the rooms and, although those rooms had more scald, all rooms developed at least some scald starting at around the same time with room 2 incidence around 10%. In this way, SRAB levels and the conditions that contributed to the relatively elevated levels, reflected only incidence and not when the disorder would actually develop. Consistent with previous results, SRAB levels most accurately predicted scald as influenced by the storage conditions of a particular room rather than the orchard where the fruit was sourced (not shown). This is similar to results from Granny Smith (below).

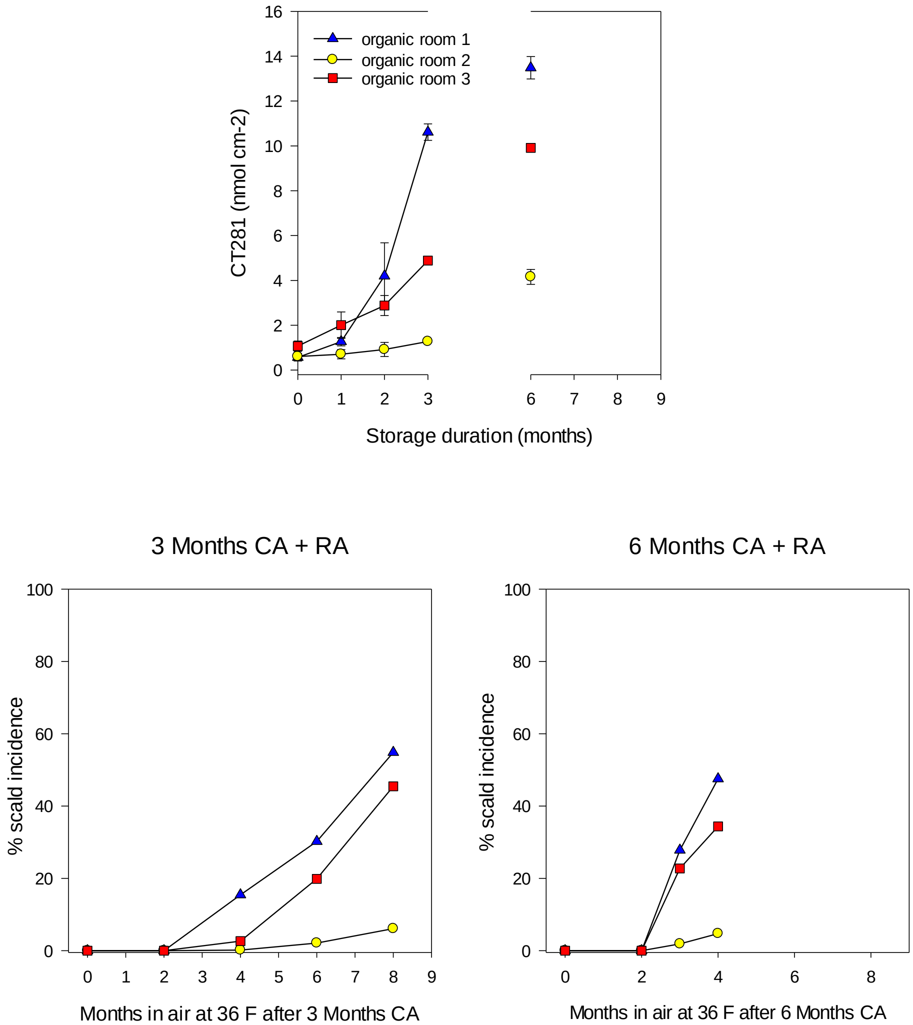


Fig. 2. (Top) Average SRAB levels measured in peel of Delicious apples stored in three commercial CA rooms containing fruit from 3 orchards each. Apples were removed from all CA storages and placed in air at 36 ºF or moved to an RCA room (0.6% O_2, 0.5% CO_2) at 3 months and, from here, moved to air at 6 months. Scald was monitored periodically up to 10 months storage to simulate a prolonged post-storage supply chain.

Scald risk assessment of Granny Smith apples

In year 1, SRAB levels increased in Granny Smith apples stored in 2 organic commercial rooms (8 lots total) but levels (281 nm) were considerably lower than those associated with high scald risk in past experiments. There was a difference in overall SRAB levels between the two rooms after 2 months of storage, although the difference disappeared following 3 months storage. Scald was detected only after 8 months+7 days at room temperature. When the room was opened for processing, sample fruit was removed and placed in an RCA room set at 0.5% O_2 possibly impacting the scald outcome as previous experiments in our test chambers and in RCA rooms have suggested, where lowering storage oxygen later in storage reduces scald.

