FINAL PROJECT REPORT

Project Title: Integration of storage technologies for fruit quality management

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Other funding sources

1. Agency Name: USDA-NIFA (competitive grant)

(Federal + non-Federal): \$2.4 million (total) over 4 years.

Notes: D. Rudell is Project Director, J. Mattheis is a Co-PI. The Standard Research and Extension Project, "A diagnostic toolbox for integrated management of postharvest apple necrotic disorders" was submitted (01/12/10) for the current funding cycle. WTFRC and AgroFresh, Inc. are cosponsors.

2. Agency Name:	AgroFresh, Inc.
Amount awarded:	\$19,000
Notes:	Funding supports 'Honeycrisp' storage research.

Total Project Funding: \$83,613

Budget History:

Item	2008	2009	2010
Salaries	\$19,605	\$20,198	\$20,808
Benefits	\$6,470	\$6,665	\$6,867
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	\$1000	\$1000	\$1000
Travel	0	0	0
Miscellaneous	0	0	0
Total	\$27,075	\$27,863	\$28,675

RECAP ORIGINAL OBJECTIVES:

The project had 4 objectives, 2 for 'Honeycrisp' storage and disorders, 2 for postharvest use of chlorophyll fluorescence monitoring technology. The objectives reflected perceived knowledge gaps in information available to the industry generated using fruit produced in Washington. Increasing 'Honeycrisp' volume suggests long term storage protocols are needed, the basis for objective 1. The use of 'Honeycrisp' as a breeding parent and the heritability of disorder (soft scald, soggy breakdown) susceptibility in light of a near complete lack of knowledge pertaining to disorder biochemistry and genetics was the basis for objective 2. While commercial technology for fruit chlorophyll fluorescence monitoring is used in a number of warehouses, questions existed related to longevity of superficial scald prevention following low O_2 storage based on fluorescence monitoring (objective 3) and the utility of this technology for assessing CO_2 injury risk.

SIGNIFICANT FINDINGS:

Objective 1. Identify postharvest protocols to maximize storage life of 'Honeycrisp' and other softscald-sensitive cultivars. Substantially met for 'Honeycrisp', not met for other cultivars.

1. 'Honeycrisp' storage regimes that included 7 days at 50 °F after harvest then 36-39 °F largely controlled development of soft scald and soggy breakdown.

2. Late harvest 'Honeycrisp' apples (starch 6.0, yellow ground color, slight greasiness) are at high risk of soft scald, soggy breakdown, internal radial browning, senescent browning, peel dimpling and greasiness within 2 months after harvest.

3. Risk of 'Honeycrisp' internal radial browning increased with decreased O_2 concentration in CA; and decreased with use of SmartFresh[®].

4. HarvistaTM-treated 'Honeycrisp' had less softscald and soggy breakdown compared to non-treated or SmartFresh®-treated fruit.

5. CA (1.5% $O_2/1\%$ CO₂) storage of 'Honeycrisp' reduced greasiness and acid loss regardless of temperature (33, 36, 39 °F) compared to fruit stored in air at 39 °F.

6. Differences in 'Honeycrisp' volatile production were evident between controls and SmartFresh® - treated fruit stored in air or CA.

7. Final storage temperature of 36-39 $^{\circ}$ F reduced soft scald and soggy breakdown risk compared to 33 $^{\circ}$ F.

8. Internal CO_2 injury developed in 'Honeycrisp' fruit stored in 3 or 5% CO_2 but not 1% CO_2 , all with 2% O_2 .

9. Based on all experiments, 'Honeycrisp' harvested with starch score 4-5 on a scale to 6, breaking ground color, held 7 days at 50 °F then 36-39 in CA (2% O_2 , 1% or less CO_2) are likely to perform well during long-term storage. SmartFresh® use can contribute to enhanced titratable acidity and lack of greasiness after storage in air or CA.

Objective 2. Characterize physiological events that result in softscald symptom development. Partially met.

1. Changes in 'Honeycrisp' metabolism were detected prior to symptom development.

2. Differences exist in volatile and non-volatile compounds between fruit that did and did not develop softscald.

Objective 3. Identify minimum storage duration required to control superficial scald by use of oxygen setpoints below 1%. Partially met.

