Project Title: Reducing CO_2-related disorders during Honeycrisp rapid CA treatment

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Report Type: Continuing Project Report

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $22,000
Total Project Request for Year 2 Funding: $85,000
Total Project Request for Year 3 Funding: $85,000

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2025
Amount: $115,317/3 yrs.
Agency Name: USDA-ARS, In-house project
Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs ($8000/yr), supplies and materials ($3000/yr), travel ($1000/yr), and 0.1 FTE (PI) and 0.1 FTE (technical).

Other related/associated funding sources: Awarded
Funding Duration: 2023 - 2027
Amount: $555,828/4 yrs.
Agency Name: USDA-NIFA
Notes: Project submitted to SCRI program on FY21 was highly scored but not funded. Reviewer concerns will be addressed with stakeholder consultation and the proposal resubmitted for FY22.

Budget
Primary PI: David Rudell
Organization Name: USDA-ARS
Contract Administrator: Sharon Blanchard
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Contract administrator email address: Sharon.Blanchard@usda.gov

Item	2022	2023	2024
Salaries (GS-7)*		43,311	45,764
Benefits (40%)		18,124	18,305
Wages	5,000	5,000	5,000
Benefits			
Equipment			
Supplies	5,000	4,565	3,931
Travel			
Miscellaneous**	12,000	12,000	12,000
Plot Fees			
Total	22,000	85,000	85,000

Footnotes: *Estimated 3% salary increase; **22% of instrument service contract

Objectives:

1. Determine influence of CO_2 levels on disorder development during rapid CA treatment.
2. Determine influence of temperature on disorder development during rapid CA treatment.
3. Monitor flesh chemistry to indicate which CO_2 level treatment conditions may elevate risk of developing soft scald/soggy breakdown or CO_2-related/other disorders.

SIGNIFICANT FINDINGS

1. Rapid CA conditioning eliminated soft scald/soggy breakdown.
2. CO_2-related internal browning incidence decreased with increasing conditioning temperature.
3. Rapid CA conditioning does not compromise quality (6 months) regardless of how long it was delayed.
4. Fruit quality was not impacted by conditioning temperature.
5. CO_2-related symptoms developed as a result of elevated CO_2 during rapid CA in all seasons where symptoms developed.
6. Internal browning associated with elevated CO_2 during rapid CA develops after transfer to long-term CA (low CO_2) storage.
7. Differences of CO_2 sensitivity was observed using cortex chemistry monitoring.
8. Bitter pit and leather blotch were more common at orchards where fruit was less mature at harvest.
9. Apples stored in atmospheres comprising higher CO_2 had higher titratable acidity and total soluble solids.

METHODS

Objective 1: Determine influence of CO_2 levels on disorder development during rapid CA treatment.

In year 3, Honeycrisp apples were harvested as close to commercial harvest as possible from 9 orchards near Bridgeport, Mattawa, Quincy, and Royal City, WA. Harvest maturity (internal ethylene concentration, firmness, starch index, titratable acidity, and soluble solids) and external/internal appearance were evaluated, and fruit were imaged. Apples were treated with 1-MCP (about 1 ppm), then stored in 2.5% O_2 and (0.5, 1, 2, 3, 5%) CO_2 for 7 days at 50 °F. Following conditioning, apples were stored for 6 months in 2.5% O_2 and 0.5% CO_2 at 37 °F upon which external and internal disorders, firmness, titratable acidity, and soluble solids were evaluated. All statistical analysis was performed using SAS version 9.4 TS Level 1M8.

Objective 2: Determine impact of initial fruit temperature during conditioning.

To determine the impacts of conditioning temperature during rapid CA, Honeycrisp apples were harvested approximately one week after commercial harvest from an orchard in Mattawa, WA. Harvest maturity (internal ethylene, firmness, starch index, titratable acidity, and soluble solids) and external/internal defects were evaluated, and fruit were imaged. Apples were treated with 1-MCP (about 1 ppm) and immediately placed in CA in 0.5% O_2 and 2.5% CO_2 at (37, 46, 50 °F) for 7 days. Due to previous difficulties in observing disorders in the apples and to establish whether disorders would develop, 2 trays were stored in a room held at 51 °F for 7 days. Following conditioning, apples were stored in 2.5% O_2 and 0.5% CO_2 at 37 °F for 6 months upon which external and internal disorders, firmness, titratable acidity, and soluble solids were evaluated.

