FINAL PROJECT REPORT

Project Title: Ripening compounds use for improved quality of fresh pear

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Total Project Request: Year 1: 29,478

Other funding sources –
CID Biosciences - $15,000 (equipment loan)

Budget 1
Organization Name: WSU **Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564 **Email address:** carriej@wsu.edu

Item	2013
Salaries	
Benefits	
Wages[a]	8,640
Benefits	838
Equipment[b]	5,000
Supplies[c]	7,000
Travel	3,000
Miscellaneous[d]	5,000
Plot Fees	
Total	29,478

Footnotes:

a. Support for two undergraduate students for 24 weeks to assist in fruit handling and analysis
b. Custom manifold to link the CID ethylene and CO_2 sensor to chambers ($5,000)
c. Ripening compounds, consumables and gas chromatography supplies ($7,000)
d. WSU instrumentation facility charges for integration of CID Biosciences equipment

OBJECTIVES

Original objectives of this project concerned gaining a greater understanding of genetic responses to 1-MCP treatment, and developing means of overcoming variability in restoration of ripening capability. Experiments were conducted in Bartlett and Anjou varieties, which comprise 95% of the fresh market. Ripening-stimulating compounds (RCs 1 and 2) were found to accelerate ethylene release in preclimacteric Bartlett and Anjou fruit treated at current industry standards of SmartFresh (1-MCP,300 and 100 ppb, respectively). In response to committee feedback concerning prelimary results, the project and objectives were amended to *Determine the physiological effect of RC1 and RC2 on short, medium and long term stored 1-MCP-treated Bartlett and Anjou fruit.*

Following subcommittee meetings, specific aims A, B, and C were developed to address the project objective as follows: (*A*) Test different concentration/time variables of ripening chemicals (RCs) for efficient ripening of SmartFresh-treated Bartlett and Anjou fruit harvested at optimal maturity stored for short, medium and long-term, (*B*) Evaluate control, SmartFresh-treated and SmartFresh (SF)/ RC-treated fruit for CO_2 levels, ethylene levels using upgraded equipment with additional data collection for scuffing and dehydration (weight loss), and (C) Collate and correlate data and perform statistical analysis.

Pursuant to this objective, we expanded experiments with Bartlett and Anjou fruit treated with SF (at 100 and 300 ppb, respectively) and untreated fruit from the 2013-2014 harvest and storage season. Unconditioned SF-treated and untreated Bartlett fruit were retrieved from Blue Star Growers. Fruit from 100-grade cases were unpacked in Pullman; all SF-treated fruit were lightly marked on the neck to indicate SF pretreatment through subsequent steps of the experiment. A total of 200 (100 SF, 100 untreated) fruit of each variety were then pooled and submerged in 8.0 liter aqueous solutions of four different treatment levels of RC1 or 2 (high concentration, middle, low, and a no-RC control). Each solution was then covered during the exposure time with plastic sheeting to minimize evaporative water loss. Submerged fruit were incubated for 24 hours at room temperature (68°F). A shorter exposure time of 12 hours was included for Anjou testing for the 2013 harvest season to characterize dose dependency. After this period, fruit were removed from RC solutions, lightly towel-dried, and placed in 6.0 liter flow-through respiration chambers held at 66°F, with a 100 mL/min dynamic flow rate for 5-6 days (Figure 1). Four replicate fruit of each unique SmartFresh pretreatment/variety/RC combination were placed into each of four replicate chambers. Fruit incubated in these chambers for 5-6 days at 66°F. Gas concentrations were measured at 8 hour intervals from each chamber in response to the 4 levels of RC dosage. Carbon dioxide and evolved ethylene were measured from headspace air in the chambers by gas chromatography. Flesh firmness, soluble solids measurements and peel tissue were obtained from a subsample of fruit from each unique treatment combination immediately after the 24 hour soak, and again following the 5-6 day incubation. Throughout the experiment, peel samples were obtained to assess ripening-regulatory and ethylene-related gene expression in response to each of the RC treatments. Peel tissue was immediately frozen in liquid nitrogen for gene expression analysis.