1. 'Granny Smith' apples stored at 0.2% O_2 above the low O_2 fluorescence deflection point + 1% CO_2 did not develop superficial scald through 7 months in CA +21 days RA + 21 days at 68 °F, or 7 months CA +10 days RA + 28 days at 68 °F.

Objective 4. Identify what if any limits for CO_2 exist during apple storage in oxygen below 1%. Partially met.

1. 'Granny Smith' apples stored in up to 3% CO₂ with 0.2% O₂ did not develop CO₂ injury symptoms through 9 months in storage plus 7 days at 70 °F.

2. No change in chlorophyll fluorescence of 'Fuji' apples was detected in CO_2 concentrations up to 10%. 'Fuji' apples from the same lot stored in 1% O_2 with 3 or 5% CO_2 developed internal browning consistent with CO_2 injury.

RESULTS & DISCUSSION

The utility of 7 days at 50 °F followed by lower storage temperature for 'Honeycrisp' apples to avoid soft scald and soggy breakdown was standard practice for most 'Honeycrisp' experiments in this project (Table 1). While some lots will not develop these chilling disorders if cooled directly to 33-36 °F after harvest, no methods are currently known to predict lot to lot susceptibility to chilling injury. Bitter pit development was not significant in any of the experiments we have conducted during the 2008-2010 period. A 7 day period at 50 °F to condition fruit to lower temperatures, first reported by Chris Watkins of Cornell University, in our experiments usually has prevented chilling injury. Some instances where the 50 °F conditioning has not completely prevented the disorders have been relatable to the final storage temperature, where disorder incidence increased as temperature decreased (Table 2). Harvest maturity is also a factor influencing chilling injury risk, with injury susceptibility increasing with later harvest. Horticultural practices that favor red color development and initiation of degreening (nitrogen and crop load management) may allow earlier maturity and harvest. In the absence of red color development, the lack of softening during maturation and storage may lead to harvest (late) and storage duration (long) decisions that compromise other fruit quality attributes including disorder susceptibility.

	2008	2009	2010
Soft Scald 7d @ 50 °F	0,13,0,2,0,0	1,0,7,5,0,9,3	4,1
Bitter pit	0, 4,0,1,0,0	0,0,0,0,0,0,0	0,0
Soft scald 33 °F continuously	80	45	1

Table 1. Incidence (%) of soft scald and bitter pit in 'Honeycrisp' apples during multiple ARS experiments in 2008-2010. Fruit were held in air at 50 °F for 7 days then at 33, 36, or 39 °F, or continuously at 33 °F (one experiment per year only).

Harvest Date	Storage °F	% Soft Scald	% Soggy Breakdown
Sep 21	33	12a	12a
	36	0b	Ob
	39	0b	0b
Oct 3	33	49a	26a
	36	10b	3b
	39	1b	1b

Table 2. 'Honeycrisp' cumulative (n=72) chilling disorders during 7 months cold storage in air. All fruit held 7 days at 50 °F after harvest. Disorder incidence recorded after 1,3,5 ,and 7 months. Means (n=18) followed by different letters are significantly different, Tukey's HSD, p<0.05.

Consequences of higher storage temperature on fruit quality are minimized within the 33-39 °F range by storing near 36 °F (Tables 3,4). After 1-7 months in air plus 7 days at 70 °F, firmness and acidity

were either enhanced or similar for fruit stored at 33 or 36 °F compared to 39 °F. Soluble solids content (SSC) is typically not responsive to cold storage conditions, and although statistically significant differences were observed related to temperature in various experiments, real differences in eating quality relatable to the magnitude of SSC variation among treatments is suspect.

Controlled atmosphere storage at 2% O₂ and 1% CO₂ slows 'Honeycrisp' ripening as measured by titratable acidity and peel greasiness. No chilling disorders or bitter pit developed on fruit in this experiment. In general, lots used in the 3 years of this project were not susceptible to bitter pit, and where the disorder was present, incidence was low and no treatment differences in bitter pit incidence were observed.

