Project Title: Reducing carbon dioxide-related postharvest disorders **(AP-19-100)**

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Report Type: Final Project Report

Project Duration: 3-Year with no cost one year extension
Total Project Request for Year 1 Funding: \$79,314
Total Project Request for Year 2 Funding: \$92,893
Total Project Request for Year 3 Funding: \$95,036

Other related/associated funding sources: Awarded
Agency Name: USDA-ARS, In-house project
Amount: \$174,719/3 yrs.
Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs (\$8000/yr), supplies and materials (\$3000/yr), travel (\$5000/yr), and 0.2 FTE (PI, co-PI) and 0.1 FTE (technical).

Other related/associated funding sources: Proposed
Amount: \$540,888/4 yrs.
Agency Name: USDA-NIFA
Notes: Pre-proposal with complimentary objectives submitted to SCRI program.

Budget 1
Primary PI: David Rudell
Organization Name: USDA-ARS
Contract Administrator: Chuck Myers
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Item	2019	2020	2021	2022
Salaries (GS-9 step 1)	52,116	53,679	55,290	
Benefits (33.3%)	17,198	17,714	18,246	
Wages (part-time employee)	10,000	10,000	10,000	
Benefits				
Equipment				
Supplies				
Travel				
Miscellaneous*		11,500	11,500	
Plot Fees				
Total	79,314	92,893	95,036	0

Footnotes: One-third instrument service contract

Objectives:
1. Develop methods to consistently identify CO_2 sensitivity.
2. Determine best cold chain practices when CO_2 sensitivity is indicated.
3. Identify fruit chemistry associated with CO_2 sensitivity.

SIGNIFICANT FINDINGS

1. A variety of internal and external browning symptoms may be attributable to CO_2 sensitivity in many of the cultivars tested.
2. 'Honeycrisp' and 'Pazazz' are sensitive to CO_2 but also develop soft scald and soggy breakdown.
3. Incidence of symptoms related to CO_2 sensitivity were reduced or eliminated by DPA drenching.
4. Reducing CO_2 exposure concentration and delaying CA impacted CO_2 disorders albeit inconsistently among cultivars.
5. Peel and cortex chemistry of typically CO_2-related symptoms is different from other peel and cortex defects.
6. Cultivars can be screened for CO_2 sensitivity using an easy, inexpensive protocol.

METHODS

Equipment and Cooperative Summary: Storage experiments, fruit quality assessment, fruit chemistry analyses using analytical instrumentation (gas and liquid chromatography-mass spectrometry), and tissue cryopreservation will be performed using facilities currently in place at ARS-TFRL, Wenatchee. Storage experiments will be conducted in our in-house CA chambers capable of maintaining both O_2 and CO_2 CA environments accurately.

Outreach (Deliverables are summarized under "Anticipated Products" Table 2): Aside from reports to the WTFRC, new information will be disseminated through presentations at industry meetings and at professional conferences, and by publications in industry publications and peer-reviewed journals. We will cooperate with WTFRC (Lead: Ines Hanrahan) to document symptoms of injury not already covered by the new WSU Apple Defect guide. Symptomatic fruit will be photographed, defect notes assembled, and associated descriptive text created. These updates will be incorporated into the existing guide as needed.

Objective 1: Develop methods to consistently identify CO_2 sensitivity

In year 1, 15 apple cultivars were harvested at approximately 2-4 weeks prior to commercial harvest and 7 days after commercial harvest. Harvest maturity (starch index and internal ethylene concentration) and external/internal appearance were evaluated, and fruit was imaged with a digital camera. Two trays of apples were drenched with an emulsion containing DPA (2000 ppm), and 2 other trays were treated with a solution containing only the inactive ingredients from the DPA emulsion (referred to as control trays). The DPA and control trays were put in separate CA chambers to avoid DPA cross contamination and set at 0.6 % O_2: 5% CO_2. After 4 months, apples were evaluated for internal and external defects. Fruit along with the external and internal defects were imaged. Symptomatic tissue was sampled, flash frozen, and cryo-preserved for chemical analysis where defects were found. Cultivars that did not develop CO_2-related symptoms in Year 1 and Year 2 were re-tested in Year 3.

Objective 2: Determine best cold chain practices when CO_2 sensitivity is indicated

Activities under this objective include 1) determining thresholds for O_2:CO_2 storage atmosphere combinations and 2) developing strategies for managing CO_2-related disorders in higher risk apples in any cold chain. Activity 3) focused on developing low-cost and simple protocols for the industry to create a high CO_2 environment to screen their own cultivars.