Objective 3: Monitor flesh chemistry to indicate which treatment conditions may elevate risk of developing soft scald/soggy breakdown or CO_2-related browning.

Honeycrisp apples were picked from the same 9 orchards in objective 1 at commercial harvest. They were treated with 1-MCP and underwent a 7-day conditioning period at 50 °F, 2.5% O_2, and one of the following five CO_2 levels: 0.5%, 1%, 2%, 3%, and 5%. During the conditioning period, apple cortex was assessed for injury and sampled for metabolic analysis at days 0 and 7 for all orchards, but also at days 2 and 4 for three of the nine orchards. Cortex samples were frozen in liquid nitrogen and ground using an analytical mill (IKA model A 11 B S001) and were stored at -80 ºC prior to targeted analysis for chemicals linked to CO_2-sensitivity (McTavish et al., 2025).

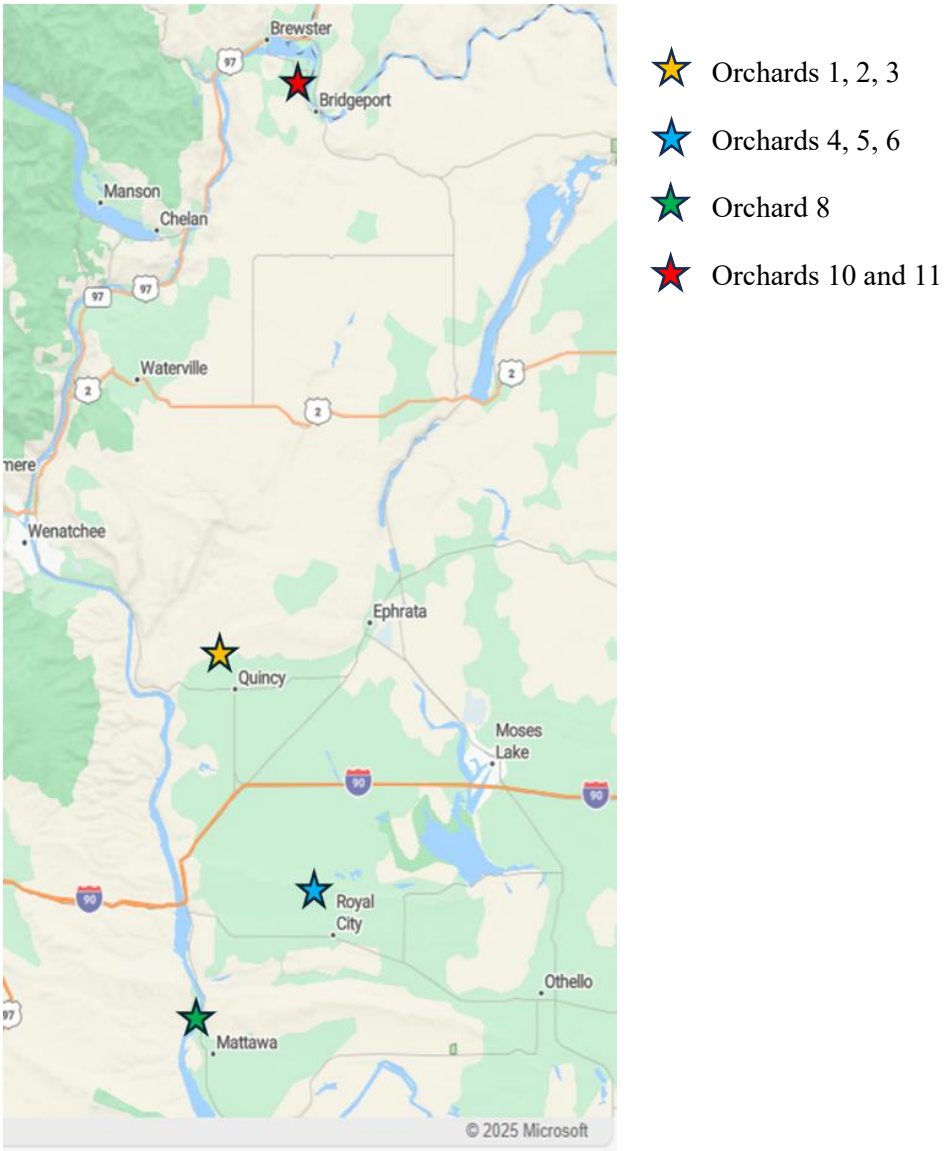

Figure 1. Distribution of orchards near four cities (Bridgeport, Quincy, Royal City, and Mattawa) of Washington State.