Month	Storage ^o F	Lbs	% TA	% SSC
Harvest		15.6	0.506	13.4
1	33	15.6ab	0.477ab	13.3b
	36	16.4a	0.498a	13.8a
	39	15.0b	0.436b	13.2b
3	33	16.2a	0.420a	13.2a
	36	16.0a	0.424a	13.0ab
	39	14.1b	0.363b	12.6b
5	33	15.1	0.379a	13.3a
	36	15.2	0.371a	13.1a
	39	14.5	0.342b	12.6

Table 3. 'Honeycrisp' fruit firmness, titratable acidity (TA) and soluble solids content (SSC) after air storage. All fruit held 7 days at 50 °F after harvest, and for 7 days at 70 °F after storage. Means (n=18) followed by different letters are significantly different, Tukey's HSD, p<0.05.

months	atmosphere	°F	% titratable acid	% soluble solids	% greasy	lbs
harvest			0.494	13.2	0	13.7
4	Air	33	0.339cd	12.7ab	4b	13.9
		36	0.358bc	12.9a	13b	14.9
		39	0.313d	12.2b	40a	14.0
	2% O ₂ 1% CO ₂	33	0.414a	13.0a	4c	14.0
		36	0.365bc	12.6ab	4c	13.8
		39	0.383b	12.6ab	0c	14.4
7	Air	33	0.290bc	12.3bc	бbc	13.8
		36	0.276c	12.1c	14b	13.8
		39	0.276c	12.8ab	83a	14.0
	2/1 O ₂ /CO ₂	33	0.372a	12.7abc	0c	13.4
		36	0.382a	13.4a	3c	13.9
		39	0.321b	12.3bc	0c	13.3

Table 4. 'Honeycrisp' fruit quality after air and CA storage. All fruit held 7 days at 50 °F after harvest, and for 7 days at 70 °F after storage. Means (n=18) followed by different letters are significantly different, Tukey's HSD, p<0.05.

'Honeycrisp' demonstrated sensitivity to low O_2 and high CO_2 during this project. Low O_2 injury was manifested as interior radial (Table 5) or diffuse and calyx end browning (Table 6). Internal and calyx end browning occurred with fruit for which the O_2 concentration was set based on chlorophyll fluorescence monitoring (HarvestWatchTM) indicating metabolic events leading to low O_2 injury may not be detectable by monitoring fluorescence. As lowering O_2 increased risk of radial browning, without substantial enhancement of fruit quality, results to date do not support CA protocols with O_2 less than 1.0%.

months	treatment	% titratable	ground	soft scald	% radial	lbs
		acidity	color 1-5	%	browning	
4	air	0.314c	5a	0	0	15.0
	O ₂ : 0.3	0.346bc	5a	0	3	14.1
	0.5	0.346bc	4b	3	3	14.7
	0.8	0.413a	5a	0	11	15.5
	1.5	0.358b	5a	3	6	14.9
6	air	0.249d	4.9a	0	0b	13.8
	O ₂ : 0.3	0.387a	4.3b	6	31a	14.9
	0.5	0.380ab	4.4ab	8	33a	14.8
	0.8	0.350bc	4.3b	0	19a	14.4
	1.5	0.325c	4.2b	0	6b	14.9

Table 5. 'Honeycrisp' fruit quality after storage, 2008 harvest. All CA treatments contained 0.5% CO₂. All fruit held 7 days at 50 °F after harvest, final storage temperature 33 °F. Fruit held 7 days at 70 °F after storage. Change in fruit chlorophyll fluorescence detected at 0.3% O₂. Means (n=18) followed by different letters are significantly different, Tukey's HSD test, p<0.05.

Month	% O ₂	trt	Peel color	% titratable	% internal	% calyx	lbs
			1-5	acidity	browning	browning	
Harvest			2.9	0.589			15.1
4	Air	Ck	4.7*	0.391	0	0	14.3
		SF	3.6	0.472*	0	3	14.4
	2	Ck	3.4	0.427	3	0	14.6
		SF	3.8	0.446	0	0	13.9
	1	Ck	3.6	0.441	3	0	14.6
		SF	3.7	0.467	22*	3	14.6
	0.5	Ck	3.3	0.449	14	9	14.6
		SF	3.7	0.490*	36*	19	14.2
7	Air	Ck	5*	0.360	0	0	14.7
		SF	4	0.461*	0	0	14.6
	2	Ck	3.4	0.419	8	0	14.4
		SF	3.1	0.423	3	0	14.2
	1	Ck	3.5	0.411	9	0	13.7
		SF	3.7	0.439*	10	17*	14.3
	0.5	Ck	3.3	0.426	17	22	14.5
		SF	3.5	0.460*	37	21	14.1

Table 6. 'Honeycrisp' fruit quality after storage, 2009 harvest. Ck: check, SF: SmartFresh[®]. Starch at harvest = 5.7 (1-6). $CO_2 = 0.5\%$ all CA treatments. All fruit held 7 days at 50 °F after harvest, storage temperature 36 °F. SmartFresh[®] applied the day of harvest. Fruit held 7 days at 70 °F after storage. Change in fruit chlorophyll fluorescence detected at 0.3% O₂. *: means (n=18) are significantly different, Bonferroni t-test, *p*<0.05.

