FINAL PROJECT REPORT
WTFRC Project Number: AP-16-101

Project Title: Reducing scald after long-term CA storage

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Budget: **Year 1**: $30,690 **Year 2**: $63,095 **Year 3**: $72,508

Collaborators: Brenton Poirier, Ed Valdez, Loren Honaas, Girish Ganjyal, Ines Hanrahan, Heidi Hargarten

WTFRC Collaborative expenses:

Item	2016	2017	2018
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental	$6,300	$6,300	$6,300
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$6,300	$6,300	$6,300

Footnotes: Costs for 1 RCA room

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510)559-5769 **Email address:** chuck.myers@ars.usda.gov

Item	2016	2017	2018
Salaries	$18,338	$39,004	$41,344
Benefits	$6,052	$12,871	$13,644
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous *		$11,220	$11,220
Plot Fees			
Total	$24,390	$75,695	$66,208

Footnotes: One-third of instrument service contract

OBJECTIVES:

1. Identify rapid, stress provoking, at-harvest treatments that reduce scald levels during a prolonged supply chain.
2. Validate changes in peel chemistry as indicators of efficacy for stress-based scald treatments.
3. Determine how at-harvest treatments that provoke stress impact other fruit quality factors.
4. Determine if post-storage reduction of ethylene action is a feasible post-storage scald control technique.

SIGNIFICANT FINDINGS:

1. Scald induction is cumulative and rapidly imposed, effective (below 0.8% O_2) CA reduces the rate of induction. Induction resumes following CA storage.
2. At-harvest delayed cold storage (2 d), intermittent warming, or hot water treatment reduces scald better on more mature fruit but only following air storage.
3. Hot water treatment following effective CA storage (3-4 months 0.5% O_2), reduces scald during the post-CA storage cold chain.
4. 1-MCP treatment following effective CA storage (3 or 6 months 0.5-0.8% O_2) reduces scald in the subsequent cold chain.
5. The CTOL test (see Blakey and Rudell, 2017) may be used to indicate whether post-storage scald treatments will be effective.
6. Cold chain temperature following CA storage for organic Granny Smith should be below 37 °F and as close to 33 °F as possible for prolonged periods and optimally not above 45 °F on retail display.

METHODS:

Equipment and Cooperative Summary: Stress treatments (excluding impingement drier) as well as fruit quality, tissue sampling, processing and analysis of SRABs using analytical instrumentation (gas and liquid chromatography-mass spectrometry) will be performed at ARS-TFRL, Wenatchee. Treatment using the impingement drier was performed at BSYSE, WSU-Pullman in collaboration with Drs. Ganjyal and Hanrahan. Pressure treatment was performed by Dr. Honaas and staff at ARS-Wenatchee. Storage experiments will be performed both at ARS-Wenatchee and in Stemilt RCA storages. New information will be disseminated through published articles in peer reviewed journals as well as poster and oral presentations at industry meetings and professional conferences.

Year 1 (includes activities outlined for Objectives 1, 2, and 3)

Year one focused on the development and characterization of temperature conditioning and stress amendment treatments for scald reduction including delayed cooling, intermittent warming, initial heat shock with an impingement oven, impact injury, and the use of chemical stressors. We also focused on optimizing post-CA supply chain conditions for scald reduction by testing the effectiveness of post-storage 1-MCP treatments and optimizing supply-chain storage temperatures. These experiments included metabolic profiling efforts, monitoring of scald risk assessment biomarkers, and assessment of quality traits and scald development.

Year 2 (includes objectives 1, 2, and 3)

Heat treatments. Granny Smith apples were harvested from Sunrise Research Orchard on October 6th (average Brix of 10.1, starch index of 2.5 out of 6) and October 20th (Brix:11.4, starch index: 3.5). Apples from each harvest were subjected to three different heat treatments prior to storage: (1) hot water submergence (118°F for 3 min); (2) warm air (100°F for 72 h); and (3) hot air (108°F for 24 h). Untreated controls were included for both storage conditions. Apples were stored in air for 6 months or CA (0.5%O2: 0.5% CO2). Scald incidence was evaluated on air stored fruit at 3 and 6 months and in CA stored fruit at 10 months (6 months post-CA).

Post-storage heat treatment and wounding experiments.
Granny Smith apples were placed into CA storage (33°F, 0.6% O_2:0.5% CO_2) immediately after harvest. After removal from CA at 3 months, apples were subjected to either hot water (118°F for 3 min) or warm air (68°F for 2 days) temperature treatments. Additional apples underwent an injury treatment of bruising with a 5.6 g ball-bearing dropped from a height of 38 cm or were punctured with a syringe. Scald incidence was evaluated on CA stored fruit at 10 months (6 months post-CA).
Post-CA supply chain optimization.
Granny Smith were sampled from 4 lots stored commercially (multiple rooms) at 0.6-0.8% O_2:1% CO_2 for 6 months. Within 2 days following removal from CA, apples were placed in 33 °F, 35 °F, or 37 °F air for two months, then moved to a simulated retail temperature of 68°F for one week. Two additional temperatures (55°F and 45°F) were used for apples that were stored 33°F. Scald evaluations were performed monthly and following the 1 week of simulated retail storage.

