FINAL PROJECT REPORT
WTFRC Project Number: PR-07-706

Project Title: Factors influencing development of d'Anjou pear scald and speckling

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Cooperators: none

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WTFRC Collaborative expenses: none
Total Project Funding:

Budget History:

Item	Year 1: 2007	Year 2: 2008	Year 3: 2009
Salaries	25,375*	26,690*	27,490*
Benefits	0	0	0
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	500	500	500
Travel	0	0	0
Miscellaneous	0	0	0
Total	$25,875	$27,190	$27,990

Footnotes: *0.5 salary for GS11 postdoctoral research associate

Objectives:

1. Characterize what if any relationship exists between fruit physiological status (including peel metabolic profiles) at harvest and lot to lot susceptibility to low O_2-induced peel speckling or superficial scald development during storage.

2. Identify changes in pear metabolic profiles that are coincident with development of speckling and superficial scald induced using postharvest environments or protocols known to enhance speckling or scald development.

3. Develop postharvest protocols to manage scald and speckling development using available or new postharvest technologies as appropriate.

The risk of peel disorder development, particularly superficial scald (scald), during storage of d'Anjou pear is a significant factor influencing postharvest management strategies for this cultivar. Issues (efficacy, expense, logistics, residues) with current disorder control strategies based on antioxidant chemical application suggest development of additional strategies less reliant on chemical use would be of benefit to the industry. Storage of d'Anjou fruit at less than 1% O_2 has prevented scald development under experimental conditions, however, the risk of development of another peel disorder, speckling, and the internal disorder pithy brown core, increase when fruit are stored at less than 1% O_2. Previous research indicates storage at less than 1% O_2 can effectively control scald while avoiding anaerobiosis and potential development of off-flavors. A comprehensive survey of peel metabolism based on a metabolic profiling approach has potential to identify perturbations in metabolic pathways that may be linked to speckling development. Development of speckling is lot specific, but factors that initiate and influence the occurrence of speckling and lot to lot susceptibility have not been characterized. Identification of factors that influence speckling development may provide a means to develop low O_2-based storage protocols that control speckling while also controlling scald. Effective speckling control strategies that allow the use of less than 1% O_2 during d'Anjou storage have the potential to alter postharvest management of this cultivar to promote retention of fruit quality while avoiding development of peel disorders.

Significant Findings:

- Ultra-low O_2 at 0.5% or lower prevented scald in all lots.
- Speckling developed on 1 in 8 lots during the first two years of the project.
- Core browning and core cavitation developed in all lots stored at or below 0.8 % O_2.
- Delaying CA up to 10 days after harvest did not prevent low O_2 core disorders.
- Fruit stored at the critical O2 concentration developed calyx browning.
- Differences in peel metabolic profiles for fruit stored in 0.4% or 1.5% O_2, or air were detected at one month and continued through 9 months.
- Some lots stored in UA through 4 months did not soften to eating ripe during 7 days at 68 ^{o}F.

Results and Discussion

Of the nine orchard lots used in the three years of this project, the O_2 concentration at which a change in chlorophyll fluorescence was detected was 0.2% for 7 lots and 0.3% for the other 2 lots. Fruit maturity at harvest based on firmness, soluble solids, titratable acidity and color was not associated with the different critical O_2 concentrations. The fluorescence signal returned to a base level following an increase in O_2 to the final setpoints (0.4 and 0.5% O_2). These values are in the same range as those observed in our previous work with Anjou pears.

Scald did not develop on fruit stored in O_2 at or below 0.5%. Scald development on fruit from all lots stored at 1.5% O_2 was lower compared to fruit stored in air but higher than fruit stored at lower O_2 concentrations. Delaying CA for up to 10 days did not affect efficacy of scald control regardless of the CA O_2 concentration, and an initial low O_2 stress at 0.1% between days 7 and 13 after harvest did not change scald efficacy or result in tissue injury.