For activity 1, 'Golden Delicious' was harvested 2 weeks before commercial maturity while 'Fuji', WA-38, 'Braeburn', 'Honeycrisp', 'Scilate', 'Pazazz', and JUICI were harvested 7 days after commercial maturity and stored in 1 of 4 CA settings at 33 °F: 0.6% O_2, 1% CO_2; 0.6% O_2, 5% CO_2; 1% O_2, 1% CO_2; 1% O_2, 5% CO_2 (36 apples per CA environment). These will be stored for 4 months, removed and internal and external injury evaluated and documented. For activity 2, 'Pazazz', JUICI, 'Scilate', 'Honeycrisp', and WA-38 were harvested at commercial maturity. Apples were treated with 1 ppm 1-MCP for 12 h. Following 1-MCP treatment, apples were placed into one of the following regimes: immediate CA, 2 weeks air (33 °F) then CA, or 4 weeks air (33 °F) then CA, with CA conditions of 0.6% O_2, 5% CO_2 at 33 °F utilized. Each cultivar had 108 apples per treatment combination. At 3 months, external disorder incidence was evaluated, and at 6 months, external and internal disorder incidence as well as fruit quality was evaluated. The remainder of cultivars determined to be CO_2 sensitive under Objective 1 were tested in Year 3, which included 'Fuji', 'Plumac', 'Golden Delicious', and JUICI (retested).

For the third activity, 360 apples each of a CO_2 sensitive cultivar (JUICI) and a non-sensitive cultivar ('Delicious') were harvested 7 days after first commercial pick. 108 apples from each cultivar were either drenched with an emulsion containing DPA (2000 ppm) or with a solution containing only the inactive ingredients from the DPA emulsion (DPA-free). The DPA and DPA-free treatment were each then separated into clear plastic bags with 36 fruits per bag. Each bag was tightly twisted shut and secured using two zip-ties to ensure the bag would not leak (Figure 4). The remaining untreated fruit were stored in boxes as a control. Bags were stored in 30 °F air with O_2 and CO_2 levels were evaluated daily, then all treatments were evaluated for external and internal CO_2 injury at 4 months.

Objective 3: Identify chemistry associated with CO_2 sensitivity

Our broad analysis of peel and cortex chemistry is ongoing and is the remaining activity we need to complete during the no cost extension. To develop a system for diagnosing peel and cortex browning caused by CO_2 sensitivity, symptomatic peel and cortex from activities outlined under objective 1 were sampled regularly with adjacent healthy tissue and healthy tissue from DPA treated fruit as control. Any browned tissue in or on DPA treated fruit was also sampled as a control to reveal any similarities or differences of chemistry caused by non-CO_2 related browning. This is expected to improve our accuracy of discerning browning injuries caused by CO_2 sensitivity from browning caused by other factors.

We also determined how increasing CO_2 levels in storage influence symptom development alongside changes in levels of chemicals linked with CO_2 sensitivity. By doing this, we confirmed the chemistries that are specific to CO_2 sensitivity. 'Pazazz' was chosen for this activity, as it was one of the most CO_2 sensitive cultivars. At harvest, apples were drenched with 2000 ppm DPA or a solution containing the inactive ingredients. Apples were then stored at 33 °F CA at 0.6% O_2 and different levels of CO_2 (0, 1, 2.5, or 5%). Peel and cortex have been sampled at harvest, 0, 2, 4, 8, and 16 weeks. This experiment was repeated in Year 3 with 'Fuji', as it was also one of the more sensitive cultivars but stores better than 'Pazazz'.