__**Year 3 (includes objectives 2, 3, and 4)**__

Delayed post-storage 1-MCP and DPA treatments following delayed CA storage and scald risk assessment
Granny Smith apples were harvested from Sunrise research orchard and placed in in-house CA (0.5% O_2:0.5% CO_2; 33°F) chambers immediately or after 2 or 4 weeks in 33°F air. Apples were removed from storage after 4 months and transferred to 37°F air storage. At 0, 14, and 28 days, 8 trays of apples from each CA regime were drenched with 2000 ppm DPA or treated for 12 h at 33°F with 1 ppm 1-MCP. Treated apples were then placed back into 37°F air storage. Scald incidence was evaluated monthly during the post-CA storage simulated cold chain.

For scald risk assessment, peel was sampled from 1 tray of control apples from each storage delay treatment at harvest, upon CA establishment, following removal from CA (4 months), and monthly thereafter until 8 months (4 months 37°F air storage). Peel (3 composite samples containing 6 apples) were processed and analyzed for CTOLs and other natural peel chemicals associated with superficial scald risk using HPLC-MS as outlined by Rudell et al. (2009).
Delayed DPA treatment during CA storage at different O_2 percentages
Granny Smith apples were harvested from Sunrise research orchard and placed in are storage (33°F) or in-house CA chambers (1% or 2% O_2:0.5% CO_2; 33°F) for up 6 months. At 0 weeks and after 2, 8, 12, 16, and 24 weeks 3 trays (18 fruit/tray) were removed from each storage, drenched with 2000 ppm DPA, and immediately placed back into storage. Apples were transferred to 37°F air storage at 6 months and stored until 8 months (4 months post-CA storage) at which scald incidence was evaluated.
Post-storage hot water or 1-MCP treatment of Granny Smith stored in commercial CA
Granny Smith apples were sampled after 4 months from 1 lot stored in a commercial CA room (800 bin; 0.8% O_2:0.6% CO_2; 33°F). Upon removal, 8 trays apples were treated for 12 h at 33°F with 1 ppm 1-MCP, treated by submerging in 118°F water for 3 min or left untreated. Temperature of hot water treated apples was evaluated prior to and following treatment. Apples were moved to 37°F air storage where scald incidence was evaluated at 8 months (4 months post-CA storage).

RESULTS AND DISCUSSION

A number of stress-inducing or acclimation treatments were employed (ozone, nitrogen dioxide, hydrogen peroxide, superoxide, non-bruising physical stressor, bruising, intermittent warming, hot water, irradiant heat) in year 1 with or without a 2 day waiting period at 68°F. Bruising, intermittent warming, hot water, and the 2 day waiting period were the only at-harvest treatments with consistent impact and only during air storage but not following CA. Treatments that effectively reduced scald after CA were reducing post-storage cold chain temperature and post-storage 1-MCP. Much of the following year focused on scald reduction during the post-CA period, repeating experiments from year 1 and determining why at-harvest treatments that reduce scald during air storage immediately after harvest do not reduce scald following ultra-low O_2 CA.

Post-storage 1-MCP and DPA treatment reduces scald during a prolonged cold chain following CA storage. Year 1 results revealed that a 12 h 1 ppm 1-MCP treatment reduced scald during a 4 month cold chain following 3 and 6 months ultra-low (ULO) O_2 (0.5% O_2) CA storage in our storage chambers. In Year 2, again scald was reduced following 3 months ultra-low O_2 CA storage as well as in multiple lots of commercially CA stored Granny Smith (0.6-0.8% O_2). Year 3 results confirmed that post-storage 1-MCP treatment is nearly as effective as pre-storage 1-MCP treatment only if less than 0.8 % O_2 CA storage (commercial rooms and in-house chambers) is established immediately following harvest. Likewise, delaying CA storage establishment for up to 4 weeks reduced scald control by post-storage 1-MCP treatment (Fig. 1). Year 3 results also demonstrated a similar effectiveness of post-storage DPA treatment. This indicates that postharvest scald control, as with immediately after harvest, is not merely based on controlling ripening but is also a function of oxidative stress.