In another experiment, SRAB levels were much higher than our previous results in all fruit except for those treated with SmartFresh where no scald was detected at the end of the trial (Fig.2). Scald was accurately predicted by monitoring SRABs.

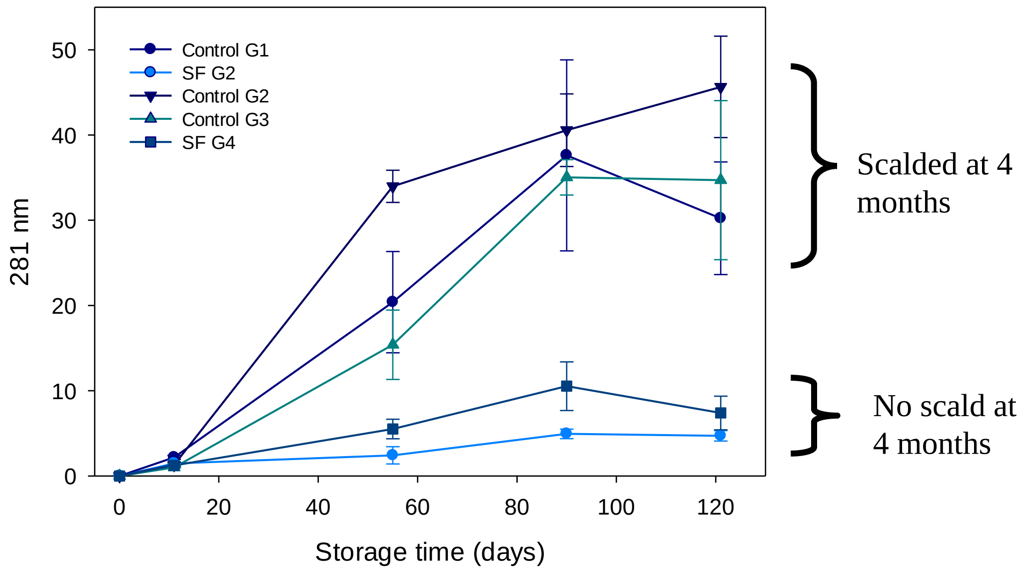
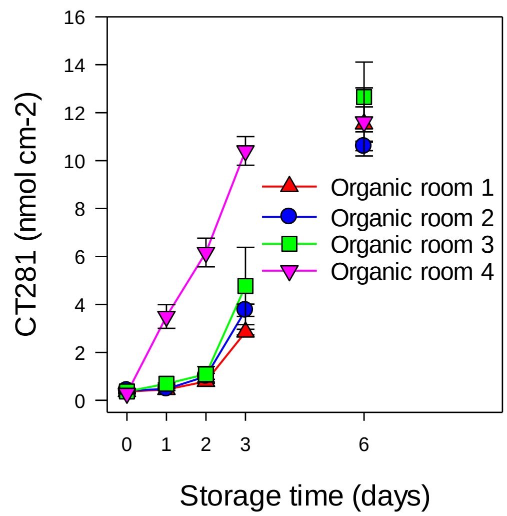


Fig. 3. Granny Smith SRAB levels and final scald incidence after 4 months commercial CA storage in an AgroFresh study using ARS methodology. Apples were chosen from a total of 4 growers treated with SmartFresh (SF) after harvest. Error bars represent standard error (n=3).

To improve upon the year 1 trials, year 2 (2014) trials employed fruit from additional Granny Smith orchards and commercial CA rooms. Average SRAB levels from all orchards within rooms were higher in one of the rooms after 1 month and continued to increase by 3 months indicating a higher risk in this room (Fig. 4, top). As in the Delicious trial, to simulate transit and retail supply chain, the samples were split and moved to air storage or the RCA room at 3 months and then from RCA to air at 7 months. Scald was first detected in all rooms at 4 months and continued to develop after removing apples after 3 months CA (Fig. 4, bottom). Scald began to develop in all rooms at 3 months following 7 months of CA. SRAB levels as early as 1 month reflected eventual scald incidence after 3 and, then, 7 months CA. Average values among orchards were very similar within the same CA room indicating factors contributed by the room (ie. room loading time, other fruit in the room, oxygen concentration) had a greater impact on SRAB levels than factors brought in from the orchard.