Peel speckling was observed on one lot in the 2008-09 season (Table 1). Speckling developed during the 7 day warm room period after removal from storage after 9 months. Symptoms were present on fruit previously stored in air (89% incidence) and on fruit stored in 0.2% O_2 (11%) or 1.1% O_2 (15%). The lack of symptoms at removal from storage is not typical compared to previous experiments. Fruit in the same experiment stored at 0.5, 0.8, or 1.4% O_2 did not develop speckling in contrast to previous reports where speckling increased as CA O_2 concentration decreased. Fruit on which speckling developed were not subjected to metabolic analysis was not part of this experiment.

Core disorders (Figure 1) were present in fruit from all lots stored at or near the critical O_2 concentration at which a change in chlorophyll fluorescence was detected (Table 2). In no case was a change in fluorescence evident after the initial O_2 increase to the final O_2 setpoint. This indicates that metabolic stress occurring at the low O_2 setpoint over the course of the experiments is not detectable by monitoring chlorophyll fluorescence. Core disorders ranging from browning of the seed cavity walls to severe cavitation were evident in as little as 2 months after harvest for many lots. While seed cavity wall browning could by some be considered a minor defect as it impacts fruit tissues typically discarded, the symptoms were very noticeable when present.

Calyx-end browning (Figure X) occurred only on fruit stored at the critical low O_2 concentration. The affected area had a russet-like appearance beginning around the calyx opening and increasing in size over time. Symptoms were observed as early as 2 months after harvest. Decay spread through the affected tissues at later (6-8 months) storage durations.

Shrivel was an issue in some experiments for fruit stored at or below the critical O_2 concentration (Table 1). For example, all fruit stored at 0.05 or 0.2% O_2 for 6 or 8 months had some shrivel in a 2008/09 experiment. Fruit stored in air or in 0.5 to 1.4% O_2 did not show shrivel. It is possible fruit stored at the lowest O_2 concentrations experienced higher water loss due to lack of cuticle development under low O_2 conditions.

Fruit from 5 of the 9 orchards stored under UA conditions softened slower relative to fruit stored in air or CA after 2 months storage. This residual impact of low O_2 CA has been observed in our previous work with pears. A possible means to overcome the low O_2- induced softening delay may be to delay CA. An experiment where half of a lot of fruit was held 7 days at 33 ^oF prior to establishment of low O_2 CA softened normally after 2 months compared to fruit from the same lot for which CA was established within 36 hours of harvest.

Ethanol accumulation during low O_2 stress has been associated with physiological disorders in other studies. We did not find a clear relationship between ethanol content and injury development in fruit stored near the critical low O_2 concentration until after symptoms were observed (Figure 2). Fruit from 5 lots were stored 0.2% O_2 above the critical O_2 concentration as determined by chlorophyll fluorescence, or were stored at 1.5% O_2. Fruit from all lots stored close to the critical O_2 concentration developed core disorders, but in only 3 of 5 lots was ethanol determined to be higher at some point during storage in the low O_2 fruit. While several lots stored using HarvestWatch technology had higher ethanol after 12 weeks compared to fruit stored in standard CA, earlier

occurrence of core browning indicates ethanol accumulation in this case may be and effect rather than a cause of the disorder.

Analyses of many fruit compounds indicated fruit could be chemically differentiated by storage treatment after one month (Figure 3). The differences in these profiles were due to a number of individual compounds including but not limited to amino and organic acids, sugars, vitamins, antioxidants, sterols, and pigments. Patterns of some individual compounds included a large reduction in vitamin C during the first 4 weeks after harvest regardless of storage regime and a more moderate reduction in vitamin E throughout the storage period. None of the storage treatments effectively slowed loss of any of these compounds over the course of the storage period (Figure 4). Core browning occurred in fruit stored in UA at 2 months and after indicating levels of these 3 compounds with anti-oxidant activity do not appear to be related to development or resistance to core browning.