RESULTS AND DISCUSSION

Objective 1: Determining influence of CO_2 levels on disorder development during rapid CA treatment

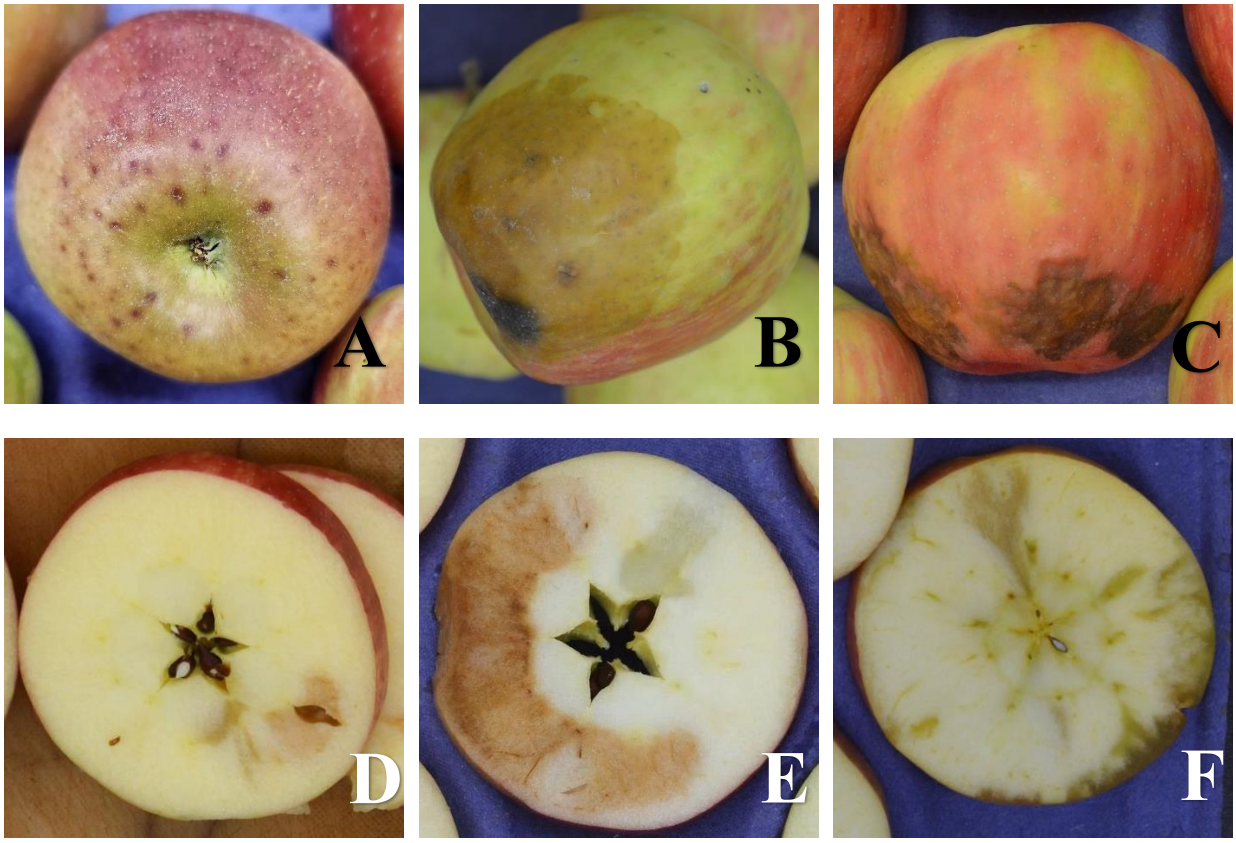

Figure 2. Disorders of Honeycrisp observed in the 2024 sampling season. (A) Bitter pit (B) Soft scald (C) Leather blotch (D) CO_2 browning and lens-shaped cavity (E) Soggy breakdown (F) Watercore. Brightness was enhanced 20% on pictures D-F.