While IEC levels were higher in Delicious than Granny Smith at harvest, indicating fruit was more mature, SRAB levels remained very similar to similarly stored Granny Smith. SRAB levels across all of the trials this year do not increase alongside IEC indicating there are other factors contribute to their generation (not shown) and, therefore, IEC would not be an accurate method for assessing scald risk. Overall, results indicate that monitoring SRAB levels is an accurate means of assessing scald risk as influenced by CA conditions after as little as 1 month CA storage.



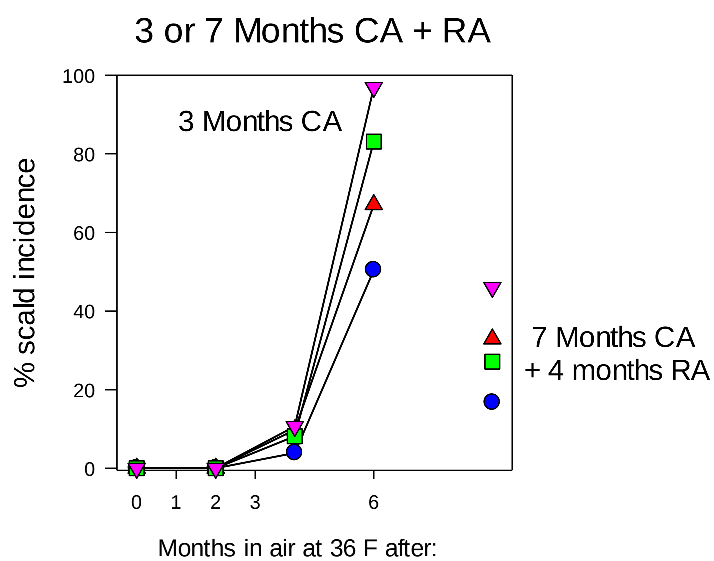


Fig 4. (top) Average SRAB levels for samples from 4 commercial organic Granny Smith storage rooms containing doorway samples from 3 orchards each. Fruit were moved at 3 months from commercial rooms into air at 36 ºF or RCA room (0.6% O_2, 0.5% CO_2, 33 ºF) and, then, to air at 7 months. Scald levels were evaluated periodically after until up to 11 months to simulate a prolonged post-storage supply chain.

Impacts of delayed CA on scald development of Granny Smith apples

In year 1, A one week CA delay lead to higher IEC, room ethylene, and SRAB levels (Fig. 5) but significant scald incidence was not observed in any of the treatments after 10 months storage. IEC continued to increase as did room ethylene for the storage period indicating that rapid CA imposition is key to controlling this event. SRAB levels were considerably lower in all of the treatments than those that preceded scald development in previous years' trials. In year 2, CA imposition was delayed for 2 weeks and fruit from 3 orchards were moved to air storage at 9 months and scald incidence monitored for up to 2 months in 36 ºF air storage. As in year 1, SRAB and IEC are already considerably higher in the room where CA was delayed (Fig. 6) but scald did not develop until 6 weeks following removal from CA and was not different among orchards or between rooms. The influence of the delayed storage imposition was lessened by long-term storage under optimal CA conditions. Interestingly, samples from one of the orchards are stored in 2 commercial rooms and are also used in the delayed CA imposition trial this year. SRAB levels at 1 and 2 months were nearly equal in Room 4 and the RCA room with 2 week CA imposition while they remain relatively the same as the initial values in the other rooms.