RESULTS AND DISCUSSION

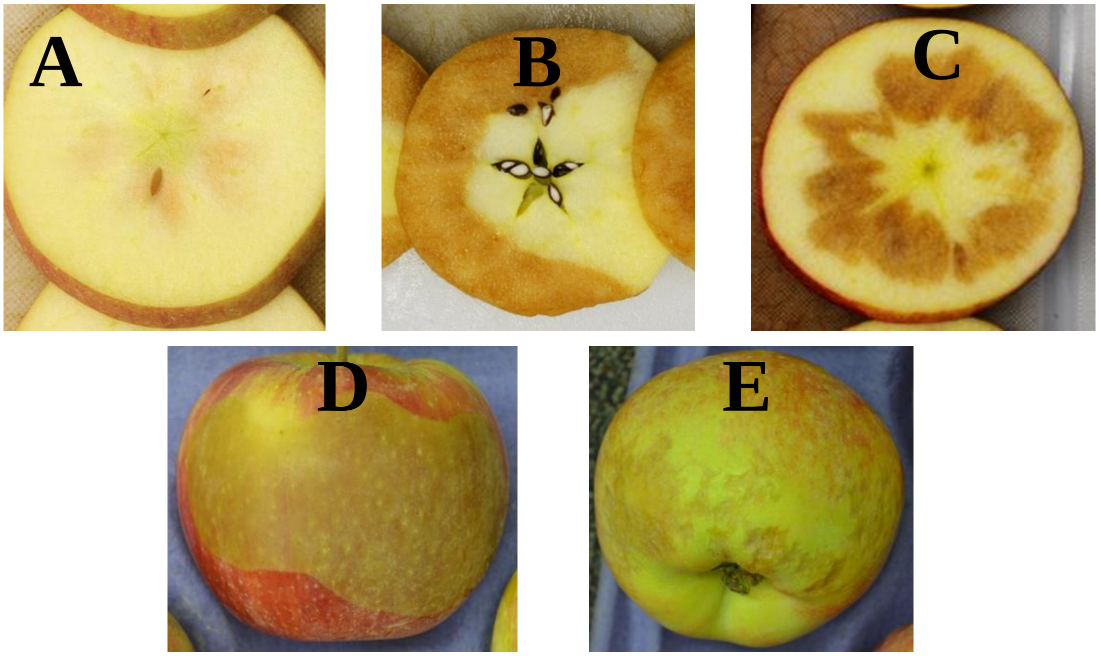

Figure 1. General categories (types) of observed disorders. (A) Lens shaped cavities ('Braeburn'), (B) non-radial browning (soggy breakdown 'Honeycrisp'), (C) radial browning ('Honeycrisp'), (D) peel browning (soft scald, 'Honeycrisp'), and (E) orange ("rugose scald") peel ('Pazazz'). C and E are typically associated with CO_2 sensitivity.

Triggering external and internal CO_2 sensitivity and distinguishing symptoms typically attributed to CO_2 sensitivity from other disorders.

A variety of internal and external symptoms were observed and recorded across many cultivars in the test. These ranged from soft scald of the peel and soggy breakdown in the cortex to the typical symptoms attributed to CO_2 sensitivity, such as orange peel ("rugose") scald on peel and lens-shaped cavities and/or radial browning in the cortex with an asymptomatic barrier immediately under the peel (Fig. 1). Symptoms were presented and discussed at a scientific roundtable to amend WSU online disorder databases in the summer of 2020.

All cultivars, other than 'Autumn Glory', developed some sort of disorder, although incidence was insignificant in many cases (Table 1). Cultivars with significant symptom development of any type on any of the treatments included 'Fuji', 'Plumac', 'Braeburn', 'Scilate', JUICI, 'Honeycrisp', 'Pazazz', 'Smitten', 'Gala', and 'Cripps Pink'. Harvest maturity impacted disorder development. 'Fuji' orange peel symptoms were more prevalent on earlier harvested fruit while most cortex disorders were either more prominent or only found in cortex of the later harvest. Internal cavities were the least impacted by harvest maturity. We observed some symptoms that were less recognizable such as severe core and peel browning of 'Fuji' (Fig. 2).

Figure 2. Softened solid brown cortex on 'Fuji'. Incidence was not eliminated by DPA drench.

Table 1. Percent incidence of different internal and external disorders in Year 1 (see Fig. 1). Radial browning and rough or "orange peel" peel texture symptoms are typically associated with CO_2 sensitivity. DPA drenches typically reduce or eliminate CO_2-related disorders. This activity was repeated in Years 2 and 3 on cultivars with no disorders in Years 1 and/or 2. Only one harvest was tested in year 3.

Cultivar	harvest	Treatment	Cavities	Non-radial browning	Radial browning	External browning	Orange peel
Golden Delicious	early	no DPA					
	early	DPA					
	late	no DPA	3				3
	late	DPA					
Gala (Year 3)		no DPA		**69**		**47**	
		DPA		**81**		**36**	
Cripps Pink (Year 3)		no DPA	8				**92**
		DPA					
Ambrosia	early	no DPA					3
	early	DPA					
	late	no DPA					
	late	DPA					
Delicious	early	no DPA	6				
	early	DPA					
	late	no DPA					
	late	DPA					
Fuji	early	no DPA	**11**	3			**14**
	early	DPA	3				
	late	no DPA	3	**86**	**86**		3
	late	DPA		**28**	**28**		
Autumn Glory	early	no DPA					
	early	DPA					
	late	no DPA					
	late	DPA					
Plumac	early	no DPA	**11**				
	early	DPA					
	late	no DPA	3	6			
	late	DPA					
Braeburn	early	no DPA	**14**	**14**			
	early	DPA					
	late	no DPA	**58**		**89**	6	6
	late	DPA		6			
Smitten (Year 3)		no DPA	**14**		**31**		
		DPA					
Scilate	early	no DPA					
	early	DPA					
	late	no DPA			**86**		**17**
	late	DPA					
JUICI	early	no DPA	**14**				
	early	DPA	3				
	late	no DPA	**33**		**67**		
	late	DPA	**19**				
Honeycrisp	early	no DPA	6				
	early	DPA					
	late	no DPA			**69**	**33**	
	late	DPA		**30**		**30**	
Pazazz	early	no DPA	3		**72**		**78**
	early	DPA					
	late	no DPA	6		**56**	**47**	**72**
	late	DPA					
WA 38	early†	no DPA		**67**		**11**	
	early†	DPA		3			
	late†	no DPA					
	late†	DPA					

†Early and late samples were harvested from different orchards. Bold text indicates significant incidence (pooled z-test, n=36, $p<0.05$).