SIGNIFICANT FINDINGS

Significant findings for Objective 1

- RC1, while effective, is challenging to incorporate in a commercial setting, and can produce undesirable peel damage at effective concentrations.
- Ethylene stimulation in SmartFresh-treated, unconditioned Bartlett and Anjou fruit after 24 hours in RCs 1 and 2 solutions results in a 2-fold increase compared to untreated fruit.
- This 2-fold threshold is exceeded after 4-5 days in SmartFresh-treated Anjou, and faster in SmartFresh-treated Bartlett.

- Ethylene stimulation is also seen in untreated, unconditioned fruit of each variety.
- A minimum of 24 hour RC exposure elicits measurable responses in SmartFresh-treated fruit of each variety.

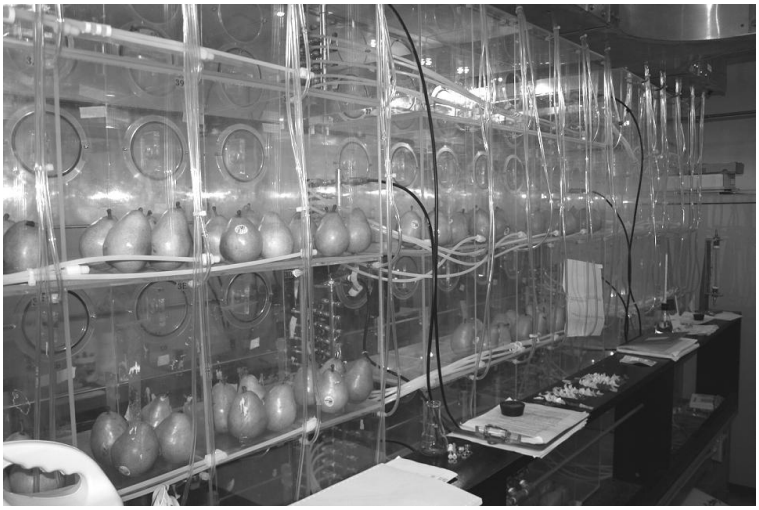

Figure 1. Anjou fruit in 6.0 liter chambers in existing flow-through respiration system.

RESULTS & DISCUSSION

The following table reviews the progress and milestones achieved for each objective outlined in the project.

Ripening Chemicals Use for Improved Quality of Fresh Pear				
Begun in first quarter, 2013	**Year 1**			
	May - July	Aug - Oct	Nov - Jan	Feb - April
Objectives and Goals	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Objective 1: Test Ripening Chemical (RC) dosage and time durations for Bartlett and Anjou fruit treated with SmartFresh				
a. Test different concentration/time variables of ripening chemicals (RCs) for efficient ripening of SmartFresh-treated Bartlett and Anjou fruit harvested at optimal maturity and stored for short, medium and long-term				
b. Evaluate control, SmartFresh-treated and SmartFresh/RC-treated fruit for CO_2 levels, ethylene levels using upgraded equipment along with scuffing and dehydration (weight loss)				
c. Collate and correlate data and perform statistical analysis				

Results of RC2 testing demonstrated effective ethylene stimulation in RC exposures in SF-treated and untreated Bartlett and Anjou fruit. Shorter exposure times of 12 hours failed to demonstrate acceleration of ethylene over basal levels from a 24 hour drench (Figure 2A-2D). For this reason, 8 and 4 hour RC exposure times were not tested. In Bartlett, the RC-dependent ethylene response exceeded a 2-fold threshold. Results of shorter RC exposures indicate a correlation with retention of

flesh firmness (Figures 3A-3D). Overall, ethylene and ripening stimulation in Anjou occurred over a longer time frame, suggesting RCs for fresh pear products may be most effectively applied prior to shipping to wholesale or retail or markets. In contrast to RC1, which exhibited responses which would make handling and commercial implementation of the chemical challenging such as excessive peel damage, RC2 treatments have elicited increases in SF-treated Bartlett and Anjou fruit over comparatively longer time frames. It is a relatively stable, benign compound which may be more amenable to use in the postharvest chain than RC1 through fogging or drenching applications. RC2 exposures in excess of or beyond 1.0mM generated undesirable peel tissue damage. This suggests longer exposures in RC2 concentration at or below 0.5mM may enhance penetration of the compound into the interior of the fruit, increasing the ethylene and ripening response. Overall, 0.5mM RC2 has generated the best balance of ethylene and ripening responses in SF-treated fruit without exhibiting undesirable peel tissue damage. We hypothesize greater penetration of RCs into the pear interior elicits stronger ethylene and ripening responses.