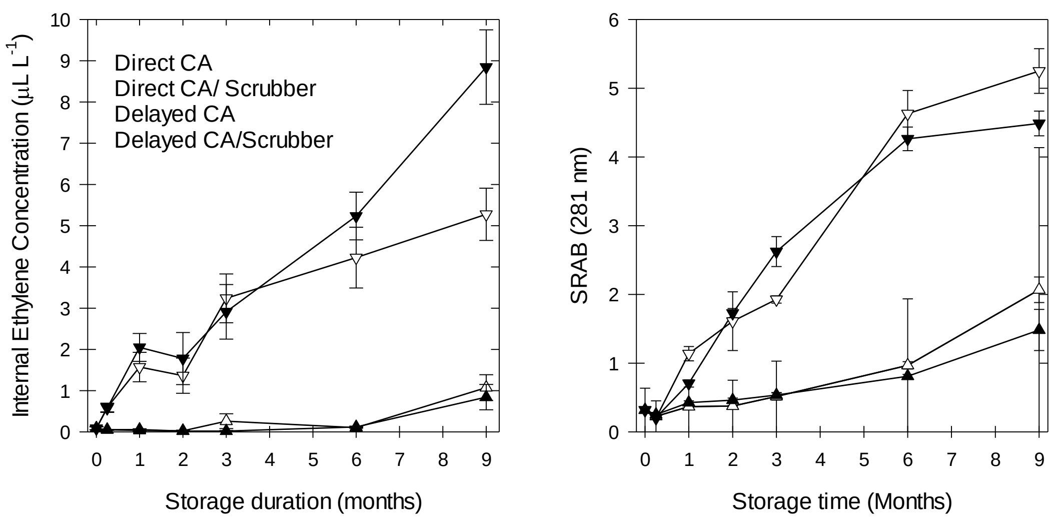


Fig. 5. (Year 1) Fruit internal ethylene concentration (left) and SRAB levels (right) over storage period in research CA rooms (30 bins, 1 orchard) set at 0.5% O_2/0.5% CO_2 immediately or following a 1 week delay. SRAB levels were higher in rooms with delayed CA imposition as were IEC values, although SRAB levels were lower than those recorded in past experiments in fruit at high risk for scald development. Error bars represent standard error (n=3 for SRAB evaluation; n=18 for IEC assay).

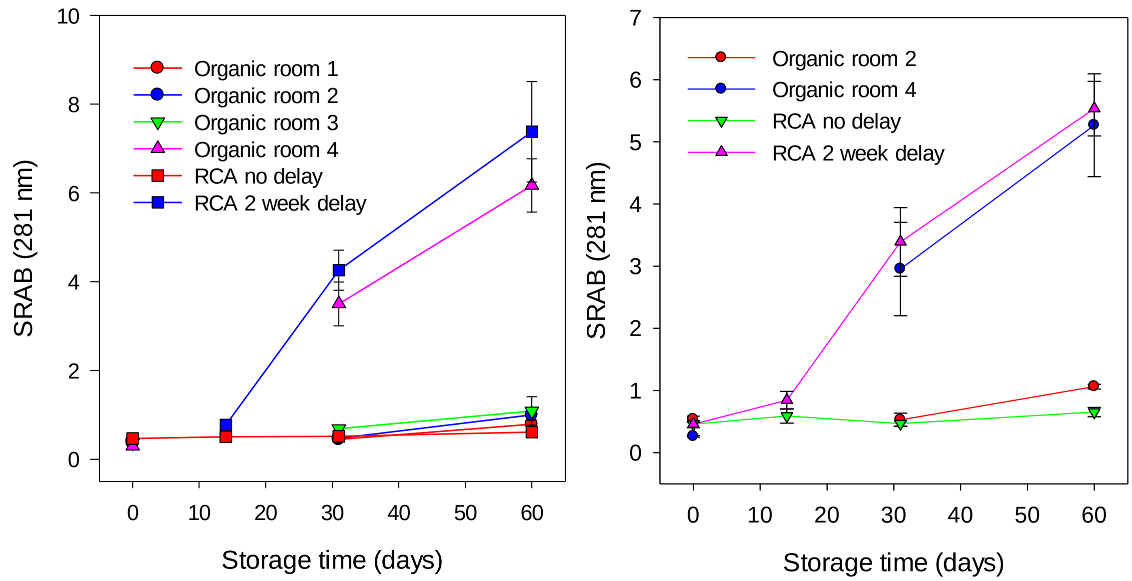


Fig. 6. (left) Average SRAB levels for samples from organic Granny Smith storage rooms and organic research CA room trials (Year 2). Averages represent samples from 3 orchards in each room. The low variability of SRAB levels among orchards in a particular room indicate factors evoked by the room have a greater impact on SRAB levels than those from the field. (right) SRAB levels of the same orchard lot stored under 4 different storage conditions including 2 commercial rooms and RCA rooms pulled down immediately or after 2 weeks to 0.5% O_2:1% CO_2. Error bars represent standard error (n=3).