As apples used for this activity were all stored in high CO_2 and low O_2, we expected DPA treatment to indicate disorders that were associated with CO_2 sensitivity. DPA typically reduces or eliminates both internal and external symptoms of these disorders. Given this criterion, disorders symptomatically attributable to CO_2 sensitivity were observed in 'Golden Delicious', 'Plumac', 'Braeburn', 'Scilate', JUICI, 'Fuji', 'Honeycrisp', 'Pazazz', 'Smitten', and 'Cripps Pink' that were not drenched at harvest with DPA emulsion. 'Fuji' developed cavities, severe browning that had "radial" appearance, and softened solid brown cortex (Fig. 2) and incidence was lowered but not eliminated by DPA treatment. 'Honeycrisp', as well as its progeny, 'Pazazz', developed both CO_2 sensitivity-related and soft scald/soggy breakdown. In 'Honeycrisp', these disorders could be segregated using DPA treatment which eliminated the radial browning symptoms but not soggy breakdown (Figure 1). In 'Pazazz', disorders were not present in DPA drenched fruit. None of the cultivars that did not develop disorders in Year 1 developed disorders when retested in Year 2. However, 'Smitten' and 'Cripps Pink' both developed CO_2-related disorders in Year 3. A high incidence of both external and internal browning was found this year in/on both control and DPA drenched 'Gala'. This peel (sometimes called "caramelization") and stem end flesh browning in this cultivar is not CO_2-related (not controlled by DPA) and has been attributed to climatic conditions and possibly the transition into cold storage.

Improving cold chain practices when CO_2 sensitivity is indicated

Activity 1 tested thresholds for O_2:CO_2 % atmosphere combinations. Lower CO_2 resulted in lower incidence of CO_2 related disorders for all cultivars, while lower O_2 levels increased disorder incidence along with elevated CO_2 although only in JUICI (Table 2). Apart from 'Golden Delicious' and WA-38, which were clean after 4 months, radial browning symptoms occurred in all other cultivars tested at 5% CO_2. At 1% CO_2, "radial browning" was not found in 'Scilate' and 'Fuji' and incidence was reduced in 'Braeburn', 'Pazazz'. In JUICI, incidence was higher 5% CO_2 than 1 % CO_2 at 0.6% O_2. Incidence in 'Honeycrisp' and 'Pazazz' was not different, potentially due to conflation of CO_2 related and non-CO_2 related disorders of which these cultivars are susceptible. "Orange peel" symptoms were found on both 'Honeycrisp' and 'Pazazz' at 5% CO_2. This further demonstrates these cultivars' high sensitivity to CO_2 injury. Disorders not related to CO_2 sensitivity (soft scald and soggy breakdown) were not influenced by differing combinations of O_2:CO_2. Soft scald was found in both 'Honeycrisp' and 'Pazazz' and "non-radial browning" in 'Scilate', 'Fuji', 'Honeycrisp', and JUICI. Cavities in the cortex tissue were found in all cultivars except 'Golden Delicious' and WA-38. Cavities were not present in 1% CO_2 for 'Fuji' and 'Honeycrisp', and reduced in 'Braeburn' and JUICI, but 'Scilate' and 'Pazazz' were not apparently linked with CO_2 % (Table 2).

Table 2. Reducing CO_2 reduces radial browning and cavities. Lower O_2 % enhances sensitivity to CO_2 in JUICI. Other disorders were not impacted by CO_2 or O_2 levels. 'Golden Delicious', 'Plumac', and WA-38 were also tested but did not develop disorders in this test. 'Cripps Pink' and 'Smitten' developed disorders in the screening test in Year 3 and were not included in this test. Radial browning, rough or "orange peel" peel texture, and cavities are typically associated with CO_2 sensitivity. Different letters indicate incidence of a symptom is different from other atmospheres for that cultivar according to a pooled z-test (n=36, p<0.05).

Cultivar	**O_2 (%)**	**CO_2 (%)**	**Soft scald %**	**Orange peel %**	**Radial browning %**	**Non-radial browning %**	**Cavity %**
Scilate	0.6	1			0.0 a	5.6 a	2.8 a
	1	1			0.0 a	0.0 a	0.0 a
	0.6	5			13.9 b	0.0 a	77.8 b
	1	5			8.3 ab	8.3 a	2.8 a

Fuji	0.6	1			0.0 a	0.0 a	0.0 a
	1	1			0.0 a	0.0 a	0.0 a
	0.6	5			11.1 b	0.0 a	44.4 b
	1	5			2.8 ab	5.6 a	13.9 c
Honeycrisp	0.6	1	19.4 a	0.0 a	2.8 a	25.0 a	0.0 a
	1	1	38.9 a	0.0 a	0.0 a	44.4 a	0.0 a
	0.6	5	38.9 a	8.3 a	8.3 a	44.4 a	2.8 a
	1	5	25.0 a	2.8 a	8.3 a	30.6 a	8.3 a
Braeburn	0.6	1			8.3 a		47.2 a
	1	1			2.8 a		16.7 b
	0.6	5			47.2 b		97.2 c
	1	5			66.7 b		52.8 a
JUICI	0.6	1			0.0 a	0.0 a	11.1 a
	1	1			2.8 a	0.0 a	5.6 a
	0.6	5			22.2 b	0.0 a	69.4 b
	1	5			5.6 a	2.8 a	38.9 c
Pazazz	0.6	1	63.9 a	61.1 a	5.6 ab		0.0 a
	1	1	50.0 a	61.1 a	0.0 a		25.0 b
	0.6	5	69.4 a	69.4 a	11.1 b		36.1 b
	1	5	50.0 a	36.1 b	8.3 ab		0.0 a