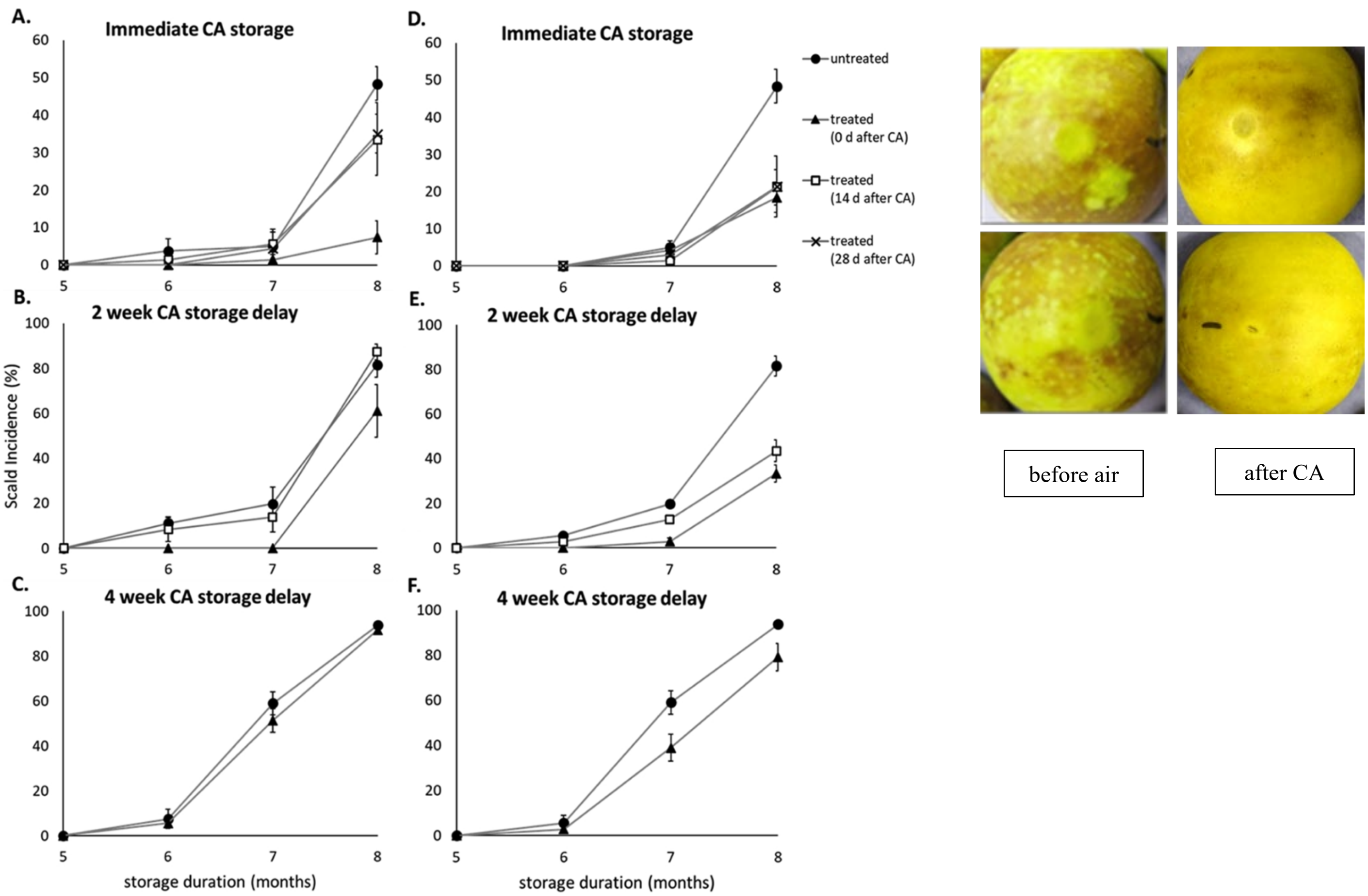

Fig. 1. Delayed (0, 7, and 14 d) CA storage (0.6 % O_2; 0.5% CO_2) establishment combined with delayed post-storage 1-MCP (left) and DPA (right) treatment demonstrates reveals that the fruit's response to chilling that results in scald reduced or stopped by ULO-CA. Error bars represent standard error (n=8 trays). Physical wounding prior to air storage or following CA storage causes scald clearing (right).

Physical wounding at harvest does not cause the same clearing on fruit stored in CA unless it occurs after CA.

Scald induction period is prolonged by ultra-low controlled atmosphere storage. Our results demonstrating the effectiveness 1-MCP and DPA treatment following immediately applied ULO contradict conventional understanding of scald control. It was previously considered difficult, if not impossible, to control superficial scald if the treatment was not applied within the first few weeks of cold storage. This typically meant assuring susceptible cultivars were drenched or otherwise treated with DPA and/or gassed with 1-MCP soon after harvest or upon placing fruit into the cold. It is well known in the scientific literature and from our previous experience that scald control using DPA or 1-MCP treatment