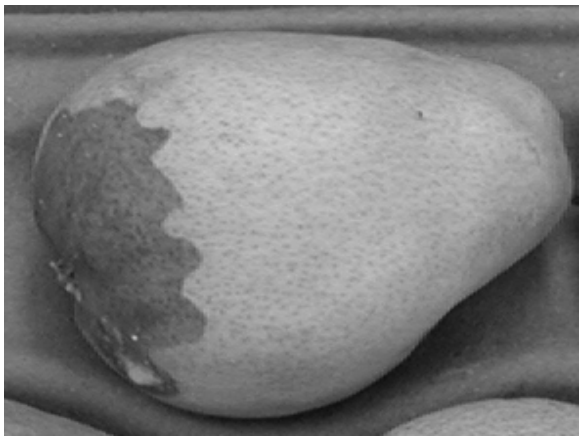
A number of peel chemicals including including ursolic acid and β-sitosterol with putative cholesterol-lowering and antioxidant capacity were found in the peel and were differentially impacted by both storage atmosphere and storage duration. A number of related compounds that may be sterols or other triterpenoids were also detected. Considerable clinical evidence in the medical literature links phytosterols with positive health effects related to their antioxidant properties. These compounds were also differentially impacted by storage environment with both increase and decreased content observed related to storage environment and storage duration. We have recently observed similar compounds in apples have patterns related to superficial scald development. More work is needed to confirm the identity of these possible sterols and to determine what if any relationship they have to peel disorders. At harvest indicators indicating the potential for scald or other disorders were not identified during this project. Additional studies with multiple lots are needed to further investigate the potential for at-harvest prediction of disorder susceptibility.

Table 1. Peel speckling on Anjou pears. CO_2 in all CA treatments =0.5%. Fruit stored 9 months at 33 ^{o}F and evaluated after 7 days at 68 ^{o}F.

% O_2	% speckling	% scald	% core browning	% shrivel
Air	89	56	0	0
0.05	0	0	0	100
0.2	11	0	22	100
0.5	0	0	24	0
0.8	0	29	24	0
1.1	15	46	6	0
1.4	0	43	29	0

Table 2. Core browning incidence(%) in Anjou pears stored in controlled or ultra-low O_2 atmospheres or air. Ultra-low O_2 concentration = O_2 concentration at which change in chlorophyll fluorescence detected + 0.2%. *:stored in 0.5% O_2. **: evaluated after 3, 6, or 9 months.

Storage	1.5% O_2, 0.5% CO_2			0.4% O_2, 0.5% CO_2			air	
Months	2	4	6	2	4	6	2	4
Orchard 1*	0%	0	0	39	6	28	0	0
2	0	0	0	11	89	67	0	0
3	0	0	0	33	39	39	0	0
4*	6	11	6	83	56	67	0	0
5	17	28	33	61	63	67	0	0
6	0	0	0	56	39	94	0	0
7	17	17	6	17	18	22	0	0
8**	0	12	29	0	6	24	0	0
9	17	0	19	50	50	50	0	0



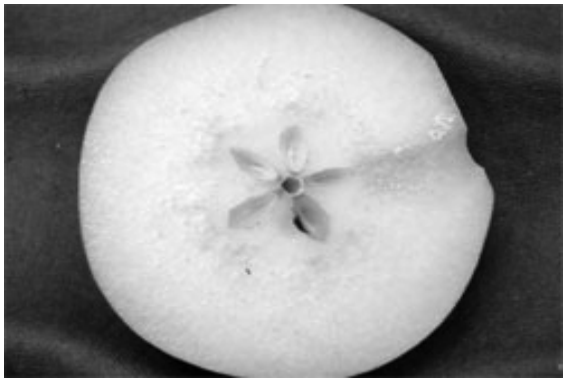
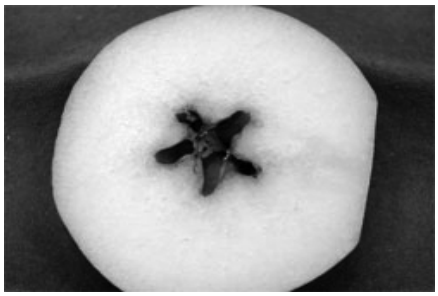
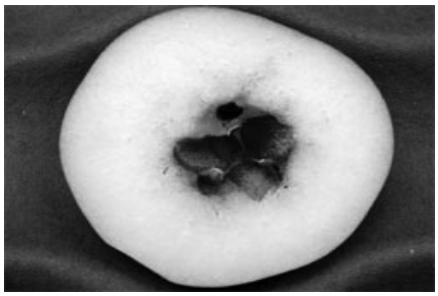


Figure 1. A. Calyx-end breakdown. B. Undamaged pear.