Honeycrisp apples were harvested immediately ahead of commercial harvest from 7 orchards and immediately after harvest for 2 (orchards 10 and 11). Apples were temperature conditioned during the initial pull down to 2.5% O_2 and different CO_2 concentrations to observe response to CO_2 levels as well as susceptibility to bitter pit, soft scald, leather blotch, soggy breakdown, and watercore. Averaged starch index values at harvest were between 4 and 6 at all orchards except orchards 3 and 10 with values of 2.08 and 3.17 respectively. Likewise, orchards 3 and 10 produced the least internal ethylene further indicating fruit from these orchards was less mature than those from the other 7 orchards.

Bitter pit, leather blotch, and watercore incidence remained insignificant on apples harvested from every orchard, but notably fruit from orchard 3 developed high frequencies of leather blotch (up to 84%), regardless of conditioning treatment (Table 1). The relatively less mature apples from orchards 3 and 10 explains the higher incidence of bitter pit in fruit originating

Table 1. Percent disorder incidence after 6 months of storage of Honeycrisp harvested at 9 different orchards and conditioned at 50 °F alongside rapid CA with 2.5% O_2 and varying CO_2 levels to display differences in disorder development among orchards. CO_2-related internal browning and lens-shaped cavities were orchard dependent and, in those orchards with significant incidence, was primarily associated with 5% CO_2 during conditioning. Other disorders were less linked with conditioning variables and were, instead, equally prevalent in all treatments as in the case of leather blotch. Data were analyzed comparing CO_2 conditioning treatments within a single orchard using either a Pearson's Chi-Square table or a Fisher's exact test if the data was sparse (cells with counts <5), followed by pairwise comparisons using Pearson's Chi-Squares (n=36; α=0.05). Letters indicate significant differences of disorder incidence within a single orchard, which were observed at some orchards for soft scald, CO_2 damage, and soggy breakdown.

Orchard #	CO_2 Treatment	Bitter Pit	Soft Scald	Leather Blotch	CO_2-relatedinternal browning and cavities	Soggy Breakdown	Watercore
1	0.50%	0 a	2.86 a	0 a	14.29 ab	0 a	0 a
	1%	2.78 a	0 a	0 a	11.11 a	0 a	0 a
	2%	0 a	0 a	0 a	11.11 a	5.56 a	0 a
	3%	0 a	0 a	0 a	5.56 a	2.78 a	0 a
	5%	0 a	0 a	0 a	30.56 b	0 a	0 a
2	0.50%	0 a	0 a	0 a	5.56 a	0 a	0 a
	1%	0 a	16.67 b	0 a	11.11 a	11.11 b	0 a
	2%	0 a	0 a	0 a	0 a	0 a	0 a
	3%	0 a	0 a	0 a	16.67 a	0 a	0 a
	5%	0 a	8.33 ab	0 a	11.11 a	8.33 ab	0 a
3	0.50%	0 a	0 a	75.00 a	2.78 a	0 a	0 a
	1%	0 a	0 a	72.22 a	2.78 a	0 a	0 a
	2%	0 a	0 a	83.33 a	2.78 a	0 a	0 a
	3%	0 a	0 a	75.00 a	0 a	0 a	0 a
	5%	0 a	0 a	77.78 a	0 a	0 a	0 a
4	0.50%	0 a	8.57 a	2.86 a	2.86 a	0 a	0 a
	1%	0 a	0 a	8.33 a	5.56 a	0 a	0 a
	2%	0 a	0 a	8.57 a	2.86 a	0 a	0 a
	3%	0 a	5.71 a	0 a	0 a	0 a	0 a
	5%	0 a	5.56 a	13.89 a	2.78 a	0 a	0 a
5	0.50%	0 a	0 a	8.82 a	2.94 a	0 a	0 a
	1%	0 a	0 a	8.33 a	0 a	0 a	0 a
	2%	0 a	0 a	3.03 a	3.03 a	0 a	0 a
	3%	0 a	0 a	8.57 a	8.57 ab	0 a	0 a
	5%	0 a	2.86 a	11.43 a	25.71 b	0 a	0 a
6	0.50%	0 a	0 a	0 a	0 a	0 a	0 a
	1%	0 a	0 a	2.78 a	0 a	0 a	0 a
	2%	0 a	0 a	2.86 a	0 a	0 a	0 a
	3%	0 a	0 a	0 a	0 a	0 a	0 a
	5%	0 a	0 a	2.86 a	0 a	0 a	0 a
8	0.50%	0 a	0 a	0 a	0 a	0 a	0 a
	1%	0 a	0 a	2.78 a	8.33 a	0 a	0 a
	2%	0 a	0 a	0 a	5.56 a	0 a	0 a
	3%	0 a	0 a	0 a	5.56 a	0 a	0 a
	5%	0 a	0 a	0 a	2.78 a	0 a	0 a
10	0.50%	2.94 a	0 a	2.94 a	11.76 a	0 a	8.82 a
	1%	11.11 a	0 a	0 a	13.89 a	0 a	2.78 a
	2%	8.82 a	0 a	0 a	17.65 a	0 a	8.82 a
	3%	2.78 a	0 a	0 a	5.56 a	0 a	0 a
	5%	5.71 a	0 a	2.86 a	8.57 a	0 a	0 a
11	0.50%	2.78 a	0 a	2.78 a	8.33 a	0 a	0 a
	1%	0 a	0 a	2.78 a	2.78 a	0 a	0 a
	2%	0 a	0 a	0 a	2.78 a	0 a	2.78 a
	3%	0 a	0 a	0 a	5.56 a	0 a	0 a
	5%	0 a	0 a	2.78 a	0 a	0 a	0 a