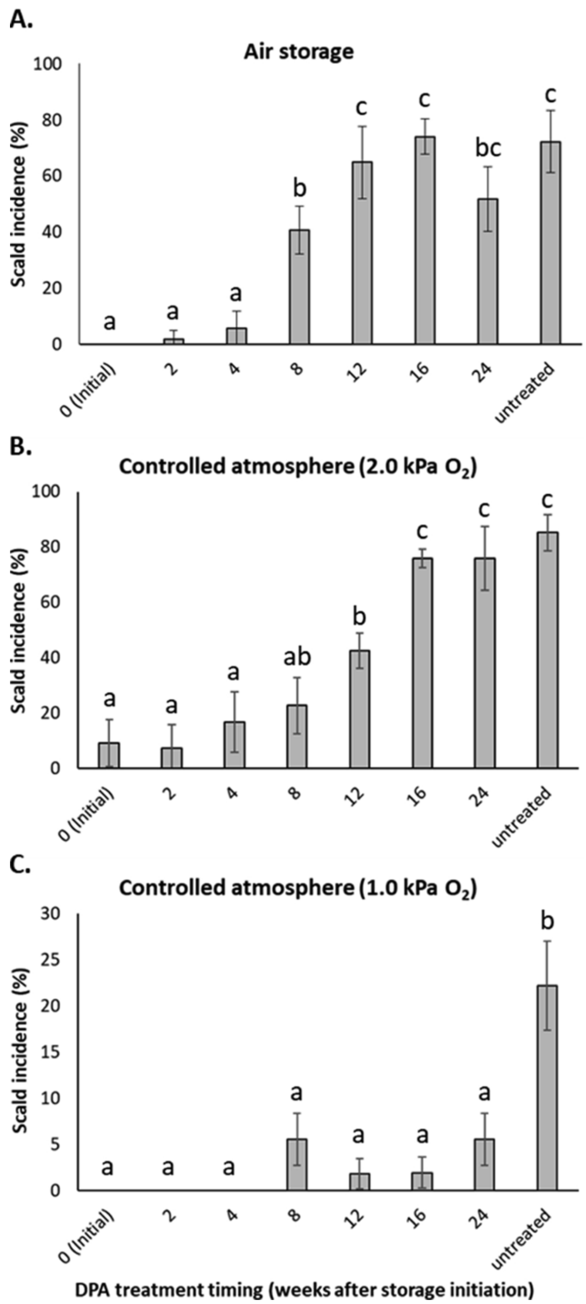

Fig. 2. Storage O_2 setpoints of below 1 % reduce scald induction and work best for controlling scald during the post-storage supply chain and providing fruit that will be receptive to post-storage scald control treatments. Reduced CA storage O_2 % increases the storage duration at which delayed DPA treatment to 'Granny Smith' remains effective at controlling scald. Apples stored under different atmospheric compositions, including air (A), 2.0 kPa O_2: 0.5 kPa CO_2 (B) and 1.0 kPa O_2: 0.5 kPa CO_2 (C) were treated with DPA throughout storage, moved to 37 °F air storage at 6 months and evaluated for scald incidence (%) at 8 months. (n=3 trays of 18 apples each, ±standard error, means with same letter are not significantly different according to Tukey HSD test at $p \leq 0.05$).

wanes after apples have been placed in air storage. Control weakens after 1-2 weeks of storage, and 1-2 months is the longest delay with any measurable control. However, in this way, we can define the period during which cumulative cold stress causes scald, and this is fairly regular year-to-year. This event can be defined as the "scald induction period" or when the invisible injury that later leads to superficial scald occurs.

When apples are stored without use of these crop protectants, scald control relies on establishing and maintaining the appropriate atmosphere, typically the lowest affordable O_2 setting that does not cause other disorders. More conventional CA settings (1% O_2 and above) do not provide the same scald control as often manifested by scalded apples as fruit approach 6 months storage. Our results show that delayed DPA application loses its effectiveness when drenching is successively delayed during storages set at sequentially higher (1.0%, 2.0%, and air storage; 33°F) O_2 % indicating that the scald induction period occurs more rapidly at higher O_2 percentages (Fig. 2). This clearly indicates that quick room loading and establishment of proper CA atmosphere before too much of the scald induction period has transpired is critical when depending on CA for scald control. However, once effective CA is established and scald induction is slowed, can scald induction begin again when controls are no longer in place?

Scald induction takes a set amount of time both before and after effective CA storage. Successively delaying 1-MCP or treatment (0, 7, and 14 d) following 4 months of 0.6% O_2 CA established at 0, 14, or 28 d revealed that the scald induction period indeed resumed once apples were removed from storage (Fig. 1). If the CA environment was established immediately and 1-MCP was applied immediately following removal from storage, scald control continued in the cold chain for up to 8 months (<10% incidence). When these fruit were treated at 14 and 28 d, they developed over 30% between 7 and 8 months (incidence) scald. Post-storage DPA similarly controlled scald, albeit slightly less efficiently. The duration of post-storage scald control diminished to 2 and 1 month if CA was established at 2 and 4 weeks, respectively. One particularly useful result was that the combination of time was about 2 months