Most prior work indicates 1-MCP exacerbates CO_2 sensitivity, although it reduces ripening while apples are not in CA. We set out to test whether a delay of CA conditions would decrease CO_2 sensitivity after treatment with 1-MCP. We used CA conditions harsh enough to cause disorders (0.6% O_2: 5% CO_2) so as to detect even the slightest sensitivity. Results indicate that delaying CA can reduce CO_2 related disorders, albeit inconsistently across cultivars, but not soft scald, soggy breakdown, or the small cavities impacting JUICI cortex with no apparent relationship with CO_2 sensitivity. After 6 months of storage, 'Honeycrisp', 'Pazazz', JUICI, 'Fuji', and 'Golden Delicous' developed external CO_2 "orange peel" symptoms (Fig. 1; Table 3). Delaying CA establishment did not affect incidence on 'Honeycrisp', but a 4 week delay reduced incidence on 'Pazazz' compared with no delay and 2 week delay. Both 2 and 4 week delays eliminated 'Golden Delicious' orange peel although "ghosting" was prominent on peel stored in the reduced CO_2 atmosphere (1 % CO_2), especially if CA was established at harvest. Also, a superficial scald like symptom was prominent in both atmospheres if CA establishment was delayed 4 weeks.

With the exception of 'Fuji', internal CO_2 related disorder symptoms were found in all cultivars tested in this activity. 'Fuji' had a high watercore incidence which can exacerbate internal browning in less than optimal storage conditions. Most cultivars had lower incidence of radial browning with increased CA delay. For JUICI, a 2 week delay reduced incidence, while waiting an additional 2 weeks (4 weeks total) did not produce any further reduction (Fig. 3; Table 3). Incidence was reduced in 'Pazazz' only following a 4 week delay of CA but had no impact on radial browning of 'Honeycrisp'. This may result from a conflation of soggy breakdown and radial browning of which both cultivars are sensitive. Internal cavities in the cortex were detected in all cultivars tested in Year 2 and were cultivar-dependent regarding the impact of delayed CA. Delayed CA did not impact cavity incidence in 'Pazazz' or 'Scilate'. Delayed CA reduced cavities in 'Honeycrisp' and WA-38. Delaying CA actually increased cavity incidence in JUICI compared to no delay or a 4 week delay. Large cavities were reduced in this cultivar in a second Year 3 trial of this cultivar. JUICI also developed small cavities that were even present at harvest, in many cases. While large and small cavities were considered together in the first trial, large cavities were considered different from the small cavities in the second JUICI trial. CO_2 during storage was kept very high for these tests, and it is possible that combinations of delayed CA and reduced CO_2 during CA would be effective for reducing symptoms on sensitive cultivars. Neither firmness nor titratable acidity were impacted by CA delay in any of the cultivars in this activity.

Figure 3. Radial browning on JUICI treated with 1-MCP with (from left to right) no delay, 2 week, and 4 week delay before CA storage at 0.6% O_2, 5% CO_2 for 6 months (Year 2)

Table 3. Delayed CA reduced orange peel in 'Pazazz' and 'Golden Delicious' and radial browning of JUICI, 'Pazazz', and WA-38 but not for other cultivars. Percent incidence of different internal and external disorders in Year 2 after treatment with 1-MCP and delays before CA storage (0.6% O_2:5% CO_2). Radial browning and orange peel symptoms are typically associated with CO_2 sensitivity. Different letters indicate incidence of a symptom is different from other atmospheres for that cultivar according to a pooled z-test (n=108, year 2 or n=90, year 3; $p<0.05$).

Cultivar	**Delay**	**Soft Scald %**	**Orange peel %**	**Radial browning %**	**Non-radial browning %**	**Cavities %**
Honeycrisp	0 weeks	11.1 a	1.9 a	16.7 a	4.6 a	13.9 a
	2 weeks	13.9 a	0.9 a	4.6 b	8.3 ab	1.9 b
	4 weeks	12.0 a	2.8 a	13.0 a	13.9 b	6.5 ab
Pazazz	0 weeks	11.1 a	45.4 a	16.7 ab		16.7 a
	2 weeks	17.6 a	50.9 a	21.3 a		17.6 a
	4 weeks	10.2 a	28.7 b	8.3 b		20.4 a
JUICI (Year 2)	0 weeks		0.9 a	88 a		16.7 a
	2 weeks		0.0 a	18.5 b		23.1 ab
	4 weeks		0.0 a	15.7 b		29.6 b
JUICI (Year 3)	0 weeks			76.9 a		30.6 a
	2 weeks			2.8 b		6.5 b
	4 weeks			5.6 b		8.3 b
Scilate	0 weeks	0.9 a		11.1 a		9.3 a
	2 weeks	0.0 a		9.3 a		3.7 a
	4 weeks	0.0 a		12 a		3.7 a
WA-38	0 weeks			4.6 a		12 a
	2 weeks			0.0 b		0.9 b
	4 weeks			0.0 b		0.0 b
Fuji	0 weeks		8.3 a	26.9 a		14.8 a
	2 weeks		2.8 a	22.2 a		9.3 a
	4 weeks		3.7 a	21.3 a		7.4 a
Golden Delicious	0 weeks		6.5 a			
	2 weeks		0 b			
	4 weeks		0 b			