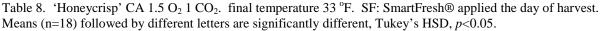
Incidence of internal browning and cavities increased with storage CO_2 concentration during 'Honeycrisp storage (Table 7). Browning symptoms from CO_2 exposure (diffuse, light brown color) were distinguishable from chilling-related soggy breakdown (solid patches, saturated tan color). Likelihood that injury is induced by CO_2 is supported by DPA use in a 2010 experiment where injury has occurred in untreated fruit but not DPA-treated (1000 ppm) fruit stored in 5% CO_2 (not shown).

Month	% CO ₂	% internal browning	% cavities
4	1	0*	0
	3	14	0
	5	25	0
7	1	0*	0
	3	15	15
	5	36	10

Table 7. 'Honeycrisp' disorders after high CO₂ CA storage. O₂ =2% all CA treatments. All fruit held 7 days at 50 °F after harvest, storage temperature 36 °F, fruit held 7 days at 70 °F after storage. *:significant linear trend, r^2 =0.94.

Use of SmartFresh® with 'Honeycrisp' slows fruit ripening resulting in higher acid retention, slower ground color change (in air storage), reduced greasiness and radial browning (Table 8, Figure 1). While the field 1-MCP formulation HarvistaTM reduced chilling disorders (Figure 1), SmartFresh® has not consistently impacted development of soft scald or soggy breakdown in our experiments. Production of ripening/senescence-related ethyl volatiles (Figure 2) is delayed by SmartFresh® treatment, an impact that may prolong the optimal eating period. Ethyl ester production increases in many cultivars including 'Honeycrisp' as ripening and senescence progress. The lack of softening in 'Honeycrisp' can be misleading as an indicator of overall fruit quality when compared with flavor and taste (i.e. good firmness and texture, overripe flavor and taste).

	Air	SF air	1.5% O ₂ /1% CO ₂	SF 1.5% O ₂ /1% CO ₂
Ground color	5.0a	5.0a	4.2b	4.3b
% titratable acid	0.258d	0.287c	0.320b	0.336a
% radial browning	0b	0b	28a	бb



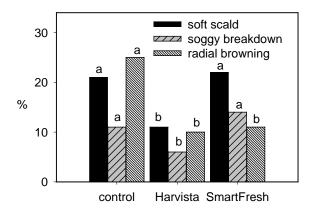


Figure 1. Cumulative soft scald, soggy breakdown, and radial browning incidence for 'Honeycrisp' fruit stored up to 6 months in air. Means followed by different letters are significantly different, Tukey's HSD test, p<0.05.

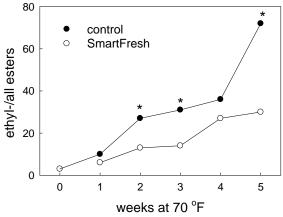


Figure 2. Ratio of ethyl esters to all esters during 'Honeycrisp' ripening at 70 °F. SmartFresh® applied the day of harvest. *: means significantly different, Bonferroni t-test, p < 0.05.

Objective 2. Characterize physiological events that result in softscald symptom development. In an experiment during which 80% of 'Honeycrisp' apples held continuously at 33 °F developed soft scald while fruit from the same lot stored 7 days at 50 °F then 33 °F did not develop the disorder, several

differences in fruit chemistry were detected. Volatile compounds indicative of overripe/senescent apples including acetaldehyde, ethanol, and many ethyl esters were produced in much higher amounts compared to fruit without symptoms. Two unique volatiles, ethyl butenoate and ethyl 2-methylbutenoate, were produced only by fruit that developed soft scald. A group of non-volatile compounds, acylated sterol glycosides, were also detected only in injured fruit. Volatiles typical of ripening 'Honeycrisp' including acetate, butyrate and propionate esters were higher in sound fruit. A number of the compounds typical of fruit that developed soft scald were detected prior to symptom development indicating a potential use as biomarkers for soft scald prediction. The butenoate esters and acylated sterol glycosides unique to damaged fruit have been found in other cultivars with other disorders (superficial scald, CO_2 injury) indicating a relationship with fruit stress response.