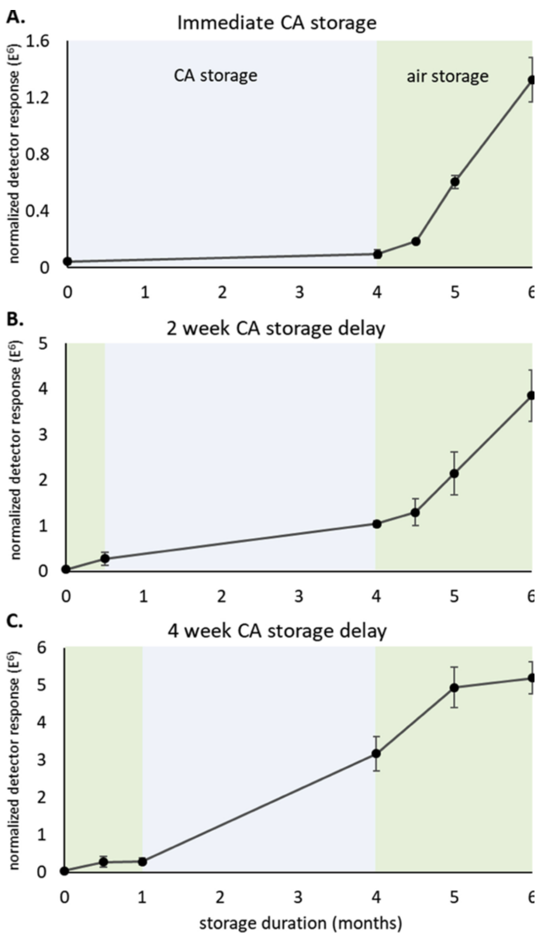

Fig. 3. Scald risk assessment using the CTOL test reveals if post-storage scald control treatments will be successful. Levels of CTOL during and following 4 months CA (0.6% O_2, 33°F) storage established immediately (A), 14 d (B), or 28 d (C) following harvest. Scald incidence during the subsequent cold chain at 37°F is presented in Fig. 1. These results show how CTOL level reflect how well the storage protocol controlled scald as well as estimated how well post-storage 1-MCP and DPA treatments would work. CTOL levels at 4 month indicate how much of the scald induction period has occurred up to that point. CTOL levels can be evaluated using the test outlined by Blakey and Rudell (2017).

regardless of whether it was before or following CA storage. Interestingly, this is the same amount of induction time indicated using delayed DPA and 1-MCP treatment during the first months of air storage. This result reveals that scald induction is a cumulative process that, once susceptible apple cultivars are harvested, requires a set amount of time under conditions that promote the process. Ultimately, these results establish some loose guidelines by which we can expect post-storage scald mitigation treatments to work. However, our results also indicate that the same tool we to determine if storage conditions are controlling scald induction may also be a means to indicate whether an apple will be receptive to post-storage scald mitigation.

Monitoring CTOL levels can indicate whether post-storage scald treatments will be effective (Fig. 3). The protocol for monitoring conjugated trienol (CTOL) levels in apple peel (Blakey and Rudell, 2017) is a direct way to monitor in scald-susceptible cultivars whether a storage environment is controlling scald given many other factors that influence disorder incidence. Our current results indicate the same test, when performed immediately following removal from storage, may be used to indicate whether post-