An inexpensive protocol for establishing CO_2 sensitivity

Placing trays of apples in sealed bags during cold air storage (see materials and methods) appears to be a viable, inexpensive means to test for CO_2 sensitivity of a cultivar (Fig. 4). Sealing trays of JUICI (CO_2 sensitive) and 'Delicious' (CO_2 insensitive) in this manner reduced O_2 to around 9% for both cultivars and elevated CO_2 to 3.1% for 'Delicious' by 9 d and 3.4% by 14 d, respectively. 'Delicious' did not develop any symptoms. However, JUICI developed radial browning only in fruit sealed in bags without DPA (Table 4). Radial browning incidence did not differ among replications validating the precision of this protocol. This protocol is inexpensive (cost of garbage bags, zip ties, and existing O_2/CO_2 meters) appears an effective means to screen new cultivars.

Table 4. Bagging apples in sealed trash bags during air storage caused radial browning in JUICI, a CO_2-sensitive cultivar. Radial browning incidence in JUICI after 2 months of storage in bags. The same letter indicates the no difference. "Cavities" in this case are the very small type found in this cultivar.

	No DPA bagged			DPA bagged		
	Bag 1	Bag 2	Bag 3	Bag 1	Bag 2	Bag 3
Radial browning	5 a	6 a	7 a			
Cavities	0 a	1 a	0 a	1 a	3 a	0 a

Figure 4. A simple procedure for screening apples for CO_2 sensitivity seals apples in trash bags, seals them with zip ties and places them in 33 °F air storage for 2 months. O_2 and CO_2 can be verified periodically using an O_2/CO_2 meter.

Different chemistries are linked with different symptoms and causes of symptoms

Our screening of peel and cortex chemistry among symptomatic and asymptomatic periphery tissue from all cultivars yielded chemical markers that may be used distinguish CO_2-related peel and cortex symptoms from those related to other horticultural and storage factors. We compared related chemical differences and changes from all of the disorder symptoms in the test by screening 720 and 588 chemicals in symptomatic and asymptomatic tissue in the peel and cortex, respectively. Many of these natural chemicals point to processes associated with temperature adaptation and oxidation which would be expected to be linked with tissue browning or processes leading up to symptom development.

Symptomatic cortex was determined to be related to CO_2 sensitivity if it was mostly eliminated by DPA drench and was reduced by reducing atmospheric CO_2. Cortex chemistry comparisons were made between symptomatic and asymptomatic tissue from all cultivars that developed internal browning or cavities. Initial evaluations focused on 'Honeycrisp' disorders as it also had soggy breakdown which could be distinguished from internal browning related to CO_2 sensitivity (Fig. 5, left). While there were many chemical differences overall, we found that certain chemicals were most indicative of tissue status and may provide the best metabolic markers. Browned tissue contained higher levels natural chemicals ostensibly associated with stress adaptation such as sphingolipids, diglycerides, phytosterol conjugates (ASGs), and vitamin E metabolism. Oxidation of vitamin E and production of some ASGs was specifically linked to CO_2-related cortex browning. This observation held true for nearly every sample with CO_2-related internal browning as supported by oxidized Vit E levels (Fig. 5, right).