The existing gas chromatography-based respiration system experienced multiple significant technical failures resulting in skipped sampling times, complete lack of data capture, and even total system shutdown. These failures required repetition of entire experiments which depleted sample inventory, and slowed the acquisition of meaningful data during an already small annual window for this work. Ethylene and CO_2 measurement is a critical component of postharvest fruit quality research. In order to understand the genes involved in various post-harvest processes and issues it is critical to access the fruit during postharvest treatments. Our collaboration with CID Bio-Science allowed us to obtain two iterations of the CID-900 instrument. We have developed completed schematics for design and implementation of an updated, more reliable, more accurate, system utilizing this instrument which would have greater data resolution, and require less maintenance. The highly sensitive, patented, and costly technology used in the instrument does not allow the company to make it available for long-term demo use. We completed side-by-side comparison of a demo CID-900 instrument against the existing GC-based system during a 4 week loan period with the cooperation of CID Bio-Science and demonstrated enhanced data resolution; the CID-900 outputs both CO2 and ethylene data every minute as opposed to every 8 hours. This is seen in Figure 4, where the CID-instrument is logging ambient ethylene at levels 30-50% lower than results from the GC-based system, and on much shorter time intervals. Similar results were seen in 0.875 liter chambers filled with a single climacteric fruit (Figure 5). With this in mind, acquisition and installation of this design remains a top priority for postharvest research at the WSU Pullman campus. Engineers at CID have actively collaborated and encouraged construction of a modular, high-throughput respiration and ethylene monitoring system for use at WSU, and have volunteered to produce the data-logging software to yield a fully automated system for future use in pear genomics research. This collaboration has involved two other postharvest labs in the department. We are also working closely with Frank Younce at WSU to design and implement a system that would fit the needs of all postharvest research at WSU.