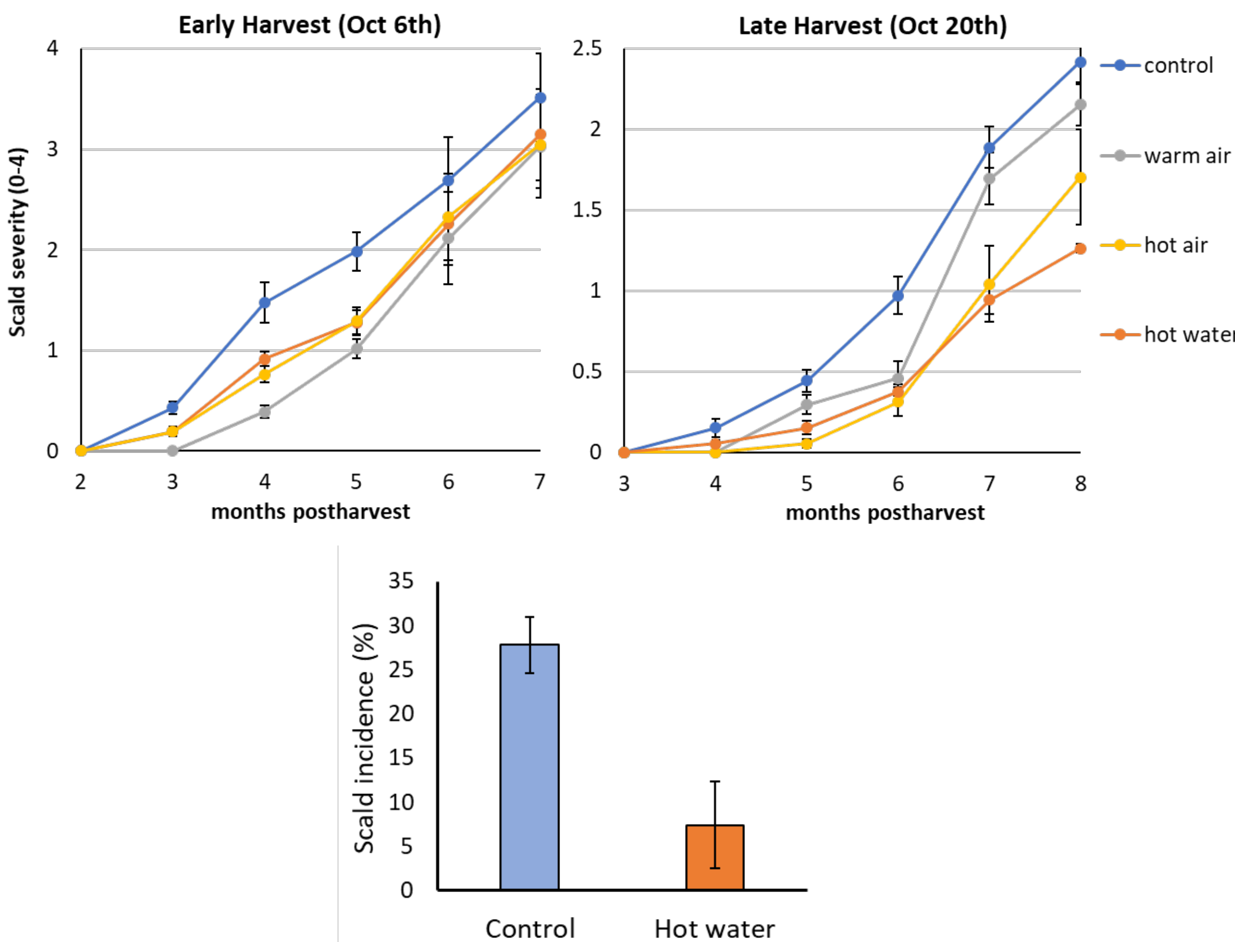

Fig. 4. At-harvest hot water, hot air and warm air treatments reduce scald during air storage but not following CA storage (0.5% O_2: 0.5% CO_2). Scald reduction using these strategies was most effective on mature fruit (Top left and top right). Post-CA-storage hot water treatment reduced scald up to 5 months following 3 months CA storage (bottom). Error bars = standard error.

storage scald mitigation treatments will be effective. Samples taken from our experiment combining delayed CA storage establishment and delayed post-storage 1-MCP and DPA treatment were tested for CTOL and other natural peel chemicals associated with superficial scald risk. CTOL (Fig. 3) and ASG (not shown) levels reflected eventual scald incidence. CTOL levels increased very little by the end of storage where effective (0.6% O_2) CA conditions were established immediately.

Post-storage (0.6% O_2–4 months) heat treatment reduces scald development. While post-storage 1-MCP treatment provides a scald mitigation tool for apples produced for a conventional market, other solutions are required for stock that is to remain in an organic or crop protectant-restricted cold chain. The consensus of our at-harvest stress and wounding treatments intended to reduce scald during a prolonged cold chain indicates that scald control using this general strategy only reduced scald development during air storage. However, as in most of our past work, once fruit were removed from CA, it would eventually develop scald after months in air at 33°F and stress or wounding treatments at harvest had little impact on latter cold chain scald where we proposed to reduce scald. It appears that scald reduction conferred by these "softer" at-harvest treatments was lost over a long period of ultra-low oxygen CA as opposed to 1-MCP and DPA which have a considerable residual impact that can control scald throughout a long cold chain. Consequently, year 2 experiments focused on determining why this was not working and if and when these sorts of scald mitigation strategies would provide any benefit. We found that scald reduction mediated by hot air, or hot water treatments was more effective for controlling scald on relatively more mature (at harvest) apples (Fig. 4). Warm air did not provide any appreciable control and hot air damaged the peel. As in Year 1, hot water impacted scald incidence during air storage and not following