C. Core browning.

D. Core browning with cavitation.

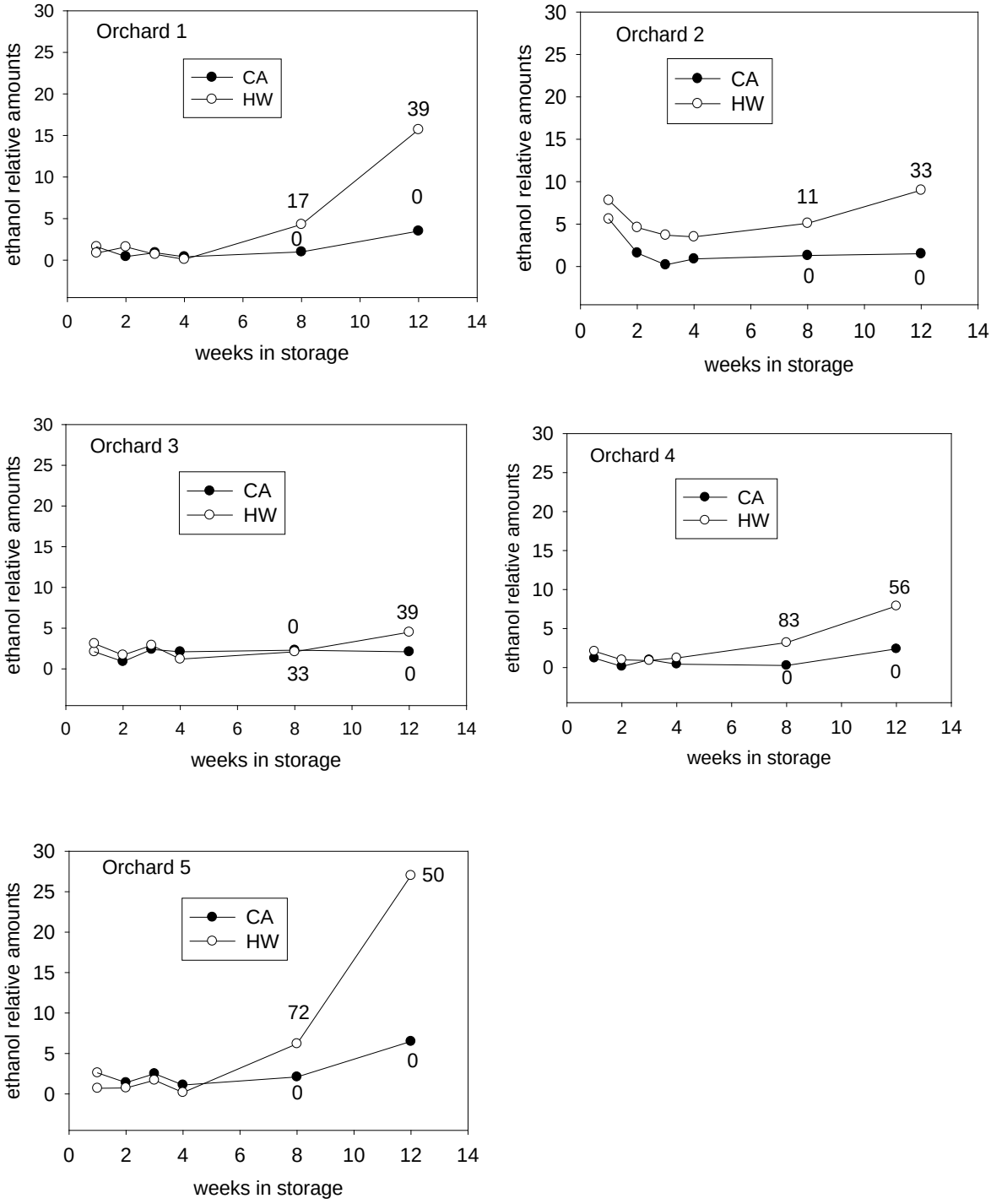


Figure 2. Ethanol content of Anjou pears after storage in 1.5% O_2 (CA) or 0.4% (orchards 2,3,5) or 0.5% O_2 (orchards 