from orchard 10 and leather blotch at orchard 3, both disorders associated with immature apples. Soft scald and soggy breakdown incidence were different among conditioning treatments for apples from

orchard 2, where the 1% CO_2 treatment had more instances of these disorders than all other treatments except the 5% CO_2 treatment (Table 1). Incidence on fruit from other orchards was insignificant. Honeycrisp from orchards 1 and 5 developed CO_2-related internal browning, primarily when CO_2 levels were adjusted to 5%.

Table 2. Fruit quality after 6 months of storage of Honeycrisp conditioned at 50 °F alongside rapid CA with 2.5% O_2 and varying CO_2 levels to display differences in CO_2 sensitivity among orchards. Firmness was not impacted by CO_2 % during conditioning. TA was elevated with CO_2% during conditioning. Data were analyzed comparing CO_2 conditioning treatments within a single orchard using ANOVA followed by Tukey's comparison or – if the data were not normally distributed or did not have equal variances – a Kruskal-Wallis test followed by Wilcoxon DSCF pairwise comparisons (n=36 for firmness and 18 for TA and °Brix; α=0.05). Letters indicate significant differences between CO_2 treatments within a single orchard, which were observed at some orchards for TA and °Brix.

Orchard #	CO_2 Treatment	Firmness (lb)	TA (g/L)	°Brix
1	0.50%	14.28 a	3.98 ab	14.48 a
	1%	14.39 a	3.79 a	14.47 a
	2%	14.59 a	3.91 ab	14.81 a
	3%	14.67 a	4.14 ab	14.72 a
	5%	14.55 a	4.39 b	14.57 a
2	0.50%	14.46 a	3.95 a	14.16 a
	1%	15.06 a	4.06 a	14.11 a
	2%	14.85 a	4.12 a	13.99 a
	3%	14.87 a	4.22 a	14.22 a
	5%	14.21 a	4.02 a	13.97 a
3	0.50%	14.42 a	5.42 a	13.19 a
	1%	14.89 a	5.61 a	13.16 a
	2%	14.97 a	5.61 a	12.96 a
	3%	14.86 a	5.56 a	12.98 a
	5%	14.94 a	5.52 a	13.23 a
4	0.50%	13.15 a	3.70 a	12.24 ab
	1%	13.84 a	4.05 ab	12.39 ab
	2%	13.12 a	4.13 b	12.13 a
	3%	13.53 a	4.16 b	12.59 b
	5%	13.45 a	3.93 ab	12.53 b
5	0.50%	14.94 a	4.80 a	13.45 a
	1%	15.44 a	4.52 a	12.91 b
	2%	15.58 a	4.52 a	13.14 ab
	3%	15.12 a	4.77 a	13.22 ab
	5%	15.16 a	4.80 a	13.12 ab
6	0.50%	14.72 a	3.50 a	13.86 ab
	1%	14.97 a	4.07 b	13.75 abc
	2%	14.66 a	3.79 ab	13.56 c
	3%	14.48 a	3.42 a	13.61 bc
	5%	14.84 a	3.54 ab	13.91 a
8	0.50%	15.94 a	5.38 a	13.64 a
	1%	15.70 a	5.46 a	13.74 a
	2%	15.58 a	5.44 a	13.58 a
	3%	15.54 a	5.46 a	13.52 a
	5%	15.66 a	5.54 a	13.51 a
10	0.50%	16.47 a	6.24 ab	17.77 a
	1%	17.16 a	6.31 ab	17.66 ab
	2%	17.77 a	6.33 ab	17.67 ab
	3%	17.52 a	6.22 a	17.88 a
	5%	17.73 a	6.79 b	17.32 b
11	0.50%	14.74 a	4.47 ab	14.09 a
	1%	15.08 a	4.67 abc	14.23 a
	2%	15.23 a	4.40 a	14.16 a
	3%	14.86 a	4.85 bc	14.41 a
	5%	15.01 a	4.90 c	14.43 a