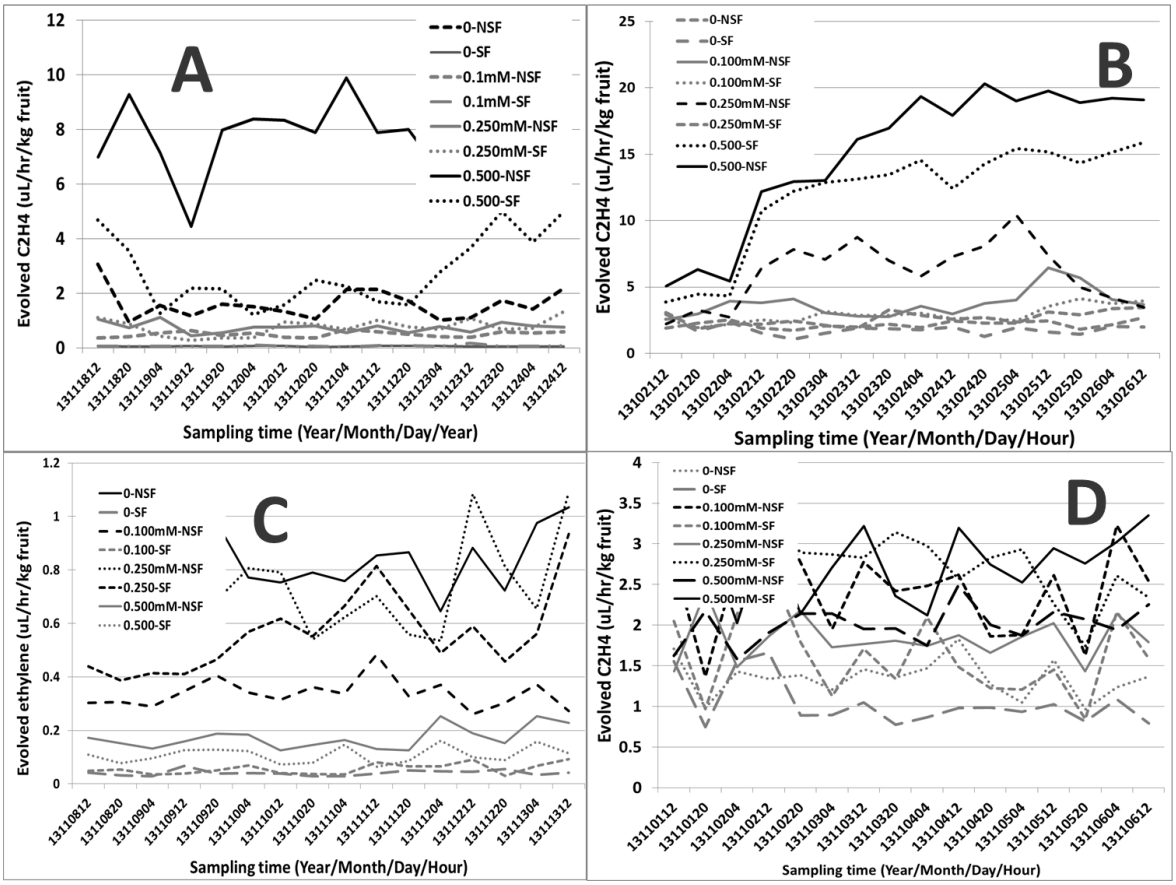

Figure 2. Evolved ethylene (A) from 12-hour RC2-exposed Bartlett, (B) evolved CO2 from 12-hour exposed Bartlett, (C) evolved ethylene from 12-hour exposed Anjou, and (D) and 12-hour exposed CO2. Ripening acceleration is dramatically lower in all experiments relative to longer drench periods.