Objective 3. Identify minimum storage duration required to control superficial scald by use of oxygen setpoints below 1%. 'Granny Smith' apples stored in air, 1.5 or 0.5% O₂ with 1% CO₂ were stored at 33 °F for up to 9 months. The low O₂ setpoint was determined by monitoring fruit chlorophyll fluorescence using the HarvestWatchTM system. Fruit were moved from CA after 3, 5, 7, or 9 months to air and held 10 or 21 days, then moved to a warm room (70 °F) and held up to 28 days. Scald symptoms were assessed weekly during the 28 day warm room period. Scald developed only on air-stored fruit after 3 months plus 7 days at 70 °F, and on air- or 1.5% O₂-stored fruit after 5 months plus 7 days at 70 °F. After 7 months, scald was present at removal from cold storage on air-stored fruit, and on fruit stored in 1.5% O₂ after 7 days at 70 °F following 10 days in air at 33 °F (Table 9). Fruit stored in 0.5% O₂ then 10 days at 33 °F in air did not scald in 28 days at 70 °F, but did scald when held 21 days at 33 °F in air after CA plus 28 days at 70 °F. Fruit were not marketable at that point due to low quality (soft, yellow, greasy). After 9 months, fruit stored in either O₂ regime developed scald when moved to air storage at 33 °F followed by 7 days at 70 °F, and all fruit was marketable at that point. This example demonstrates the low O₂ benefit (at 0.5 compared to 1.5% O₂) for scald control is detectable through 7 but not 9 months CA.

Month	Treatment	Days in RA at	Days at 70 °F after RA until	Scald Incidence
		33 °F after CA	superficial scald symptoms	%
7	Air	10	0	67
	O ₂ : 1.5%	10	7	100
	0.5	21	28	22
9	Air	10	0	72
	O ₂ : 1.5%	10	7	100
	0.5	10	7	25

Table 9. 'Granny Smith' scald incidence following storage. Fruit moved from CA to 33 $^{\circ}$ F in air, then to 70 $^{\circ}$ F after 7 or 9 months.

A similar study conducted with 'Delicious' apples was unsuccessful as no fruit developed scald.

Objective 4. Identify what if any limits for CO_2 exist during apple storage in oxygen below 1%.

Experiments were conducted with 'Granny Smith' and 'Fuji' apples. Although normally at low risk of CO_2 injury under CA, 'Granny Smith' was used due to the utility of CA in O_2 less than 1% for scald control. 'Fuji' was used due to its high CO_2 injury susceptibility.

'Granny Smith' apples were stored in air or CA with 0.3 (determined with HarvestWatchTM) or 1.5% O₂ combined with 1,3, or 5% CO₂. No change in chlorophyll fluorescence was detected related to CA CO₂ concentration. No symptoms of CO₂ injury were observed on fruit evaluated after 3,6, or 9 months plus 7 days at 70 °F (Table 10). Fruit stored at 0.5% CO₂ were greener compared to fruit stored in 1.5 or 3% CO₂. Fruit stored in CA developed less core- and senescent browning compared to air-stored fruit (except for fruit stored in 1.5% O₂, 0.5% CO₂).

% O ₂	% CO ₂	Peel color	% CB	% SB	% scald	lbs
air	air	1.9a	13a	92a	100a	11.4b
0.3	0.5	1.0c	4b	0b	Ob	18.0a
	1.5	1.9a	0b	0b	Ob	17.3a
	3.0	1.5b	0b	0b	Ob	17.4a
1.5	0.5	1.0c	13a	0b	83a	17.8a
	1.5	1.8a	Ob	0b	79a	16.6a
	3.0	1.7a	0b	Ob	Ob	17.4a

Table 10. 'Granny Smith' color, disorders, and firmness after 9 months storage at 33 °F. Low O_2 setpoint determined by monitoring fruit chlorophyll fluorescence. Fruit held 7 days at 70 °F after removal from storage. Means (n=18) followed by different letters are significantly different, Tukey's HSD, p<0.05.