There were no significant differences in fruit firmness among treatments at any orchard after 6 months of storage. Conditioning treatment did impact titratable acidity and soluble solids concentrations (Table 2). Significant differences of titratable acidity among treatments occurred following storage of apples from orchards 1, 4, 6, 10, and 11. The majority of these differences were linked with higher CO_2 % during conditioning, including stepwise increases with CO_2 levels in a few cases rather than only the extremes. CO_2 level during conditioning impacted soluble solids concentrations for fruit from orchards 4, 5, 6, and 10. Most of these differences were between adjacent treatments in CO_2 concentration rather than extremes.

Objective 2: Determining impact of initial fruit temperature during conditioning

Honeycrisp apples were harvested from orchard 8 (objective 1) about 2 weeks after commercial harvest. Apples were conditioned at various temperatures to observe disorder incidence rates response. Average starch values of 5.89 and an IEC of 40.71 ppm at harvest indicate these apples were well into the climacteric stage of ripening. Firmness and titratable acidity were consistent across treatments, but soluble solids varied when comparing the temperature treated apples to the control treated apples (Table 3). All temperature conditioned apples (37, 46, and 50 °F) had significantly more soluble solids compared to the air treated apples. There were no significant differences in apple quality between the temperature treatments.

Table 3. Fruit quality after 6 months of storage of Honeycrisp conditioned at 2.5% CO_2 and 0.5% O_2 and varying temperatures. The air stored, unconditioned comparison is listed at the bottom. Results demonstrate that temperature treatment following 1-MCP treatment had little influence on fruit quality. Data were analyzed comparing treatments using ANOVA followed by Tukey's comparison or – if the data were not normally distributed or did not have equal variances – a Kruskal-Wallis test followed by Wilcoxon DSCF pairwise comparisons (n=36 for firmness and 18 for TA and °Brix; α=0.05). Letters indicate significant differences between treatments, which were observed among the °Brix values.

Treatment	Firmness (lb)	TA (g/L)	°Brix
37 °F	13.61 a	4.04 a	13.14 ab
46 °F	13.92 a	4.09 a	13.20 ab
50 °F	13.92 a	4.17 a	13.42 a
Air	13.64 a	4.03 a	12.50 c

There were no instances of bitter pit, leather blotch, or watercore in any of the apples sampled from orchard 8 at the late harvest (Table 4). Apples conditioned at 37 °F were more sensitive to CO_2 than those conditioned at 46 °F. Also, apples conditioned at any temperature developed more CO_2-related internal browning and lens-shaped cavities than those stored in air without conditioning. There was no significant incidence of soggy breakdown and soft scald.

Table 4. Percent disorder incidence after 6 months of storage of Honeycrisp conditioned at 2.5% CO_2 and 0.5% O_2 and varying temperatures. The air stored, unconditioned comparison is listed at the bottom. Results indicate that lower temperatures during conditioning elevate CO_2-sensitivity. Data were analyzed comparing treatments using either a Pearson's Chi-Square table or a Fisher's exact test if the data was sparse (cells with counts <5), followed by pairwise comparisons using Pearson's Chi-Squares (n=36; α=0.05). Letters indicate significant differences of disorder incidence.