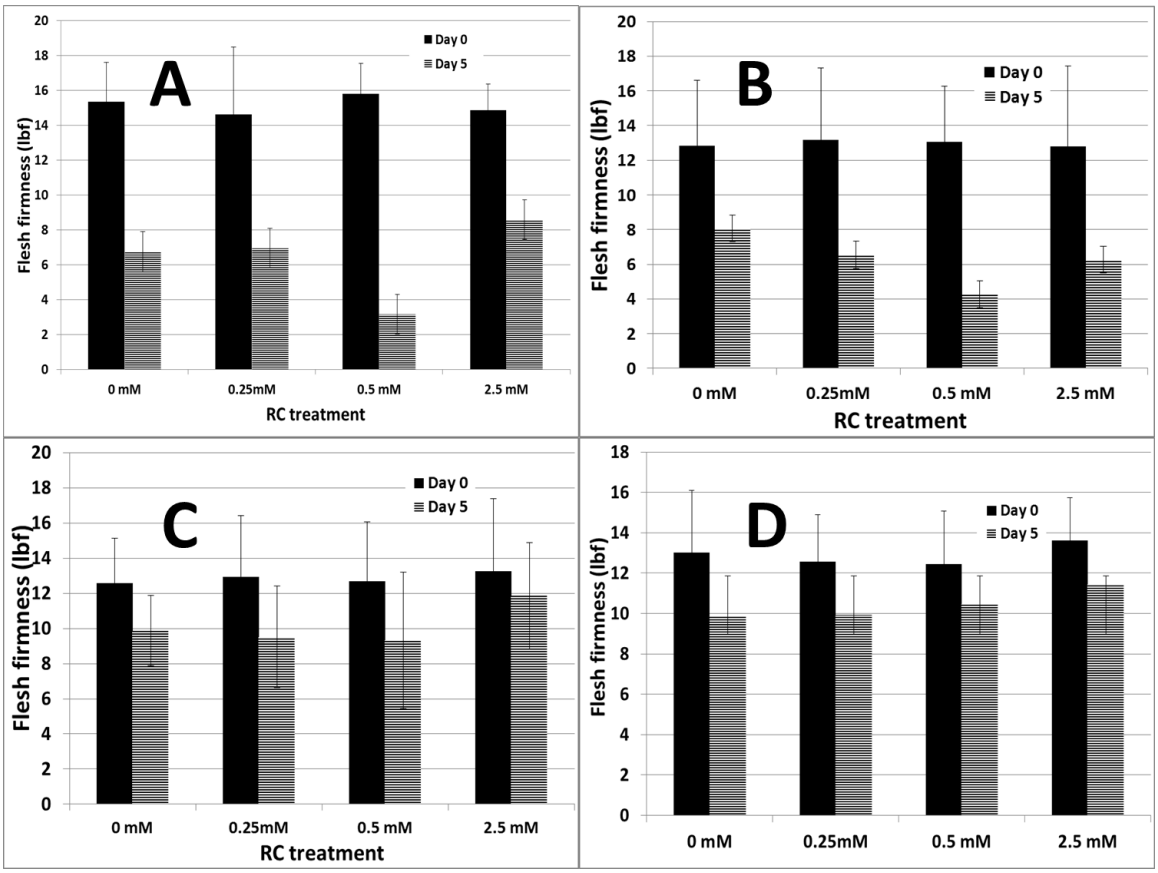

Figure 3. Flesh firmness reduction in SmartFresh-treated, RC2 exposed Anjou fruit from the 2013 season. (A) and (B) represent firmness reduction in Bartlett and Anjou (respectively) after 24 hour RC2 exposure. (C) and (D) represent firmness reduction in Bartlett and Anjou (respectively) for 12 hour RC2 exposure. Error bars represent standard deviation from the mean. The 12 hour exposure prevents significant losses in flesh firmness associated with ripening.