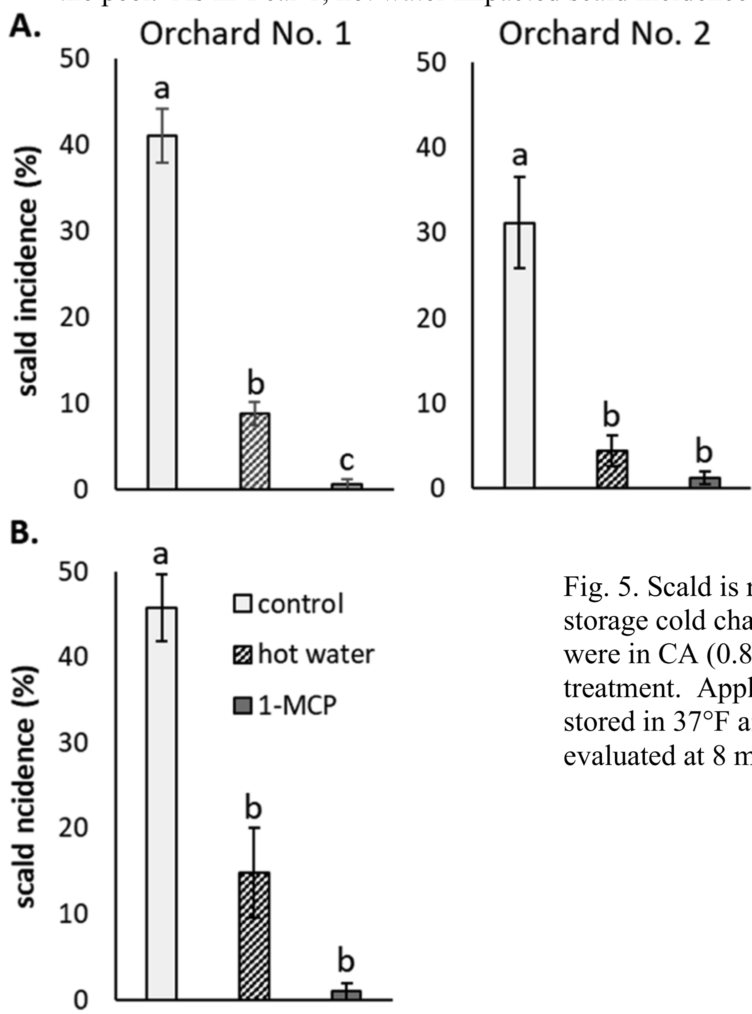

Fig. 5. Scald is reduced over a 4 month post-commercial CA storage cold chain by both 1-MCP and hot water treatment. Apples were in CA (0.8% O_2: 0.6% CO_2, 33°F) for 4 hot water or 1-MCP treatment. Apples were stored for 4 months in CA, treated, then stored in 37°F air for an additional 4 months. Scald incidence was evaluated at 8 months.

CA storage during a prolonged cold chain. However, applied hot water after removal from 3 months CA, reduced scald beyond 5 months in air at 33°F (Fig. 4). In Year 3, we sought to confirm that hot water treatment immediately following 4 months commercial and in-house CA effectively controlled scald. Hot water controlled scald up to 8 months at 33°F in air (Fig. 5). Fruit firmness and titratable acidity were no different among controls and treatments (not shown). Scald control by post-storage hot water treatment was equal to that of post-storage 1-MCP treatment on 1 of 2 commercially stored lots and the in-house stored lot. Results indicate that post-storage hot water treatment may be a viable scald control that could be adapted for controlling scald over a long post-storage cold chain for apples produced for a crop protectant restricted market.

Post-CA storage temperatures are critical for scald reduction in a crop protectant restricted cold-chain. Results from the previous year indicated that after commercial CA storage the optimal supply-chain temperature for minimizing scald was somewhere between 33-37°F (Fig. 6, left). Our most recent experiments determined to optimize supply-chain temperature and study the impact of subsequent retail temperature on scald development using 4 organic Granny Smith lots after six months of commercial CA storage (0.6-0.8% O_2). Consistent with the previous year's experiment, lowering the storage temperature from 37°F to 33°F decrease scald development, but the largest benefit came from decreasing the retail storage temperature (Fig. 6, right). A moderate decrease in scald was observed when temperatures were decreased from 68°F to 55°F after only one week, and further decreasing the temperature to 45°F resulted in a dramatic (~2.5-fold) reduction in scald occurrence. A great deal of effort is expended to maintain apples at the lowest possible temperature during storage, but dramatic increases in scald development arising from increased retail temperature may ultimately nullify the benefits of CA and low storage temperatures.