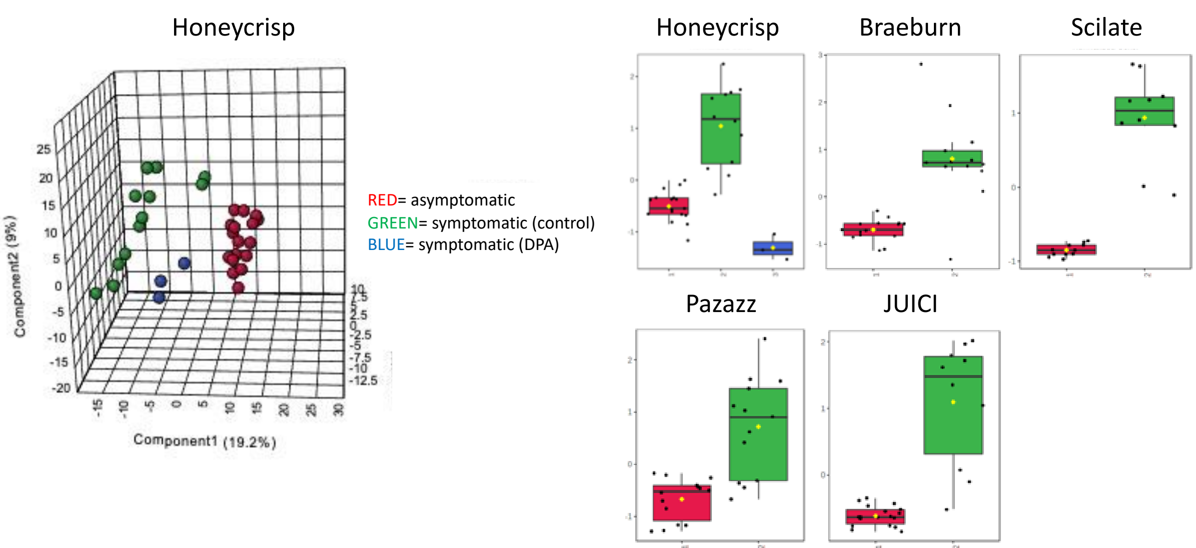

Figure 5. (Left) Analysis (principal components analysis) of 588 cortex chemicals show that asymptomatic (both control and DPA drench) as well as symptomatic tissue of control (CO_2 browning) and DPA drenched (soggy breakdown) 'Honeycrisp' all differ. There are many chemicals responsible for these differences in the multiple CO_2-sensitive cultivars analyzed. One example is an oxidized form of Vitamin E that is elevated specifically in symptomatic cortex linked with CO_2-sensitivity in 5 cultivars (Right). Vitamin E levels decrease alongside the increase of this metabolite indicating a potential process linked with symptom development.

To determine if any levels of any of these metabolites changed prior to symptom appearance, we stored DPA drenched and undrenched apples for up to 4 months under different CO_2 levels, sampling peel and cortex periodically. We studied 'Pazazz' in this way in Year 2. As disorder levels were extreme for this cultivar, we repeated this study with 'Fuji' in Year 3. Internal browning was first detected at 4 weeks especially in apples stored under 5 % with lesser amounts in those stored under 2.5 % CO_2 in control but not DPA drenched apples. As with in the initial selection, chemicals that were higher or lower in symptomatic compared with asymptomatic tissue were also lower in 'Fuji' in this study (not shown). Vitamin E oxidation was elevated in control fruit indicating that DPA impeded the process as is indicated by the ratio of oxidized Vitamin E to Vitamin E. However, this process was only impacted in asymptomatic tissue by CO_2 levels once adjacent high levels of internal browning were present at 8 weeks. Similar increases of levels were observed with other highlighted chemicals. Monitoring these chemicals may distinguish different injuries but would not provide an early, pre-symptomatic indication of CO_2-sensitivity as they all appear to be directly linked with the symptoms.

We also can distinguish "typical" CO_2 sensitivity-related peel symptoms such as dimpled or "orange peel" from healthy (Fig. 6A) peel or peel with other defects such as soft scald (Fig. 6B). Further refinement of the search identified chemicals whose levels are higher or lower depending upon whether the symptoms are related to CO_2 sensitivity (Fig. 6C).

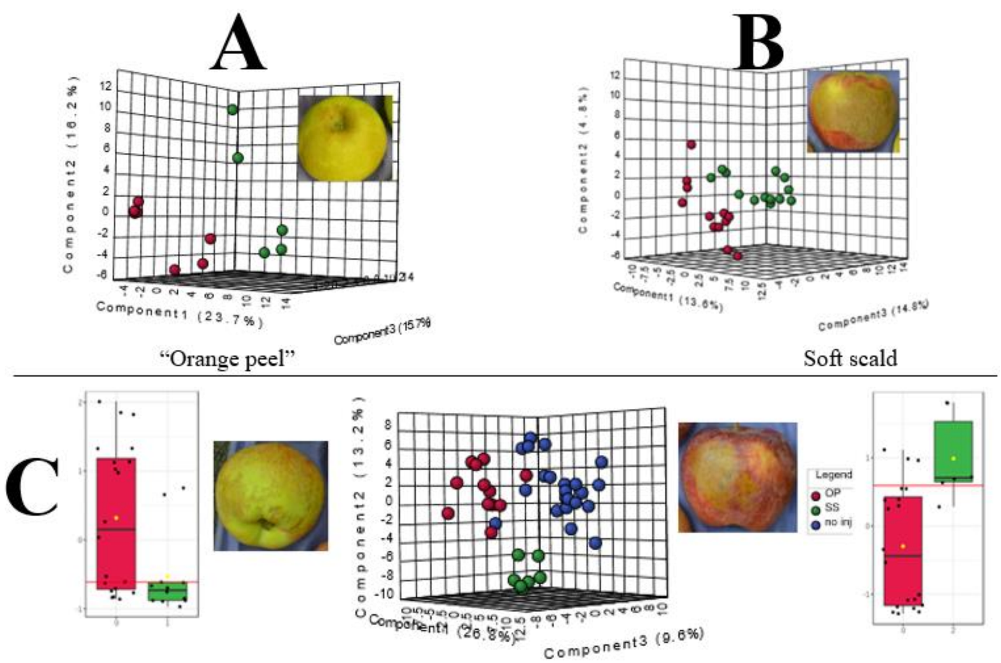

Figure 6. Peel chemistry of CO_2 sensitivity-related symptoms including A) orange peel (rugose scald) of 'Golden Delicious' B) soft scald of 'Honeycrisp' were different from periphery tissue. C) Chemistry of "orange peel" (red dots) and soft scald (green dots) were different in 'Pazazz' which developed both. Threonic acid, a natural peel chemical, is found in lower quantities in injured peel (green bar) than peripheral peel (red bar) in "orange peel" and in greater quantities than peripheral peel in soft scald.