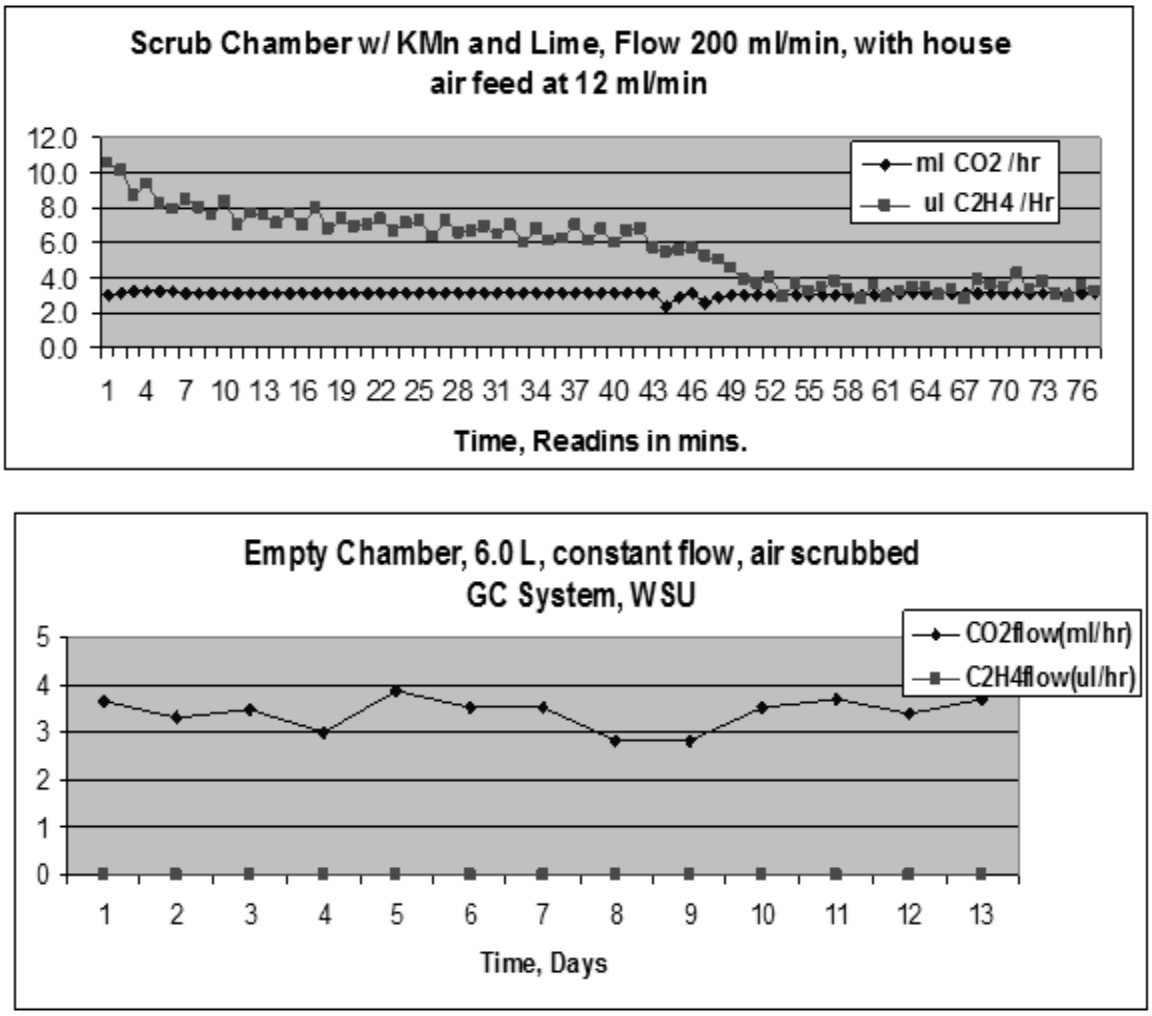

Figure 4. Blanking calibration in GC based system (top) and CID-900 instrument (bottom) with 400 mL/min dynamic flow through sample chamber. Note continuously lower ethylene values than GC-based technology.