At harvest scald risk assessment

For Year 3, Granny Smith apples were harvested 2 weeks before, at, and 2 weeks after commercial maturity from 3 different locations (Basin and Wenatchee area). Orchards were relatively the same maturity across all three harvests with average starch index ranging from 1.2-1.5 at H1 and 2.1 – 2.6 by H3. IEC was low or undetectable as is typical of Granny Smith at harvest. Scald incidence, SRAB levels, and peel samples are taken after 0, 1, 2, 3, 4 and 6 months 33 ºF air storage and at 6 months (0.6 kPa O_2, 0.5 kPa CO_2) CA storage. Scald incidence continued to be monitored following 6 months CA on fruit kept in 36 ºF air. Peel samples were evaluated at-harvest for differences in peel chemistry (800+ natural peel chemicals screened) associated with scald risk. In air storage, scald symptoms began to appear between 3 and 4 months on apples from all orchards and harvest timings (Fig. 7). Scald incidence was less on fruit from the Mattawa location from all harvests. Scald incidence at 4 months decreased with harvest date only on fruit from the Mattawa orchard. Scald incidence at 6 months remained less on fruit from the Mattawa location for the final harvest. Scald levels were also lowest in the Mattawa orchard following 6 months CA+ 5 months air (not shown). Scald incidence was not reflected by the relative harvest maturity and, instead, was related to other undetermined factors.

SRAB levels did not entirely reflect differences of scald incidence among orchards and harvest maturities after air and CA storage but not earlier (not shown). This is consistent with our earlier work which indicates that this test does not consistently reflect scald risk among orchards held under the same storage conditions. Instead, we suggest that, when using only CA storage to control scald, the CA conditions have the greatest influence over whether a lot will scald or not, regardless of its susceptibility going into storage and this test shows its greatest value in monitoring SRAB levels during the first 3 months to indicate whether storage conditions are controlling scald. Six month SRAB values using this method also did not accurately assess scald risk and, as we have reported before, should not be used to assess scald risk.

Evaluating natural peel chemicals at harvest yielded compounds may be positively or negatively associated with scald risk at 4 months. To find out which natural chemicals are associated with scald at harvest, 800+ peel chemicals were evaluated. A consensus of results from different statistical modeling techniques found those chemicals most associated with the Mattawa orchard at any harvest compared to the other two orchards (Fig. 7). Chemicals most associated with fruit that were at the highest risk were of a particular origin, the isoprenoids, while those associated with fruit having the lowest risk were primary oils and fats, many of which reside in the peel wax. These results are supported by an earlier preliminary study. As mentioned above, like superficial scald incidence, these associations apparently also have very little relationship with standard estimations of harvest maturity such as starch index and internal ethylene.