1,4) with 0.5% CO_2. HW: Harvest Watch: chlorophyll fluorescence monitoring equipment used to determine low O_2 setpoint.

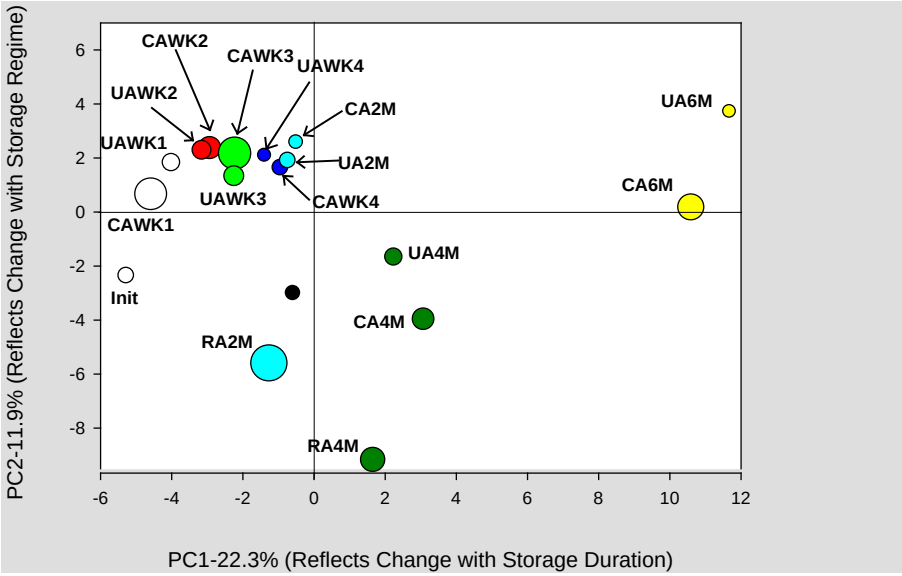
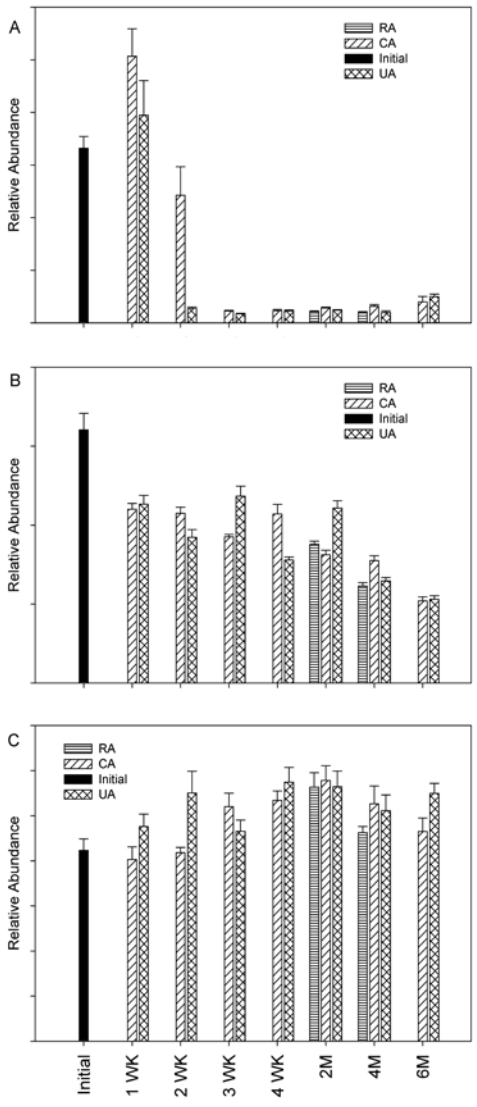