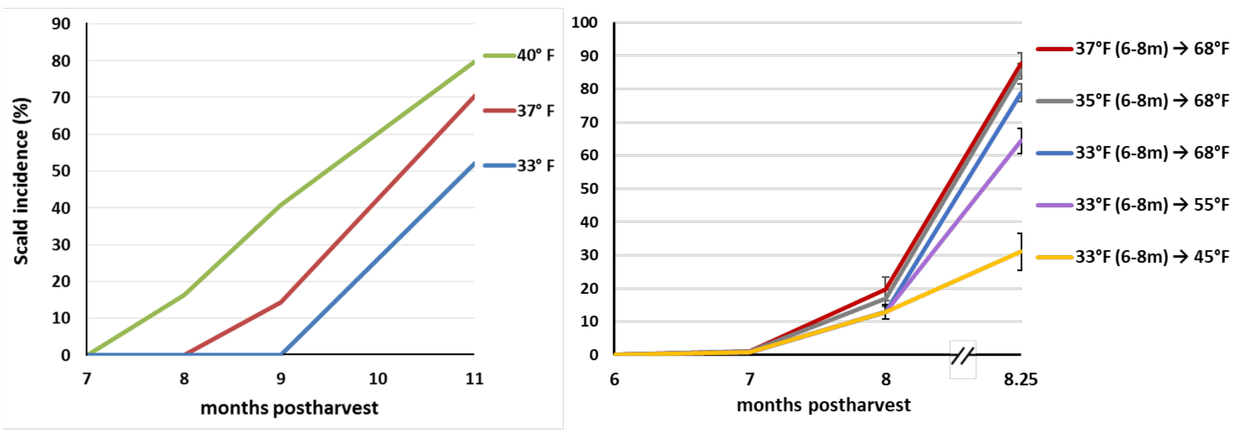

Fig. 6. Cooler temperatures reduce scald on commercial Granny Smith lots following storage at 0.6-0.8% O_2:1% CO_2 for 6 months. During year 1, 33, 37, and 40 °F was tested (left). In Year 2, we focused on determining the optimum temperature between 33 and 37 °F for 2 months post-storage and, then, best simulated retail temperature between 45 and 68 °F for 1 week starting at 8 months after harvest.

Conclusions

Where control options are limited by regulation or customer requirement and cold chains can last months beyond removal from storage, scald control strategies rely on advanced CA storage technologies coupled non-crop protectant-based controls such as acclimation and hot water. When scald is controlled using ULO, the induction period is prolonged or even delayed until after storage. This may afford new options with respect to post-storage 1-MCP treatment if a shift in marketing strategy is necessary but also makes it necessary to apply acclimation or temperature treatments that can reduce scald after removal from CA. A critical step when employing post-storage scald control strategies is estimating how much of the induction period has occurred during CA storage as any delay in CA atmosphere establishment, period of sub-optimal O_2 settings, or extended storage period may negatively influence any post-storage scald treatment. Our results indicate that scald induction is cumulative and only occurs where crop protectants are not used or CA conditions are not optimal (not less than 1%). Given our current information, we expect that the entire cumulative scald induction to be approximately 2 months sub-optimal conditions before and/or after storage. While this could be used as a general guideline to estimate scald-free life in the post-storage cold chain, we do not know of how pre-harvest conditions, such as chilling hours received, may influence this factor. A more exact approach may be using the protocol for monitoring scald risk (Blakey and Rudell, 2017) following CA storage to assess how much scald induction has occurred prior to and during storage. Finally, cold chain temperature, including retail storage is especially critical for maintaining scald free fruit where crop protectants are not used.

Publications

Blakey, R. and D.R. Rudell. 2017. Superficial scald risk assessment assay for apples. WSU Extension Bulletin FS287E. (www.extension.wsu.edu/publications/)

Poirier, B.C., Mattheis, J.P., and D.R. Rudell. 2020. Extending 'Granny Smith' apple superficial scald control following long-term oxygen controlled atmosphere storage. Postharv. Biol. Technol. 161 https://doi.org/10.1016/j.postharvbio.2019.111062

Project Title: Reducing scald after long-term CA storage (AP-16-101)

Executive Summary

Keywords: Superficial scald, apple, cold chain, hot water, scald risk assessment, post-storage scald mitigation

Abstract: Combinations of CA and other novel and established treatments were used to find protocols that reduce or eliminate scald following long-term CA where crop protectants are restricted. Hot water treatment following CA (below 0.8% O_2), immediately established after harvest worked best. Likely effectiveness of post-storage treatment could be indicated.

Project outcomes:

1. A protocol for reducing superficial scald up to 4 months following effective CA storage.
2. New evidence that shows superficial scald induction is cumulative and continues following CA storage.
3. New evidence that post-storage superficial scald treatments including both crop protectants and hot water treatment are effective.
4. A method for estimating the degree to which scald induction has occurred and the potential effectiveness post-storage scald mitigation treatments.
5. Temperature recommendations for the post-CA storage cold chain.

Significant Findings:

1. Scald induction is cumulative and rapidly imposed, effective (below 0.8% O_2) CA reduces the rate of induction. Induction resumes following CA storage.
2. At-harvest delayed cold storage (2 d), intermittent warming, or hot water treatment reduces scald better on more mature fruit but only following air storage.
3. Hot water treatment following effective CA storage (3-4 months 0.5% O_2 CA storage), reduces scald during the post-CA storage cold chain.
4. 1-MCP treatment following effective CA storage (3 or 6 months 0.5-0.8% O_2 CA storage) reduces scald in the subsequent cold chain.
5. The CTOL test (see Blakey and Rudell, 2017) may be used to indicate whether post-storage scald treatments will be effective.
6. Cold chain temperature following CA storage for organic Granny Smith should be below 37 °F and as close to 33 °F as possible for prolonged periods and optimally not above 45 °F on retail display.

Future Directions:

1. Test hot water treatment on packing line for non-crop protectant post-storage scald control
2. Improve existing and develop new post-storage scald risk assessment tests that accurately indicate the degree to which scald induction has occurred.
3. Develop at-harvest superficial scald risk assessment tests that accurately reflect chilling hours and other factors impacting susceptibility.