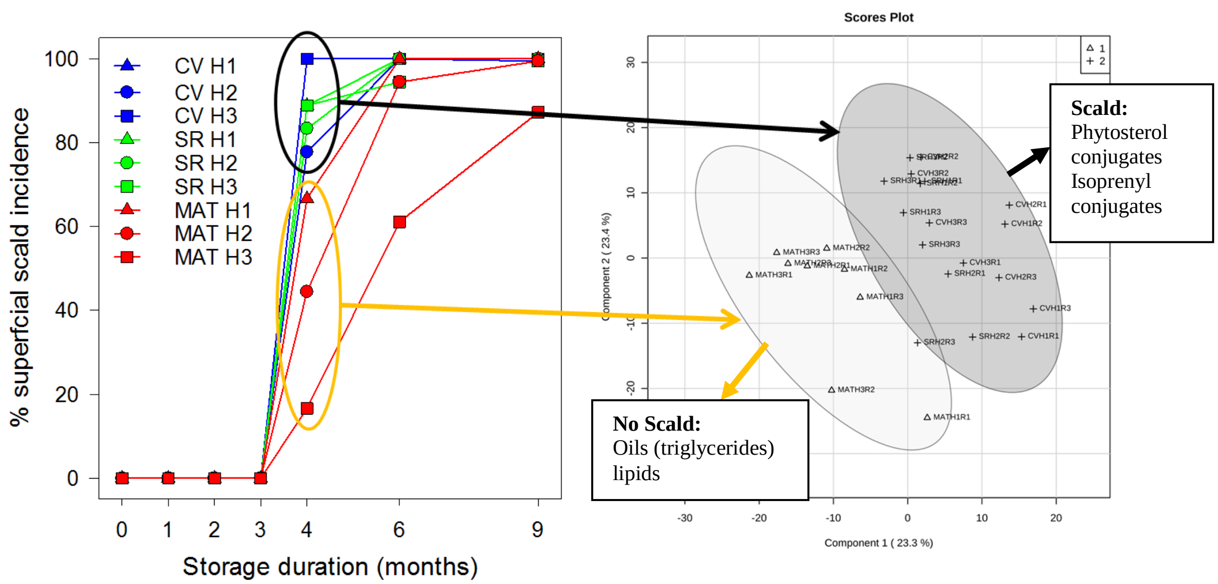


Fig. 7. (A) Superficial scald incidence on Granny Smith during 33 ºF air storage from Columbia View (CV), Sunrise (SR), and an orchard located near Mattawa (MAT). (B) Screening of ~800 peel chemicals at harvest indicates the low scald orchard had levels of a number of these metabolites that were different. These included higher levels of oils and lipids in peel less likely to get scald and a class of other non-polar compounds, called isoprenoids, in peel more likely to develop scald.

There is considerably more work required to devise a test that could use these sorts of metabolites as for scald risk assessment. Challenges potentially include difficulty of analyzing these compounds, likely requiring an agricultural services company's expertise, and additional validation required to determine accuracy. The existing SRAB storage monitoring protocol actually interrogates the fruit once the stress has been imposed, "asking" the fruit whether the storage conditions are sufficiently controlling the biological processes caused by chilling that lead to scald. However, it is likely that at-harvest risk assessment could be included as one basis for diagnosis of scald risk very early in the storage and evaluation of compounds like these may be one mean for achieving this goal.

Identification of SRABs that previously uncharacterized natural wax components in plants

We identified some of the SRABs that are collectively estimated using the spectrophotometric method revealed new components of apple wax not previously reported. The novel components are fatty acyl esters of secondary and primary farnesols. Two of these esters are SRABs and are ostensibly synthesized by an active process. All of these components, including farnesene, are mostly or entirely found in the apple wax. It has been widely accepted that the oxidative process is abiotic, or occurs during storage as a result of exposure to air rather than metabolically. This new evidence indicates that this process, which is closely linked with scald development, may be an actual component of metabolism where farnesene is enzymatically oxidized and then esterified. We have also found evidence that high levels of these compounds at harvest are associated with soft scald risk in Honeycrisp. The role of these compounds in wax structure is unknown as is where in the cell these compounds are synthesized and how they arrive at the surface and are incorporated into the wax layer. However, given their association with scald and soft scald, understanding the role and biosynthesis of these novel wax layer components may be critical to understanding why apples scald and may even be found to be a useful target for phenotyping.

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. Around 25 scald risk assessment biomarkers or "SRABs" were initially discovered and validated using a wide variety of conditions known to impact scald development including crop protectants, harvest maturity, temperature conditioning, harvest maturity, and CA oxygen level. Initial tests using test chambers and research CA rooms indicated that monitoring SRABs may 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 chose a relatively inexpensive and easy means of monitoring a few of these SRABs that correlated well with more costly and rigorous analyses. It was unknown whether the tests would remain accurate in full sized, loaded CA rooms or with other cultivars. Accordingly, our current work addressed these issues and other issues related to the practicality of SRAB monitoring.

Project outcomes:

1. An effective means to verify if postharvest crop protectant and CA controls are effectively controlling scald during storage.
2. Scald risk assessment tools validated for Granny Smith and Delicious.
3. Scaled down monitoring method that could be incorporated into industry QC protocols.

Significant Findings:

1. Monitoring SRAB levels indicates which CA room will have the highest scald incidence for Delicious and Granny Smith as early as 1 month into storage.
2. Low and unchanging SRAB levels while in CA indicate that apples will not scald while in those CA conditions.
3. When scald risk is high, room conditions can be checked and changed or fruit marketed according to assessed risk of each room.
4. We devised a scaled-down protocol to monitor SRAB (281 nm) that could be used in the industry as part of a quality control regime.
5. SRAB levels increase with higher O_2 levels in CA storage.
6. Delaying CA imposition results in enhanced ethylene and SRAB levels.
7. CA conditions and room environment are the most important factors in scald control
8. SRAB monitoring can be used to monitor how multiple factors associated with room loading, impacts of other fruit in the same room, and room atmosphere/integrity affect scald risk.
9. Identification of previously unidentified natural apple wax components that are also accurate SRABs.
10. Identification of additional chemistries linked with scald risk at-harvest.

Future Directions:

1. Treatment approaches that diminish scald incidence where apples are unprotected during post CA storage distribution and retail, especially for organic apples.
2. Continued validation of storage monitoring SRAB-based tools and defining their utility.
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.