Figure 3. Separation of Anjou peel metabolic profiles based on storage duration and storage atmosphere. Fruit were stored up to 6 months in air (RA) or 0.5% CO_2 with 1.5 (CA) or 0.5 (UA) % O_2 at 33F.

Figure 4. Content of Anjou pear (A) vitamin C, (B) vitamin E, and (C) β-carotene during storage. Fruit were held at 33 ^{o}F in air (RA), 1.5% O_2 with 0.5% CO_2 (CA), or 0.5% O_2 with 0.5% CO_2 (UA). Samples were collected the day fruit were removed from storage.



Executive Summary

Monitoring chlorophyll fluorescence did not predict development of Anjou internal disorders. While some lots did not develop scald when stored based on identification of the critical O_2 concentration at which a change occurred in chlorophyll fluorescence, the risk of core disorders was high for fruit stored at less than 1% O_2. Other potential issues to be addressed prior to commercialization of this technique include a lack of early season (2-4 months after harvest) softening as well as calyx breakdown and shrivel should the critical O_2 concentration be underestimated. Implementation of low O_2 storage for scald control will require further research to identify risk factors at harvest or during storage that can provide a means to avoid low O_2 induced disorders while affording scald control. The lack of typical peel speckling in multiple lots and seasons is puzzling but was offset in these studies by the propensity for core disorder development in fruit stored under low O_2. The lack of peel speckling in these studies in our opinion does not allay the risk of this disorder that has been documented to occur under low O_2 storage. This is due to the lack of known system-wide changes in Anjou production practices that would have eliminated susceptibility to this disorder. Until more information regarding speckling and its causes are known, additional research focused on Anjou responses to low O_2 are likely to encounter the disorder.

Ethanol has long been associated with fruit disorders ranging from off-flavors to a variety of peel and internal browning or breakdown. The association has typically been observed due to an accumulation of ethanol in fruit with one or more quality problems. High concentrations of ethanol in fruit are typically the product of periods of O_2 stress when the O_2 concentration is too low to support normal metabolism. However, ethanol is usually present during normal ripening, a fact that prevents establishment of an unquestionable cause and effect relationship between ethanol accumulation and browning disorder development. The studies conducted for this project do not support a direct relationship between ethanol and subsequent development of injury as the largest accumulation occurred after injury began to be observed. While further studies of ethanol and related metabolism are needed in relation to development of commercial low O_2 storage for Anjou, the current results indicate at least that monitoring of ethanol alone is likely to not be sufficient to avoid disorder development.

Metabolic analyses revealed a number of compounds not known to be present in pear fruit that based on clinical trials have positive effects on human health. These compounds are sterols and are referred to as phytosterols in the relevant medical literature. Our work showed the compounds we think are sterols exhibit various patterns during storage in relation to duration and atmosphere. Both increased and decreased trends were found for different compounds, and further work to characterize these compounds in relation to storage environments as well as the onset and progression of disorders may provide a means develop at harvest or during storage information that can predict and/or diagnose disorders. This information, particularly at harvest, would have utility in postharvest management to assist in decisions related to at-harvest treatment for scald. Similar compounds in apple fruit have recently been identified by Dave Rudell as acylated sterol glycosides and their metabolism appears to be very responsive to low temperature as well as controlled atmospheres. There is evidence to support a role for some of the apple compounds as a means to resist stress or as indicators stress has occurred. Based on those results, future work with pears to determine if similar properties exist among the pear compounds found to date may be a means to explore ways to mitigate the negative responses to low O_2 storage.