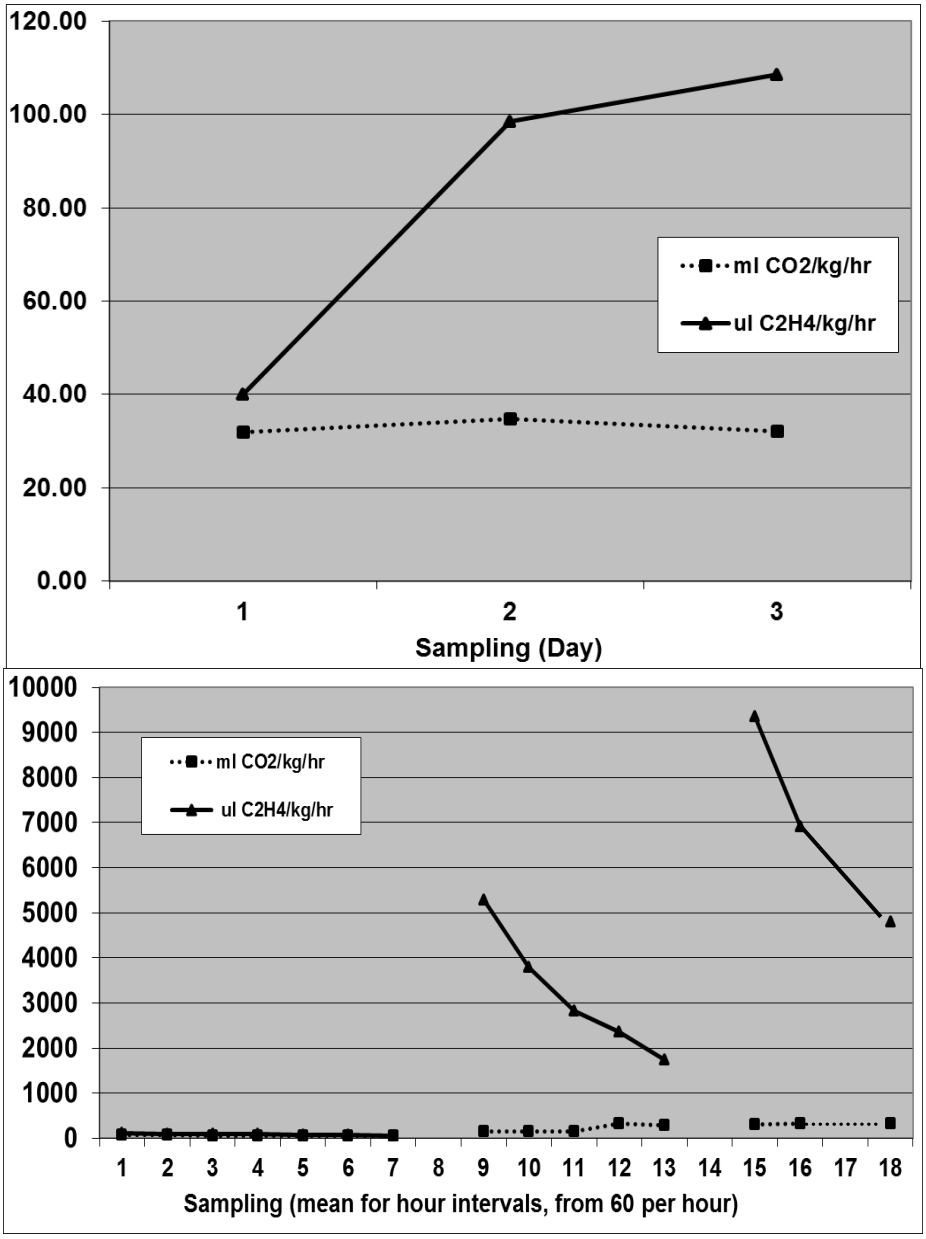

Figure 5: Comparison of existing gas-chromatography based respiration monitoring (Top) and CID-900 real-time ethylene and carbon dioxide monitoring instrument (Bottom). A dynamic flow was established in the headspace of the chamber measured by the CID-900 instrument between sampling events 7 and 9 at 400 mL/min flow rate. Note enhanced resolution in data from CID-900 instrument in comparison.

The aim of this project was to determine the physiological effects in Bartlett and Anjou pear in response to RC1 and 2 treatment. Despite industry adoption, Bartlett and Anjou pear exhibit variable ripening ability in response to SmartFresh and common postharvest storage regimes. Evaluation of responses to these RCs is critical in order to gauge their utility for future use as a companion product in the postharvest chain to 1-MCP products such as SmartFresh. The ability to accelerate ethylene release and ripening in 1-MCP treated pear products can minimize loss of quality through storage and transport and guarantee complete control over fruit quality throughout the postharvest chain. While access to the CID instrument was limited, we were able to test its accuracy, resolution and potential for use in an upgraded flow-through respiration system for future pear genomics study. We quickly

realized further testing of RC1 may not best address the goals of the project. In contrast, RC2 holds greater potential for use in the postharvest chain of fresh pear compared RC1 due to capability of handling longer response times without loss of fruit peel quality. Ethylene production exceeded a 2-fold threshold in Bartlett and Anjou after 24 hour RC2 exposure times only. Flesh softening was not accelerated in response to reduced RC2 exposure. Greater response trends were seen in Bartlett and untreated (SmartFresh) fruit in general. As a whole, this work has demonstrated a novel molecular mechanism to induce ethylene production in unconditioned, SmartFresh-treated pear in a controlled manner. RC2 concentrations in excess of 1.0 mM generally elicited undesirable peel damage. Increasing RC2 concentrations to this point do not result in significantly larger ethylene and ripening responses in Bartlett and Anjou fruit. A 0.5 mM RC2 drench elicited a balance between the strength of these responses and undesirable peel damage.

References

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2. Chen and Spotts. 2005. Int. J. Fruit Sci. 5(3):3-17.
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EXECUTIVE SUMMARY

This work has narrowed effective concentrations and exposure times for future ripening acceleration responses to be optimized. More work is needed to quantify the impacts of fruit maturity, genotype, RC combinations and strategies for penetration of RCs into the fruit interior.

- RC1, while effective, is challenging to incorporate in a commercial setting, and can produce undesirable peel damage at effective concentrations.
- Ethylene stimulation in SmartFresh-treated, unconditioned Bartlett and Anjou fruit after 24 hour drench in RC2 exceeding a 2-fold increase over untreated fruit.
- 2-fold threshold is exceeded after 4-5 days in SmartFresh-treated Anjou, faster in SmartFresh-treated Bartlett.
- Ethylene stimulation is also seen in untreated (SmartFresh), unconditioned fruit of each variety.
- A minimum of 24 hour RC exposure elicits measurable responses in SmartFresh-treated fruit of each variety.

Future directions

Results of this project have revealed a novel means to control ethylene release in unconditioned and SmartFresh-treated fresh pear products. To gain increasingly accurate data, void of sampling gaps due to system malfunctions, we will be implementing a flow-through respiration and ethylene monitoring system with greater sampling capacity and resolution. This will finally allow the amount of sampling throughput and resolution needed to gauge RC responses in various levels of pear maturity, additional exposure times, with different penetrants, etc., given the narrow window of time each year in which this data can be collected. Based on findings of data from existing experimental infrastructure, we aim to capture additional RC responses based on these considerations, while working with input from industry partners. Further experimentation in with various penetrants and agitation times will likely enhance the intensity and response time to RC2 exposures. Together, this information will provide knowledge required for effective RC use current and emerging fresh pear markets.