'Fuji' fruit harvested with slight-moderate watercore, starch score 4.2 (18 fruit average) were stored at 33 °F in 1% O_2 with 0.5, 1.5, or 5% CO_2 . In 1% O_2 , no change in fruit chlorophyll fluorescence was detected at CO_2 concentrations up to 10%. Symptoms of CO_2 injury (core browning, cavitation) were observed after 1 month storage for fruit stored at 5% CO_2 (Table 11). As with 'Honeycrisp' (Table 7), risk and development of CO_2 injury was not indicated by chlorophyll fluorescence assessment.

% CO ₂	% core browning	% cavitation	
0.5	0	0	
1.5	0	0	
5.0	27	11	

Table 11. 'Fuji' apple internal disorders after 1 month storage. Fruit were held 7 days at 33 °F in air, then in 1% O_2 with 0.5, 1.5, or 5% CO_2 . Fruit held 7 days at 70 °F after removal from storage.

EXECUTIVE SUMMARY

Project results indicate 'Honeycrisp' postharvest quality, usually not limited by firmness/texture, is best managed by optimizing harvest maturity, avoiding chilling injury, and recognizing low O_2 or high CO_2 can induce damage in CA. Late maturity and harvest increases postharvest disorder risk, therefore horticultural practices that enable maturation and red color development to occur relatively early will contribute to successful postharvest management. How best to achieve early maturation remains to be defined, however, experience with other cultivars indicates nitrogen and crop load management are important factors regulating fruit development. 'Honeycrisp' also appears sensitive to pre-harvest stress, particularly temperature, that may contribute to diffuse browning we have observed in some trials and in samples brought to the lab by fieldmen. This tendency has been noted in other North American production areas as well. Situations where harvest is delayed due to lack of red color development can increase risk of postharvest disorders.

'Honeycrisp' can also be sensitive to post-conditioning storage temperature, and CA environments with very low O_2 or high CO_2 . Low temperature and low O_2 sensitivity do not appear to be quality management issues due to 'Honeycrisp's slow softening characteristic, and utility of 36 °F and 2 % O_2 for reduced loss of acidity, greasiness delay, and slower ground color change. Ripening-related volatile production is also slowed at moderate O_2 concentrations, a factor we feel is important for long-term storage quality due to delay in production of ethyl volatiles that can contribute to over-ripe flavor and taste. 'Honeycrisp' high CO_2 sensitivity to be less than that of 'Braeburn' and 'Fuji', and while controllable by DPA, reasonable CO_2 storage management is likely to be sufficient to control CO_2 induced disorders.

Use of 1-MCP as SmartFresh® can slow 'Honeycrisp' ripening similar to CA alone, and some SmartFresh® CA combination benefits are evident. SmartFresh® does not appear to enhance chilling disorder susceptibility, however, as with other CO₂ sensitive cultivars, CO₂ management of SmartFresh® treated 'Honeycrisp' fruit should receive close attention.

'Honeycrisp' temperature management, particularly during the conditioning period after harvest, is an area where further research may be productive. Are fewer days at temperatures higher than 50 °F needed to acclimate fruit to avoid chilling injury? Higher temperatures typically cause more fruit acid to be consumed and less acid is a factor limiting edibility of fruit from long-term storage.

Characterization of metabolic events preceding and following chilling injury development are providing insight into the disorder development process. Further work is needed to determine if monitoring of specific compounds has utility as predictive or diagnostic tools. Information to date raises many questions about the disorder process, particularly the role of acylated sterol glycosides in stress response and injury. Information from this project was used in the successful SCRI grant proposal, and further work in this area is continuing with SCRI funds.

Monitoring fruit chlorophyll fluorescence as a function of storage O_2 concentration is increasingly used by industry for CA storage management. Our results indicated the longevity of 'Granny Smith' scald control from low O_2 storage is enhanced as room O_2 content decreases through 7 but not 9 months. While our results do not indicate fruit stress from high CO_2 is detectable using the HarvestWatchTM system, further work is needed to determine safe CO_2 concentrations in rooms where O_2 is managed using chlorophyll fluorescence based information for O_2 content. While its has long been known that apple fruit CO_2 sensitivity increases as O_2 content decreases, specific safe O_2/CO_2 combinations for individual cultivars remain to be determined.