To summarize, we have identified CO_2 sensitive cultivars as well as some potential means of mitigating CO_2-related disorders. As expected, DPA drenching was mostly effective at eliminating both internal and external symptoms of CO_2 sensitivity. The ineffectiveness of DPA for controlling some disorders also highlights where CO_2 sensitivity was not the cause of internal and external defects in some instances (soft scald and soggy breakdown), primarily for 'Honeycrisp' and its progeny, 'Pazazz', or for 'Gala' where similar symptoms have different causes. Symptoms of these disorders are often confused and are not mitigated using the same strategies. Other conditions that reduced CO_2-related disorders included reducing CO_2 during storage and, in the case of JUICI, ‘Pazazz’, ‘WA 38’, and ‘Golden Delicious’, delaying CA for up to 4 weeks following 1-MCP treatment at harvest. However, to date, this delaying CA establishment has inconsistently reduced disorders. Besides finishing CO_2 disorder mitigation storage studies, our no cost extension will entirely focus on finishing our peel chemistry analyses confirming peel chemistry specifically linked with CO_2-related disorder symptoms and the period leading to symptom development. To date, we have identified natural chemical differences that appear to be linked with symptoms related to CO_2 sensitivity. We are continuing to specifically confirm associations with CO_2-sensitivity. We expect that monitoring these chemicals during storage can indicate if CO_2 stress is occurring or if symptoms are related to CO_2 sensitivity.

Project Title: Reducing carbon dioxide-related postharvest disorders (AP-19-100)

Executive Summary

Keywords: cold chain, CO_2 sensitivity, postharvest disorder diagnosis, postharvest disorder mitigation, risk assessment

Abstract: Progenitors of many newer apple cultivars are sensitive to elevated CO_2 during storage. Consequently, there may also be an enhanced risk of CO_2-related disorders of the peel and flesh. The appearance of CO_2-related symptoms can vary by cultivar, and some cultivars can even develop similar symptoms not related to elevated CO_2 levels, confounding diagnosis and subsequent mitigation. We selected 15 cultivars to determine if they are sensitive to CO_2 during storage, symptom appearance, means for identifying CO_2-related disorders, and how to best mitigate CO_2-related disorders of these cultivars. Of those, 11 were sensitive, developing "orange peel" injuries and/or a variety of internal injuries. 'Honeycrisp' and a progeny, 'Pazaaz', develop internal and external disorders related to both CO_2-sensitivity and cold storage, alone. Diphenylamine drenches eliminated CO_2-related symptoms in nearly every case. Reducing CO_2 to below 1% also reduced disorder incidence, unless CO_2-senstivity was severe or other chilling-related disorders were also present. Delaying CA establishment for 2-4 weeks following 1-MCP treatment at the beginning of storage could reduce incidence of CO_2 related disorders without losing firmness or acidity. When multiple disorders developed, levels of specific peel chemicals were elevated when symptoms were related to CO_2 sensitivity. In summary, we determined that CO_2 sensitivity can cause disorders in many of these cultivars, and a combination of reducing levels of CO_2 during storage and delaying CA establishment from 2-4 weeks (only when 1-MCP can be used) can reduce these disorders in the most sensitive cultivars, especially when DPA drenching is not used.

Project outcomes:

1. Indication of CO_2 sensitivity of prominent cultivars.
2. Inexpensive protocol for specifically screening cultivars for CO_2 sensitivity.
3. Mitigation protocols for reducing disorders of CO_2 sensitive cultivars.
4. Identified chemistries specifically related to CO_2-related disorders.

Significant Findings:

1. A variety of internal and external browning symptoms may be attributable to CO_2 sensitivity in many of the cultivars tested.
2. 'Honeycrisp' and 'Pazazz' are sensitive to CO_2 but also develop soft scald and soggy breakdown.
3. Incidence of symptoms related to CO_2 sensitivity were reduced or eliminated by DPA drenching.
4. Reducing CO_2 exposure concentration and delaying CA impacted CO_2 disorders albeit inconsistently among cultivars.
5. Peel and cortex chemistry of typically CO_2-related symptoms is different from other peel and cortex defects.
6. Cultivars can be screened for CO_2 sensitivity using an easy, inexpensive protocol.

Future Directions:

1. Determining when CO_2 scrubbing is most necessary and to what degree for different cultivars.
2. Determining genetic factors associated with CO_2 sensitivity during storage.