Treatment	Bitter Pit	Soft Scald	Leather Blotch	CO_2-related internal browning and cavities	Soggy Breakdown	Watercore
37 °F	0 a	0 a	0 a	30.56 a	0 a	0 a
46 °F	0 a	0 a	0 a	11.11 b	2.78 a	0 a
50 °F	0 a	0 a	0 a	13.89 ab	0 a	0 a
Air	0 a	5.56 a	0 a	2.78 bc	2.78 a	0 a

Objective 3: Monitoring flesh chemistry during conditioning period

Honeycrisp cortex was collected from the apples from all locations used for objective 1 before and after the conditioning period and, for 3 of the 9 orchards, an additional two times during the conditioning period. The purpose for sampling cortex during the conditioning period was to monitor levels of natural chemicals found to be associated with risk of CO_2-related internal browning in our previous project (McTavish et al., 2025). This was attempted in 2023 at one orchard but disorder incidence was low, hence it was repeated in 2024 using multiple orchards.

CO_2-related browning risk was analyzed during the conditioning period for orchard 5. Cortex was analyzed for changes in levels of risk-associated natural chemicals. These are chemicals related to cell membrane integrity that were also identified earlier by us as associated with superficial scald risk. The relationship of one natural chemical group, the acylated steryl glycosides (ASGs), changes in ratio with its related steryl ester (SE). We are monitoring this ratio during conditioning to determine if risk caused by the environment during conditioning will lead to browning during long-term storage. If CO_2 levels were adjusted to 5%, apples from orchard 5 produced a higher ASG:SE ratio of these compounds than those conditioned in an atmosphere comprising 1% CO_2 (Figure 3). The spike in ASG:SE is potentially correlated with long-term disorder development, as 25.71% of the apples from orchard 5 conditioned in 5% CO_2 exhibited CO_2-related injuries and those conditioned in 1% CO_2 showed none (Table 1). This analysis is ongoing and expected to be completed for all samples from all orchards regardless of storage outcome with the intent of determining if these changes are linked with risky conditions or strictly with eventual symptom development.

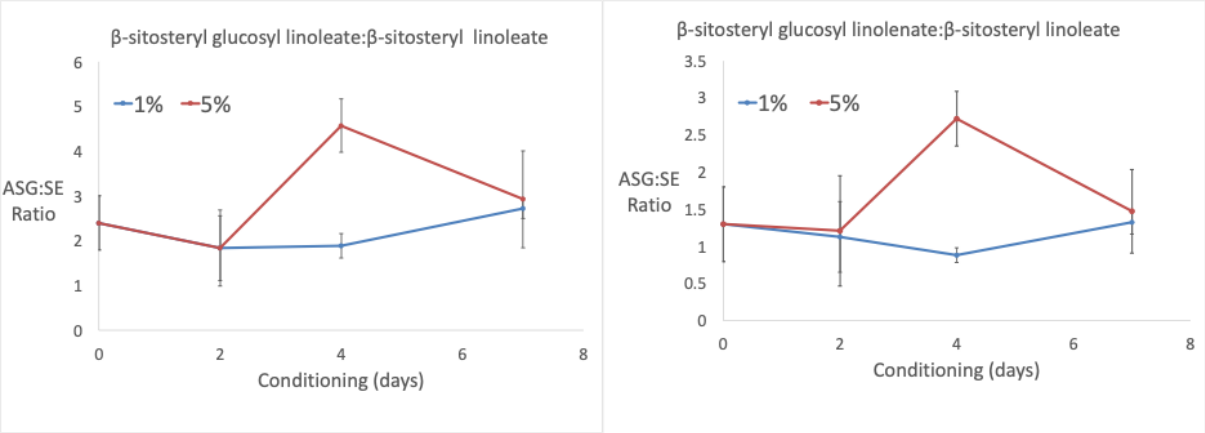

Figure 3. Natural chemicals levels increasing during rapid CA conditioning of Honeycrisp in conditions more likely to cause apples to develop internal browning. Apple cortex chemistry was analyzed during the asymptomatic conditioning period to determine if changes in CO_2-risk related chemistry would reflect disorder outcome. This was true for orchard 5 where there was a spike of the ASG:SE ratio between 2 and 7 days. Analyses are ongoing for the remainder of samples in this and other orchards. Error bars indicate standard error (n=6).

References:

McTavish, C., Klarer, E., Milne, S., Valdez, N., Mattheis, J., Leisso, R. and Rudell, D., 2025. Risk assessment candidates for carbon dioxide-related internal browning of apple during controlled atmosphere storage. Postharvest Biology and Technology, 230:113840.